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Modelling the impact of two intertidal primary consumer mollusc species on microphytobenthic biomass

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A thesis submitted in fulfilment for the degree of Doctor of Philsophy to University of Dublin,Trinity College

Department of Zoology
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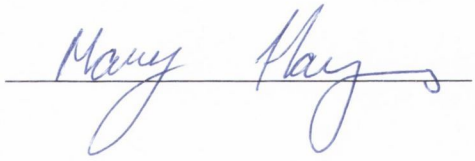

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Summary

As many marine intertidal benthic primary consumers are relatively small in body size, their individual impact on the environment is generally negligible. The combined influence of the extremely high numbers of individuals that can occur in certain habitats can have very significant impacts on their surroundings however. This study examined the potential impact of two dominant primary consumers on the primary productivity at two contrasting intertidal soft sediments habitats in Dublin Bay. Differences in sediment grain size, physical exposure and resultant primary production were recorded between sites.

Temporal and spatial surveys of the distribution, abundance and population size structure of the two consumer species, the infaunal bivalve *Tellina tenuis* and the surface deposit feeding gastropod *Hydrobia ulvae*, were conducted. Their distribution and abundance were compared with that of sediment chlorophyll *a* at the study sites, which is a useful indicator of relative primary production. The concentration of phaeopigment, the degradation products of chlorophyll, was also recorded. A carefully planned examination of the spatial variability in population parameters was conducted to facilitate the design of a medium- to long-term sampling programme. Abundance, size-distribution and photopigment concentration varied significantly between the low and high shores at both sites, being higher on the latter with only a few exceptions. High heterogeneity in the spatial distribution of all parameters examined was evident, with patchiness in photopigments being most marked. Seasonal minima and maxima in primary consumer and producer abundance were identified. Both consumer species studied occurred in high densities for much of the year, although seasonal patterns in abundance were observed. The mudflat site studied supported higher concentrations of photopigments and primary consumer abundance than the sandflat study site.

Aging and size-frequency analysis were conducted to identify distinct cohorts within the primary consumer populations. Differential growth rates, disturbance marks and failure to grow under laboratory conditions prevented completion of this task for *Tellina*, but not

for *Hydrobia*. *Hydrobia* displayed a relatively short growth phase in the field, with the main growth period restricted to only 2 months of the year. Two distinct recruitment pulses were identified in spring and autumn, but these merged by the end of the year due to differential growth and mortality. Recruitment pulses of *Tellina* were weak and seldom detected. Laboratory experiments highlighted conspecific density in particular as a strong regulator of *Hydrobia* growth, although food and temperature also imposed an effect. The adults and juveniles of this species displayed a preference for fine-grained sediments with high food content.

Isotope analysis suggested that the deposit feeder *Hydrobia* utilised both microphytobenthic and microphytoplankton food sources in varying proportions between seasons, while *Tellina* grazed more on the former. As *Hydrobia* cannot feed directly from the water column, this suggested that significant deposition occurred at the mudflat site. Attempts to quantify the feeding rates of both species in field and laboratory conditions were unsuccessful despite numerous attempts. Controversy surrounding the feeding strategy employed by *Tellina* could not be resolved in the laboratory despite the use of techniques suitable for detection of filter or deposit feeding. Previously published grazing rates were combined with field and laboratory data to estimate the total grazing impact of the consumer species on chlorophyll *a* at their respective sites. Size-specific grazing rates were derived and combined with field data to estimate daily population grazing potential of chlorophyll *a*. These estimates were adjusted to be more comparable to feeding rates and durations achievable in the wild. Despite very high consumer abundance and combined grazing rates, the constraints placed on feeding activity suggested that chlorophyll *a* associated food sources may not be limiting at these sites for a large proportion of the year.

Chapter 1. General Introduction

Primary consumers represent a vital component of many marine ecosystems, as they provide a pathway for nutrient transfer between primary producers and higher trophic levels. In many cases species within the latter group cannot directly utilise primary production themselves. Fluctuations in the abundance of primary consumers will therefore determine nutrient availability for secondary and tertiary consumers. There is some debate as to whether the level of primary productivity within a habitat is determined by the grazing activity of primary consumers, or whether the alternative 'bottom-up' control mechanism, where primary production regulates the abundance of the primary consumers, occurs. Either case can probably be considered overly simplistic for most habitats where environmental variability and interaction with other trophic levels is likely to occur. Changes in the distribution and abundance of most species in the marine environment will be determined by a complex suite of independent and interacting biological and physical processes that act over various temporal and spatial scales (Shaffer & Cahoon, 1987; de Jong & de Jonge, 1995; Santos *et al.*, 1996; Sandulli & Pinckney, 1999; Blanchard *et al.*, 2001).

Primary consumers utilise a wide range of food sources in the marine environment including a) unicellular protists, bacteria, and b) multicellular algae (seaweed) and vascular plants. The former represent the majority of photosynthetic potential within the sea and are divided into the protozoans and the unicellular algae. The latter is divided into organisms that inhabit marine sediments, the microphytobenthos, and those that can be found suspended in or at the surface of the water column, the microphytoplankton. Dinoflagellates, and in particular diatoms, constitute most of the unicellular algae in temperate marine systems. Although light and the biomass of primary producers are the most important factor determining total photosynthesis (Colijn, 1982; Cole & Cloern, 1987; Pinckney & Zingmark, 1991a; de Jong & de Jonge, 1995; MacIntyre *et al.*, 1996; Meyercordt *et al.*, 1999; Guarini *et al.*, 2000b; Perekins *et al.*, 2001; Serodia *et al.*, 2001; Kocum *et al.*, 2002a), the wide variety of marine habitats and the processes affecting them results in significant variation in the spatial and temporal distribution of primary productivity. Nutrient availability, turbidity, environmental temperature and salinity can also significantly affect primary production (Aston, 1980; Rasmussen *et al.*, 1983; Sundback & Jonsson, 1988;

Cammen, 1991; Underwood & Paterson, 1993; Heip *et al.*, 1995; Santos *et al.*, 1996; Nozais *et al.*, 2001). As light, temperature and nutrient availability are normally higher closer to shore, the shallow waters of nearshore subtidal, intertidal and estuarine habitats are generally more productive than the open ocean (Nelson-Smith, 1977; Barnes & Hughes, 1988; Charpy-Rouaud, & Sournia, 1990). Owing to a need to inhabit areas in proximity to food resources, the majority of macro-benthic primary consumers are, therefore, distributed in shallow shelf waters also. For example, the microphytobenthos are principally confined to sediments within the photic zone due to photosynthetic requirements, and so this also typically defines the distribution of primary consumers that utilise this food source.

Many species of intertidal benthic diatoms occur throughout the year, whereas phytoplankton do not (Admiraal, 1984). Due to annual fluctuations in water column nutrient load and temperature, the abundance of phytoplankton generally display a greater degree of seasonality when compared with the phytobenthos (Soetaert & Herman, 1995; Goto *et al.*, 1998). The former often attain higher production values during peak productivity, but the latter tends to sustain relatively consistent production levels with less pronounced seasonality over the entire year (Baillie & Welsh, 1980). Generally nutrient loads do not become rapidly or severely depleted in sediments due to their constant remineralistion by microbial and bacterial action (Sullivan, 1999; Decho, 2000). Sediment nutrients are also supplemented by deposition of detritus from the water column. Detritivores are particularly abundant in marine sediments, and represent an important link in recycling nutrients to lower levels of the trophic food web (Boaden & Seed, 1993). Although the microphytoplankton consists mainly of centric diatoms and the microphytobenthos of pennate diatoms, the constant deposition and re-suspension with physical tidal and wave action results in a constant flux between media. Baillie & Welsh, (1980) estimated that a resuspension of only 10-15% of mudflat sediments to a depth of 1mm would account for total chlorophyll *a* levels in the American estuary they studied.

Microphytobenthic organisms exist in a wide range of sub-habitats in the intertidal environment due to broad salinity and light tolerances. A number of diatoms have been shown to display salinity tolerances between 0 and 40‰ (Admiraal, 1977). Benthic microalgae photosynthesise over a wide range of temperatures and light

intensity (Blanchard & Montagna, 1992; Blanchard *et al.*, 1997; Goto *et al.*, 1998). Little or no photoinhibition occurs and they are adapted to withstand full sunlight at low tide or very low intensities such as can be experienced at high tide and when turbidity is significant. Blanchard & Cariou-Le Gall (1994) recorded photoinhibition in the laboratory at light intensities likely to occur at temperate latitudes, but no inhibitory effect was observed *in situ*. Some studies have recorded that no photosynthesis by microphytobenthos occurred when tidal flats became immersed (Joint, 1978). This is not a universal occurrence, however, (Kromkamp *et al.*, 1995; Guarini *et al.*, 2000a) and it is probable that the turbidity and suspended load, and therefore the depth of light penetration within estuaries significantly influences whether microphytobenthic production occurs at high tide.

The sediment properties for a particular substrate also influence the potential production of microphytobenthos. The 2-dimensional nature of hard substrates does not provide the additional habitat afforded by 3-dimensional soft sediments, offering only the opportunity for surface colonisation. The hydrodynamic influence in a particular area, combined with localised geology, will define the distribution of benthic sediments of varying grain size. Finer particles are more readily suspended in the water column, and are only deposited at lower current velocities (Thornton *et al.*, 1995). Therefore, fine-grained sediment types, such as mud and very fine sand, are generally present in low energy environments where physical perturbation is negligible (Elliott *et al.*, 1998). This results in relatively low surface sediment mobility. Much greater physical action is required to suspend, transport and deposit larger, heavier grain sizes, and as a result these sediments are relatively mobile when under the influence of tidal and wave action. Mobile sediments are less favourable for colonisation by microphytobenthos due to the resultant physical disturbance, potential for removal from the photic zone and the direct abrasive action of hard substrate particles (Hargrave *et al.*, 1983). More stable substrata are, therefore, more suitable for colonisation by free-living epipelic diatoms, which constitute the major component of the microphytobenthos (Hopkins, 1963). Additionally, the grain size of particulate sediments defines the total surface area available for colonisation by epipsammic diatoms, those that bind to sediment particles. Epipelic diatoms are the dominant microphytobenthos in most intertidal soft sediments (Admiraal, 1984; Underwood & Smith, 1998).

Particulate matter, including detritus, nutrients and planktonic organisms, are deposited from the water column to sediment surfaces more frequently and in greater quantities in low energy environments. For example, mudflats are considered to be more depositional habitats when compared with more dynamic sites such as sandflats, due to lower current and tidal velocities. These higher levels of deposition in mudflat sites increase their productivity compared with sandflat areas. Normally fine-grained sediments with high silt/clay content are richer in microphytobenthos than sandy sediments (Hopkins, 1963; MacIntyre *et al.*, 1996; Barranguet *et al.*, 1997; Elliott *et al.*, 1998). Higher densities of photosynthetic organisms and a greater variety of species also increase the production of more sheltered sites, where there is less chance of re-suspension and relocation due to current and tidal effects (Fielding *et al.*, 1988). The presence of large amounts of detritus and its degeneration to nutrients by detritivores and bacteria also increase the productivity of mudflat sites.

Natural diatom assemblages are effective in preventing resuspension of sediments and increasing resistance to erosion (Sullivan, 1999). The microphytobenthos increase sediment stabilisation by excretion of extracellular polymeric substances (EPS) during vertical migrations and the formation of dense diatom mats (Palmer & Round, 1965; Admiraal *et al.*, 1982; Admiraal, 1984; Smith & Underwood, 1998; Austen *et al.*, 1999; Underwood &Paterson, 1993; Lucas *et al.*, 2000). These mats form on the surface of mudflats under low current and tidal velocity conditions if the emersion period is sufficiently long. Resuspension of microphytobenthos occurs gradually and continuously over a range of current velocities in sandy sediments, but in silt/mud sites diatom mats display strong resistance to erosions at relatively high current speed (Lucas *et al.*, 2000). Therefore, sediment stability is often directly related to the chlorophyll *a* content (Underwood *et al.*, 1995; Underwood & Smith, 1998; Austen *et al*, 1999; Paterson *et al.*, 2000; Yallop *et al.*, 2000). Although sandy sites have less surface microphytobenthic biomass compared with mud sites, more chlorophyll *a* is resuspended above the former at higher current velocities due to the greater depth of sediment erosion at these sites (Lucas, *et al.*, 2000). Resuspension and disruption of diatom mats has also been related to precipitation (Brotas & Catarino, 1995).

Highly productive areas that produce excess energy components that are transported to other areas are termed 'source' habitats. The transported component may be nutrients, detritus, primary producers or larvae and adults of higher trophic levels. In contrast, 'sink' areas are those that do not produce sufficient resources to maintain status and receive them from a donor source habitat. The study of the transfer of larvae between spatial distinct areas, metapopulation analysis, has received considerable attention in recent years (Roughgarden *et al.*, 1988; Fogarty *et al.*, 1991; Caley *et al.*, 1996). Prevailing hydrodynamics generally dictate the direction of transport in the marine environment (Eckman, 1983; Young *et al.* 1998), and will also determine sites of suspension and deposition when the resource component is passive. It should be appreciated that areas that are sinks for some factors can be sources for other components. For example an area that receives large inputs of planktonic recruitment of a species but does not contain reproducing adults would be termed a sink, but would be termed a source if the benthic forms subsequently migrate to other areas.

Anthropogenic inputs can have major affects on the location and status of sources and sinks. For example point sources of pollution such as sewage treatment plants and power stations can increase nutrients and temperature in the local environment. The effects can be widespread due to dispersal by currents and tides. A ready supply of nutrients can have a positive effect in the environments as they, and in particular nitrogen (Thornton *et al.*, 1995), can limit production, growth and reproduction in natural conditions. High levels of anthropogenic nutrient inputs can have very negative effects however. Very high levels of primary production and the resultant decomposition of detritus can cause anoxic conditions to occur in both the water column and underlying sediments, resulting in local extinctions and decreases in diversity. Archambault *et al.*, (2001) recorded a recovery in assemblages after closure of sewage outfalls with increases in number of species compared with areas where outfalls remained active. Persistent nutrient enrichment that does not result in such events will, however, change the stable isotopic signature of all individuals in the surrounding ecosystem (Tucker *et al.*, 1999).

Microphytobenthos are a primary carbon source in estuarine food webs (Sullivan & Moncreiff, 1988; Montagna *et al.*, 1995). Their contribution to coastal production and

importance as a food source for higher trophic levels is generally underestimated (Sullivan, 1999). Benthic algae can be utilised by deposit feeding species but may also represent an important food source for filter feeders due to tidal resuspension (Baillie & Welsh, 1980; Varela & Penas, 1985; de Jonge & van Beusekom, 1992; Conde *et al.*, 1999). Shaffer & Sullivan (1988) recorded that benthic diatoms represented an average of 74% of water column microalgae. Intertidal flats are colonised by microphytobenthos composed mainly of pennate diatoms, cyanobacteria and blue-green algae (Cadee & Hegeman, 1977; Colijn & de Jonge, 1984; Sullivan & Moncreiff, 1988). The abundance of benthic microalgae within the upper 5 to 10mm of the sediment surface varies from about 10^5 to 10^7 cells cm^{-3} (Admiraal *et al.*, 1982; Baillie, 1986; Delgado, 1989) depending on location, season and sediment properties (MacIntyre *et al.*, 1996). The photosynthetic pigment chlorophyll *a* is used as an index of phytoplankton and phytobenthos biomass in most investigation due to the difficulty and cost of direct cell counts however (Heip *et al.*, 1995; MacIntyre *et al.*, 1996). Examining chlorophyll *a* concentrations of sediments allows the simultaneous measurement of the motile (epipelic) and non-motile (epipsammic) algae plus any settled phytoplankton. Although the chlorophyll content in each organism will vary depending on species and life cycle stage (Gould & Gallagher, 1990; Cloern *et al.*, 1995; Hansen *et al.*, 2000), chlorophyll *a* concentration is a useful index of the photosynthetic potential of a population. Measurement of actual primary production is difficult, time consuming and numerous methodological problems exist in the various techniques used to quantify this variable (Goto *et al.*, 1998; Krompkamp & Underwood, 1999). Microphytobenthic primary production in sediments can be measured using bell jars or benthic chamber techniques, ^{14}C uptake in slurries or oxygen microelectrodes (Heip *et al.*, 1995). There are, however, problems with each of these methods. There is no clear view whether gross or net productivity is measured in the bell jar technique and using slurries destroys microgradients in sediments (Colijn & de Jonge, 1984; Jonsson, 1991; Heip *et al.*, 1995; Underwood & Krompkamp, 1999). Oxygen microelectrodes are time consuming and sensitive to very patchy small-scale variability in the distribution of microphytobenthic biomass (Revsbech, 1983; Brotas *et al.*, 1990). As a result the extrapolation of measurements taken on such minute spatial scales to whole site areas is controversial (Shaffer & Onuf, 1985; Shaffer & Cahoon, 1987; Pinckney & Zingmark, 1993a). As a result, the

quantification of sediment chlorophyll *a* concentration is commonly used as an indication of potential productivity.

The concentration of phaeopigments, the degradation products of chlorophyll *a,* in sediment increases at higher irradiances and in the presence of grazing species (Bianchi *et al.*, 1988). Depositional sediments generally have high phaeopigment contents due to regular flux from the water column. Baillie & Welsh (1980) recorded significant variation in phaeopigment concentration with changes in the tide cycle. Ultimately, detritivores and bacteria degrade phaeopigments further into constituents that will be available to autotrophs. Although some studies have used phaeopigment concentration as an index of primary consumer grazing pressure, spatial and temporal changes in tidal deposition and re-suspension, and the activity of detritivores probably decouples this relationship in many cases. The ratio between chlorophyll *a* and phaeopigment concentration is a useful indicator of the level of active photosynthesis within sediments. Higher ratios indicate active photosynthesis, and lower ratios greater degradation. Lower levels do not necessarily indicate less photosynthesis if deposition of phaeopigments from the water column and/or primary producer 'turnover' is significant.

The combined influence of many of the physical and biological factors listed above determines the spatial and temporal variability in the microphytobenthos over varying scales. When top-down regulation of the phytobenthos occurs, the distribution and abundance of grazing species will also elicit a strong influence on the occurrence of the former. The relationship between primary producer and consumer abundance are not generally described for most habitats as they are masked by the influence of other regulatory factors however. Although food availability can determine the population size of consumers when limiting, other factors can determine the threshold when it is not (Aljetlawi *et al.*, 2000; Gosling, 2003). Food quality can also affect the abundances of primary consumers (Rossi, 2002). Varying density-dependent and independent factors will determine the carrying capacity of a habitat under changing environmental conditions. Intra- and inter-annual variation in these parameters can alter existing population size and significantly affect reproductive and recruitment success (Bowman & Lewis, 1977). Substrate type, hydrodynamics, temperature, aerial and physical exposure and salinity are amongst the main physical factors

eliciting regulatory influence on both population size and abundance (Elliott & Kingston, 1987; Bianchi, 1988; Kalejta & Hockey, 1991; Gaudy *et al.*, 2000; Gosling, 2003). Resource limitations other than for food, intra- and inter-specific competition, predation, physiological tolerances and constraints on reproductive output/timing can also elicit regulatory effects (Barnes & Hughes, 1988; Smith *et al.*, 1996; Gaudy *et al.*, 2000; Roberts *et al.*, 2000; Gosling, 2003). A combination of factors may simultaneously limit population size at any given time, but these may change over relatively short durations. The relative effects of these factors will be species-specific, and influence individuals of different life history stages within the same population to varying degrees (Montagna *et al.*, 1995). As some level of interaction occurs between many species within the same habitat, changes in the population size of one species may significantly affect that of another (Gaudy *et al.*, 2000). The combined influence of all regulatory factors often results in patchily distributed populations that are highly dynamic in nature. It is particularly difficult to determine the factors regulating population dynamics when high spatial and temporal heterogeneity occurs (Hassell, 1987).

A number of different feeding strategies are utilised by primary consumers to ingest microphytobenthos. Deposit feeders either ingest whole sediment particles or graze on their surface if too larger to ingest (Lopez & Levinton, 1987). Epipsammic flora are ingested from the surface of the sediment grains along with any epipelic species in the immediate area. Filter feeding consumers remove food items suspended in the water column, resting on the surface of sediment and from the interstitial spaces between sediment grains without consuming the sediment itself. Some primary consumers are capable of feeding using deposit and filter feeding strategies as required (Hughes, 1970; Wolff, 1983; Hummel, 1985). Primary producers are exploited most efficiently by primary consumers and therefore the biomass of the latter represents a pathway for energy transfer to higher trophic levels. With the exception of the autotrophs, primary consumers are generally numerically dominant within the trophic food web, containing very high numbers of relatively small sized individuals (Ruesink, 2000). Organisms generally become larger and fewer in number at higher trophic levels. Benthic herbivorous invertebrate species are either motile and move on or through the sediment to ingest organic matter, or else are sessile and capture food from the water column or in burrows by the use of water

currents. The types and densities of benthic feeders will vary with substrate type. Deposit feeders tend to be more abundant in fine-grained sediments where there are higher levels of organic matter and sediments are less mobile (Rossi, 2002). High sediment mobility inhibits the presence of epifaunal motile species and burrow-dwelling individuals, and so infaunal filter feeding organisms are generally more dominant in these environments.

Variation in the food sources utilised by primary consumers can reduce niche overlap and inter-specific competition. Species that feed on the microphytobenthos can utilise different sizes or species of primary producers. For example, Bianchi & Levinton (1981) recorded that the mud snails *Ilyanassa obsoleta* and *Hydrobia totteni* both grazed filamentous blue-green microalgae but coccoid blue-green algae were only utilised by the former. Hagerthey *et al.* (2002) concluded that the mud snail *Hydrobia ulvae* and the co-occurring amphipod *Corophium volutator* preferentially consume different taxa within natural microphytobenthic assemblages. Extracellular in addition to intracellular digestion can also lower the impact of interspecific competition. For example the amphipod *Corophium volutator* is capable of digesting a considerable amount of material that has passed through the gut of the mudsnail *Hydrobia ulvae* (Lopez & Levinton, 1978). Additionally, the mud snail *Hydrobia ulvae* consumes phytobenthos in a slightly higher size range than that of the co-occurring amphipod *Corophium volutator*. Although the upper size range utilised by the latter overlaps with the lower of the former, the variation in overall size range of dietary items consumed by each species reduces competition (Morrisey, 1988a). The variation in feeding techniques used by different species may also reduce competition for food resources (Bianchi *et al.*, 1988), especially when species are capable of expressing more than one strategy that result in the ingestion of varying food items. For example, the polychaete *Nereis diversicolor* can act as a deposit feeder, a suspension feeder, a scavenger and a predator (Wolff, 1983). The bivalve mollusc *Scrobicularia plana* has been observed to exhibit filter and deposit feeding behaviours in varying proportions (Hughes, 1970). Different species exploit different vertical distributions in the sediment due to their adaptations to aerobic/anaerobic conditions. For example, McMahon & Wilson (1981) observed 3 infaunal bivalve species co-occurring in sandflats in Dublin Bay but at different depths. If these subsurface species also feed at different depths, then overlap in food utilisation may again be

reduced. Additionally, differences in the food sources utilised by different individuals within the same population may reduce intra-specific competition for food resources (Wilson,1975; Polis, 1984). Ontogenetic variation in dietary components consumed is common for many species due to physiological changes or simply increasing mouth and gut size (Hentschel, 1998). Changes in the quality of dietary items consumed also regularly occur with increasing size, with smaller individuals generally consuming higher quality food types (Lopez & Levinton, 1987).

The higher concentrations of fine particles and organic matter in mudflat systems cause compaction of the sediment and the resultant reduction of interstitial spaces can result in poor water drainage. As a result the depth of the oxic layer in these sediments can be quite shallow (Barnes & Hughes, 1988). In more sandy environments oxygen can penetrate further into the sediment. Coarse-grained sediments have large interstitial spaces, good water drainage, aeration and generally low organic matter compared with fine-grained sediments (Elliott *et al.*, 1998). This has an affect on the distribution of benthic invertebrates, with higher ranges and abundance of organisms generally present in more fine-grained sediments. The depth to which burrowing organisms can exist will depend on their ability to connect to the surface oxygen supply. For example, the drawing of oxygenated water into burrows by infaunal animals can enable them to live at depths up to one metre (Barnes & Hughes, 1988). Photosynthetic activity of microphytobenthos raises the concentration and penetration depth in surface sediments (MacIntyre *et al.*, 1996; Barranguet, 1997; Meyercordt & Meyer-Reil, 1999). The activity of burrowing and tube-building species also increases the surface area available for diffusive exchange of oxygen into anoxic sediments (Rhoads & Boyer, 1982). Bioturbation by deposit feeders can also change the properties of sediment due to mixing, resuspension, erosion, or redeposition (Page *et al.*, 1992; Galois *et al.*, 2000; Josefson *et al.*, 2002; Orvain *et al.*, 2004). High abundance of deposit feeders has a negative effect on sediment stability as their feeding reduces the biomass of microalgae, which causes a reduction in cohesive properties (Admiraal, 1984) and results in the production of faecal pellets that are more easily eroded than sediment grains (Rhoads & Boyer, 1982; Austen *et al.*, 1999; Reithmuller *et al.*, 2000). Sedimentation rates can also increase in the presence of filter feeding organisms through bio-deposition of faeces and pseudofaeces.

Deposit feeders can have a negative effect on suspension feeders when their activity causes the suspension of fine particles that can clog the filtering structures of the latter (Wolff, 1983). Reworking of the sediment by deposit feeders can also lead to burial of the newly settled larvae of suspension feeders to depths where they cannot persist. High density of suspension feeders can quickly deplete the water column of food and other suspended items. Such efficient filtering of the water column by suspension feeders can result in ingestion of larval stages that may be consumed or incorporated into pseudo-faeces (Wolff, 1983). For example, successful recruitment of *Scrobicularia plana* in one year can prevent settlement of subsequent conspecific cohorts or other species by these means (Barnes & Hughes, 1988).

Increased environmental temperature can have both negative and positive effects on the early life and adult stages of many marine invertebrates. The growth rate of pelagic larvae is generally increased at higher temperatures (Barnett, 1985), which significantly shortens the duration of the vulnerable planktonic phase. Faster development generally results in smaller body size at settlement however. Robinson & Tully (2000a) stated that this resulted in higher post-settlement mortality for certain species. Increased temperature and metabolic rate generally results in higher levels of natural mortality, and may also increase the activity of predator species. Food requirements generally increase with metabolic rate while oxygen is depleted more rapidly from the environment. Temperatures below the thermal tolerance of species will result in rapid and high mortality of all life history stages (Decker & Beukema, 1999). Larger individuals with energy reserves will generally be more able to tolerate extremes of temperature and other environmental stressors such as high/low salinity, desiccation or starvation, which in turn may affect the distribution of different sized individuals within the same population.

Predation of primary consumers can be a major top-down mechanism controlling population size (Ebenhoh *et al.*, 1995; Knox, 2001). Although the impact of marine predators is reduced in the intertidal zone by greater physical stress, the feeding activity of wildfowl and other air-breathing predators can remove any spatial refuge from predators that would otherwise exist (Boaden & Seed, 1993). As sheltered estuarine and intertidal habitats have relatively high productivity they are often

nursery areas for juvenile predatory species that can significantly impact on primary consumer abundance on a seasonal basis. Particularly strong recruitment of these predatory species may therefore result in inter-annual variation in primary consumer abundance. For example, recruitment failure of 3 bivalve species in the Netherlands over a 15 year period was linked to increased abundance of the predator *Crangon crangon* (Beukema & Dekker, 2005). Primary consumers utilise a number of different active and passive defence mechanisms to mitigate the effects of predation however. Cryptic coloration and patterning are particularly common for surface dwelling species. The secretion of an inert external shell can act as a barrier to both biological and physical stress. Burrowing, migration and remaining inactive also reduce predation effects.

Patterns of settlement and recruitment are major determinants of the abundance and distribution of benthic marine species (Keough & Downes, 1982; Gaines *et al.*, 1985; Gaines & Roughgarden, 1985; Hughes et al, 1999, van der Meer *et al.*, 2001). Most marine invertebrates produce dispersive planktonic offspring that can feed on highly productive seasonal phytoplankton blooms, and many species time larval release to coincide with these events (Barnes & Hughes, 1988). Although initial larval production is related to the size of the adult stock and fertilisation success, processes acting on larvae after release significantly affect subsequent larval abundance and settlement. Density independent and density-dependent physical and biotic factors act on the larval population throughout development. Physiological stresses, starvation and predation are believed to contribute to high levels of larval mortality (Pechenik, 1987; Rumrill, 1990). Oceanographic processes can lead to the retention of larvae in the locality of release, transport larvae away and then return them at a later stage of development, or permanently remove them to new areas (Alldredge & Hammer, 1980; Eckman, 1983; Shanks, 1983; Swearer *et al.*, 1999). Larvae delivered to new areas may encounter exploitable habitats unoccupied by conspecifics or grounds that are unsuitable for either settlement or subsequent development. Although the later planktonic stages of many species are able to influence their geographic location through behavioural migrations, unfavourable hydrodynamic conditions can result in delivery to sub-optimal habitats (Rumrill, 1990). With the exception of generalists most species express varying degrees of substrate specificity. This will be dictated by the resource requirements of the early benthic phase. Patchiness in larval supply and

settlement can result in inequalities in subsequent benthic distribution (Eckman, 1983; Roughgarden *et al.*, 1988).

Early post-settlement mortality is probably one of the most important determinants of future population size (Keough & Downes 1982; Connell, 1985; Gosselin & Qian, 1997). Some benthic invertebrates settle haphazardly to a wide range of substrate types with differential mortality shaping subsequent distribution (Palma *et al.*, 1998; Robinson & Tully, 2000b), while others are extremely selective during the post-larval stage and only metamorphose to the benthic form when optimal habitats are encountered (Doyle, 1975; Strathman *et al.*, 1981; Cobb *et al.*, 1983; Cobb *et al.*, 1997). Adults of the former species type generally have very high fecundity and invest limited resources in the production of planktotrophic offspring, while the latter produce far less lecithotrophic plankton with much greater parental investment (Barnes & Hughes, 1988; Cobb *et al.*, 1997; Palma *et al.*, 1998). In the most extreme cases, parents retain offspring during almost all of their development and release them when they are sufficiently advanced to be in benthic form. Direct developers of this kind are normally extremely habitat-specific, and would probably suffer very high mortalities at the end of a dispersive planktonic phase due to the low probability of being delivered to a suitable site. Species that are highly selective at settlement have been shown to reject unsuitable benthic substrates and re-enter the water column to disperse again, although a final selection cannot be postponed indefinitely (Connell, 1961; Strathman *et al.*, 1981). Although the physical properties of the preferred sediment are believed to be the most important determinant of substrate selection, particularly its suitability in affording shelter and required resources (Stevens & Kittaka, 1998; Steele, 1999), other factors can influence larval choice. The presence or absence of conspecifics (Crisp, 1974), predator species (Boudreau *et al.*, 1993), current speed (Robinson & Tully, 2000a) or certain types of algae (Forward *et al.*, 1996) has been shown to influence settlement behaviour of some species. A combination of physical and chemical signals probably allows more selective larvae to determine habitat suitability. Regardless of the suitability of a settlement habitat, post-settlement density-dependent and independent mortality shapes the distribution pattern of many marine invertebrates however. Resource limitation, predation and intra-or inter-specific competition are thought to be the main causes of mortality during the early life history stages (Keough & Downes 1982; Gosselin & Qian, 1997).

In common with most species, the growth of primary consumers is generally higher during the early life history stages and decreases with increasing size. Changes in growth rate are often associated with the attainment of maturity when a proportion of the energy budget will be redirected from somatic to reproductive growth. Although the growth of many species is continuous, the seasonality in environmental parameters in temperate waters usually results in similar fluctuations in growth rate. As the metabolic rate of marine invertebrates is primarily linked to environmental temperature, and to some extent food availability, growth rates are generally higher in the summer and lower in the winter. In most cases growth is confined to a particular period when conditions are favourable, with cessation of growth occurring for much of the rest of the year. Growth restriction can also occur due to density-dependent factors such as resource limitation or interference competition (Cobb, & Tamm, 1974). The growth rate of a species is important in defining the period of development required to mature and recruit to the adult spawning stock.

A large proportion of flats exposed by the tide are defined as potential conservation areas protected by Annex I of the E.U. Habitats Directive 1992. Additionally, many are designated as Special Protection Areas (SPA) due to their importance as feeding grounds for native or migratory wildfowl. Significant portions of intertidal mudflats and sandflats are designated Special Areas of Conservation (SAC) in European waters. Dublin Bay, located on the eastern seaboard of Ireland is designated as an SAC divided into 2 management units 'North' and 'South'. The North Dublin Bay SAC, the focal point of which is North Bull Island, has many habitat types that are protected including saltmarshes, dune systems and intertidal lagoons. The North Bull Island site is also a wildfowl sanctuary and a Ramsar Convention site of international importance. Both the North and South intertidal areas are important sites for wildfowl species with large numbers present throughout the year including oystercatcher (*Haematodus ostralegas*), dunlin (*Calidris alpine*), redshank (*Tringa tetanus*) and also migratory brent geese (*Branta bernicla*). The high levels of primary production that occur in intertidal and estuarine habitats within the bay also provide a nursery ground for numerous juvenile fish species many of which subsequently recruit into the Irish Sea.

Bays and estuaries are generally major centres of economic activity and population density with a large range of uses from recreation to the disposal of industrial wastes. The municipal sewage and other waste of a population of ~1.7 million in Dublin City must be disposed of with as little impact on the environment as possible. European regulations dictate that the effectiveness of the measures used be monitored to ensure water quality and ecosystem health, particularly when affected areas contain one or more SAC. Bio-monitoring of benthic invertebrate populations is a useful tool for assessing the long-term environmental conditions of particular habitats. Benthic invertebrates are generally quite long-lived in comparison with pelagic species and also relatively sedentary. It is often extremely difficult to define the range of pelagic species, which may be exposed to environmental conditions within many areas. As the range of benthic species is more easily defined and they generally remain within these habitats for most life history stages, they can integrate the effects of the surrounding environment over long periods of time making them useful 'indicator' or 'index' species (Gray & Pearson, 1982; see Keegan, 1991 for review; Agard *et al.* 1993). As sediments generally act as buffers to the more extreme changes that can occur in the water column over relatively short durations, data obtained from monitoring substrate parameters could be considered a more appropriate method for determining medium to long-term trends. As particulate sediments are often depositional in nature, changes in water quality will also be expressed in sediment profiles. The establishment of time-series monitoring programmes have been shown to have application in the modelling of a range of perturbations in the marine environment, from acute pollution incidences (Pearson, 1975; Gee *et al.*, 1985) to the effects of climate change (Genner *et al.*, 2004). Environmental monitoring programmes must be conducted with suitable consideration of temporal and spatial heterogeneity in physical and biological parameters at appropriate scales (Hughes et al, 1999; Serodio & Catarino, 2000). When sufficient data are collected with an understanding of the processes that regulate the population dynamics of indicator species, models can be developed to estimate and predict probable changes under varying environmental constraints. Such models have been shown to be highly effective in the process of coastal zone management.

This thesis consists of field and laboratory-based experiments that examine the population dynamics and impact of the dominant primary consumer species at two intertidal sites. The spatial distribution of the two species and chlorophyll *a*, an indicator of relative primary production and food availability, at the mud and sandflat systems that they inhabit are described in Chapter 2. Chapter 3 examines seasonal changes in sediment chlorophyll *a* concentrations and densities of the two primary consumers. Analysis of the age structure of the two populations using field data and laboratory experiments to determine size-specific growth is presented in Chapter 4. The substrate specificity of one of the primary consumers was investigated in Chapter 5. Chapter 6 investigated if microphytobenthos were the main food source of the primary consumers and attempted to used field and laboratory-based experiments to quantify actual feeding rates. The final chapter used modelling techniques to combine data from preceding chapters with previously published data to estimate the impact of the primary consumers on primary production at the study sites. The aim of this thesis was to quantify the flow of energy from primary producers to primary consumers in the two study sites to determine whether food availability and consumer population size regulated each other and to assess the suitability of the primary consumers as indicator species for their respective habitats. A thorough examination of the spatial and temporal variation in *Hydrobia* and *Tellina* and sediment chlorophyll *a*, an index of food availability, would establish site-wide effects and relationships between these variables. Behaviour of each species in response to changes in temperature, food availability and sediment type would enable modelling the effects of possible ecosystem change.

Chapter 2. Spatial variability in the distribution and abundance of two herbivorous intertidal mollusc species and sediment chlorophyll *a*.

2.1 Introduction

Spatial variability in the distribution and abundance of species within the marine environment is defined by biological and physical parameters. These regulatory parameters influence species from broad geographic to micro-habitat scales, their dynamic nature having the potential to alter the spatial range and number of individuals present over relatively short temporal durations. Non-uniformity in the influence of biological and physical parameters in the marine environment commonly results in a high degree of spatial heterogeneity in the distribution and abundance of species in the water column, and to a greater extent the benthos (Shaffer & Cahoon, 1987; Pinckney & Sandulli, 1990; Santos *et al.*, 1996; Sandulli & Pinckney, 1999; Galois *et al.*, 2000; Haubois *et al.*, 2004). Although large areas of seabed can be covered by the same unbroken habitat types, particularly in deeper waters, many shallow water inshore areas are characterised by a mosaic of different habitat types that may vary over 10s of meters or less (Robinson & Tully, 2000b). Although changes in the distribution of actual physical sediment types is relatively common in shallow waters due to transport mechanisms associated with water movements, changes in population community structure driven by biological and physical environmental variability are very regular occurrences. These variations may be relatively predictable and associated with seasonality in temperate waters, or driven by stochastic events that also play an important role in shaping benthic species distribution and abundance (Cadee, 1980; Shaffer & Onuf, 1983; Brotas & Catarino, 1995; Heip *et al.*, 1995; MacIntyre *et al.*, 1996; Underwood & Krompkamp, 1999; Rossi, 2002). As the habits, habitats and physiological tolerances of species often vary at various stages of their life history, variation in environmental parameters can impose quite different influences on the spatial distribution of various components of a population.

Temperature, salinity, light penetration, depth, substrate type and hydrodynamics are generally considered to be the most influential of the physical factors regulating the spatial variability in species distributions (Heip *et al.*, 1995; MacIntyre *et al.*, 1996;

Underwood & Krompkamp, 1999). Environmental temperature, salinity and depth will define the potential horizontal and vertical range of a species depending on its physiological tolerances to variations in these parameters (Aljetlawi *et al.*, 2000; Gosling, 2003). Although light penetration may not strongly influence heterotrophic feeders, it does define the distribution of the majority of benthic primary production to which primary consumers and on which the lower trophic feeders are dependent. Physical transport mechanisms and exposure define not only the distribution of particular sediment types through depositional flux but also that of nutrients, primary consumers and the planktonic larvae of benthic marine organisms (Eckman, 1983). Fine sediment grains are mainly deposited in areas with low physical energy inputs and so sediments with a high proportion of silt/clay content only occur in sheltered environments, while larger particles are generally found in localities with higher energy status (Thornton *et al.*, 1995). The size of grains available for re-suspension and deposition, and the occurrence of hard substrate types will be influenced strongly by a combination of physical exposure and local geology. Although physical transport mechanisms strongly influence the distribution of nutrients and primary producers in the water column in the same way as for sediment grains, they have less influence on those bound within sediments. The currents associated with tidal action still results in the regular suspension and deposition of phytoplankton, phytobenthos and detritus to and from marine sediments however (Baillie & Welsh, 1980; Varela & Penas, 1985; Shaffer & Sullivan, 1988; de Jonge & van Beusekom, 1992; Brotas & Catarino, 1995; Conde *et al.*, 1999; Lucas *et al.*, 2000). As greater deposition and lower sediment mobility occur at low energy sites, these generally support higher densities of primary producers, consumers and detritus (Hopkins, 1963; Hargrave *et al.*, 1983; Fielding *et al.*, 1988; MacIntyre *et al.*, 1996; Elliott *et al.*, 1998). Highly mobile sediments are not suitable for colonisation by motile deposit-feeding epifauna and are generally inhabited by infaunal suspension feeders, the former commonly occurring in lower energy environments. Although the planktonic larvae of many marine inverts are capable of influencing their position, hydrodynamics strongly influence their ability to select suitable habitats at settlement, and may deliver them to sub-optimal habitats (Gosling, 2003). Hydrodynamically driven patchiness in larval supply and settlement can, therefore, result in inequalities in subsequent benthic distribution (Eckman, 1983; Gaines *et al.*, 1985; Gaines & Roughgarden, 1985).

Patchiness in larval supply and subsequent benthic distribution can be further complicated by species-specific habitat-specificity at settlement (Keough & Downes, 1982; Roughgarden *et al.* 1988). The post-larvae of some species display high competence in selecting a settlement substrate that will maximise the potential for subsequent growth (Connell & Jones, 1991; Perkins-Visser *et al.*, 1996) and survival (Strathman *et al.*, 1981; Keough & Downes, 1982), while others settle haphazardly into numerous habitats regardless of suitability (Palma *et al.*, 1998; Robinson & Tully, 2000b). Although a number of environmental cues are used by the former to locate optimal substrate types, the capacity of a habitat to provide shelter or other required resources are the most common. The availability of suitable shelter is known to be an extremely important factor in mitigating both early post-settlement and adult mortality for benthic invertebrate species (Gosselin & Qian, 1997). Post-settlement and early benthic phase mortality is known to influence the subsequent distribution and abundance of many benthic marine species. Suitable shelter from physical and biological stressors is particularly important in the intertidal zone, where regular intermittent exposure to the air and both terrestrial and marine predators can occur. Although most molluscan species have the added protection of an inert protective shell, many still require the additional shelter afforded by the habitat. Periodic or permanent burrowing into 3-dimensional particulate sediments can significantly alleviate the affect of these stressors.

Intra- and inter-specific competition/interaction, predation and resource availability can all influence the distribution and abundance of benthic species (Gosling, 2003). Limitations in space and food resources are generally considered to be the most common regulatory factors in intertidal and estuarine environments (Elliott & Kingston, 1987; Kammermans, 1994; Aljetlawi *et al.*, 2000; Rossi, 2002). Pelagic primary consumers generally experience food shortage during much of the year due to the strong seasonality in phytoplankton productivity associated with temperature and nutrient limitation, which in turn causes wide fluctuations in the distribution and abundance within pelagic food webs (Cole & Cloern, 1987; Charpy-Rouaud & Sournia, 1990; Kocum *et al.*, 2002a). Although benthic primary production, the majority of which is the responsibility of the microphytobenthos, does not generally

reach the peaks in production observed for the phytoplankton, lower fluctuations in sediment temperature and the year-round availability of nutrients generally result in less fluctuation in productivity. Previous studies have estimated that the lower but more prolonged and constant primary production in the benthos in fact results in greater annual productivity when compared to that of the phytoplankton (Goto *et al.*, 1998; Underwood & Krompkamp, 1999). Although this may result in a lower frequency and severity of food limitation in benthic habitats, the broad-scale spatial distribution of primary consumers is still likely to reflect that of the food resource. This may not be the case at finer spatial scales however, particularly if food is not limiting. There is some debate as to whether 'bottom-up' regulation of primary consumers abundance or 'top-down' regulation of microphytobenthos occurs under natural conditions. In many situations, neither case may apply during some or all periods of the year if food availability does not limit consumer population size and distribution and/or grazing pressure does not affect the abundance of the microphytobenthos. Many other environmental parameters are known to determine thresholds to both autotrophic and heterotrophic production.

Suspended phytoplankton can be transported considerable distances both vertically and horizontally in relative short periods of time, and therefore can be influenced by parameters in a wide range of locations that are difficult to define (Platt *et al.*, 1990; McMahon *et al.*, 1992; Shiomoto, 2000; Kocum *et al.*, 2002a & 2000b). As microphytobenthic organisms are relatively immobile in comparison due to their strong affiliation with the benthos, and are affected by environmental change, they are useful indicator species. The abundance of microphytobenthos within the upper 5 to 10mm of surface sediment varies from about 10^5 to 10^7 cells cm^{-3} (Admiraal *et al.*, 1982; Baillie, 1986; Delgado, 1989) depending on location, season and sediment properties (MacIntyre *et al.*, 1996), but this can be altered significantly through natural or anthropogenic influences. The photosynthetic pigment chlorophyll *a* is used as an index of microphytobenthos biomass in most investigations of benthic primary production due to the difficulty and cost of direct cell counts (MacIntyre *et al.*, 1996). This pigment provides a useful index of relative photosynthetic potential of a population and avoids the technical difficulties associated with quantifying the actual process of primary production. As it is also the most abundant photopigment in living microalgae it is a useful tracer of primary production derived organic carbon

(Ingalls *et al.*, 2000). Additionally, monitoring phaeopigments (mainly phaeophorbide *a* and phaeophytin *a*), the degradation products of chlorophyll, gives a good indication of the rate of photosynthetic 'turnover', grazing activity and deposition of detritus (Cadee & Hegeman, 1977; Cadee, 1980; Cartaxana *et al.*, 2003). Difficulties arise in the practical use of these parameters as monitoring tools in the field however, as their spatial distribution and concentration is subject to almost constant change by physical and biological variables. For example, Baillie & Welsh (1980) recorded that chlorophyll *a* and phaeopigment concentration varied significantly between successive tidal events. As the spatial distribution of primary consumers tends to be less transient than that of the microphytobenthos and are sufficiently low in the food chain to be rapidly affected by changes in the environment they are often considered useful alternative indicator species. Although more than one primary consumer may be present within a habitat, direct or indirect interaction between-species generally results in changes in the population parameters associated with one affecting another.

Spatial variability in the distribution of species must be considered when devising field sampling protocols to ensure that robust estimates of population rate parameters are obtained. The appropriate spatial scales of study will be determined by the nature of the investigation and the patchiness of the variable to be measured within the required sampling area (Shaffer & Onuf, 1985; Hughes, 1999). Failure to consider the inherent variability in the spatial structure of various population parameters can result in erroneous and unrepresentative results (Hassell, 1987). This is particularly important when variation in the spatial distribution of various life history stages is considered, as data collected from one area will not be representative of the entire population (Haubois *et al.*, 2004). The aim of this study was to compartmentalise and describe spatial variability in the distribution and abundance of two numerically dominant primary consumer mollusc species, and determine whether these correlated to that of the microphytobenthos. This information was required to design an effective protocol for the establishment of a medium- to long-term monitoring programme for these potential indicator species that would account for the inherent spatial variability in their distributions.

2.2 Materials and Methods

2.2.1 Study site and species

Dublin Bay is located on the eastern coast of Ireland, opening into the Irish Sea. The Bay is 10km wide across the mouth and covers an area of approximately 100km^2. Much of the bay can be considered a low energy environment as the mouth faces away from prevailing winds and fetch is short across the Irish Sea. As a result, the majority of the intertidal region contained within the bay, which accounts for approximately 20% of the total area (Roth & Wilson, 1998), consists of soft sediment sand and mudflats with shallow gradients, although there are limited areas were harder substrata occur. Two contrasting study sites were selected to represent these substrate types (Figure 2.1). The mudflat study site was located at South Bull Lagoon, behind North Bull Island, which is located to the north of Dublin Bay. With the exception of the narrow tidal lagoon mouth opening into Dublin Bay, the site is protected on all sides by raised land. Fetch is reduced to several hundred meters of sheltered water as the lagoon mouth faces toward the back of the bay, this further preventing physical disturbance at the site. As the lagoon is bordered by land on both long axes and at the east end opposite the lagoon mouth, two physical gradients exist at the site; vertically (from high to low shore) and horizontally (from the entrance to the enclosed end of lagoon). High shores occur on both sides and to the rear of the site, ebbing waters draining to form a central creek that meanders from the back to mouth of lagoon. At low tide the whole mudflat is exposed, with the exception of the creek, for approximately 1-2hr. Flooding tides take approximately 4hr to cover the mudflat to the high shore edges, one of these consisting of a seawall the other a salt marsh/dunes complex. The lagoon is completely immersed at high tide for approximately 1hr. The dominant primary consumer, the gastropod mud snail *Hydrobia ulvae*, is a relatively small (~8mm max shell height) surface dwelling deposit feeder. The species is relatively mobile due to its ability to crawl on the surface of the mud and float at the water surface on mucus strands.

The second sampling site, Blackrock, was selected to be representative of the numerous sandflats in the bay. Located approximately 10km south of Bull Island and facing the mouth of Dublin Bay, the Blackrock site is considered to be relatively

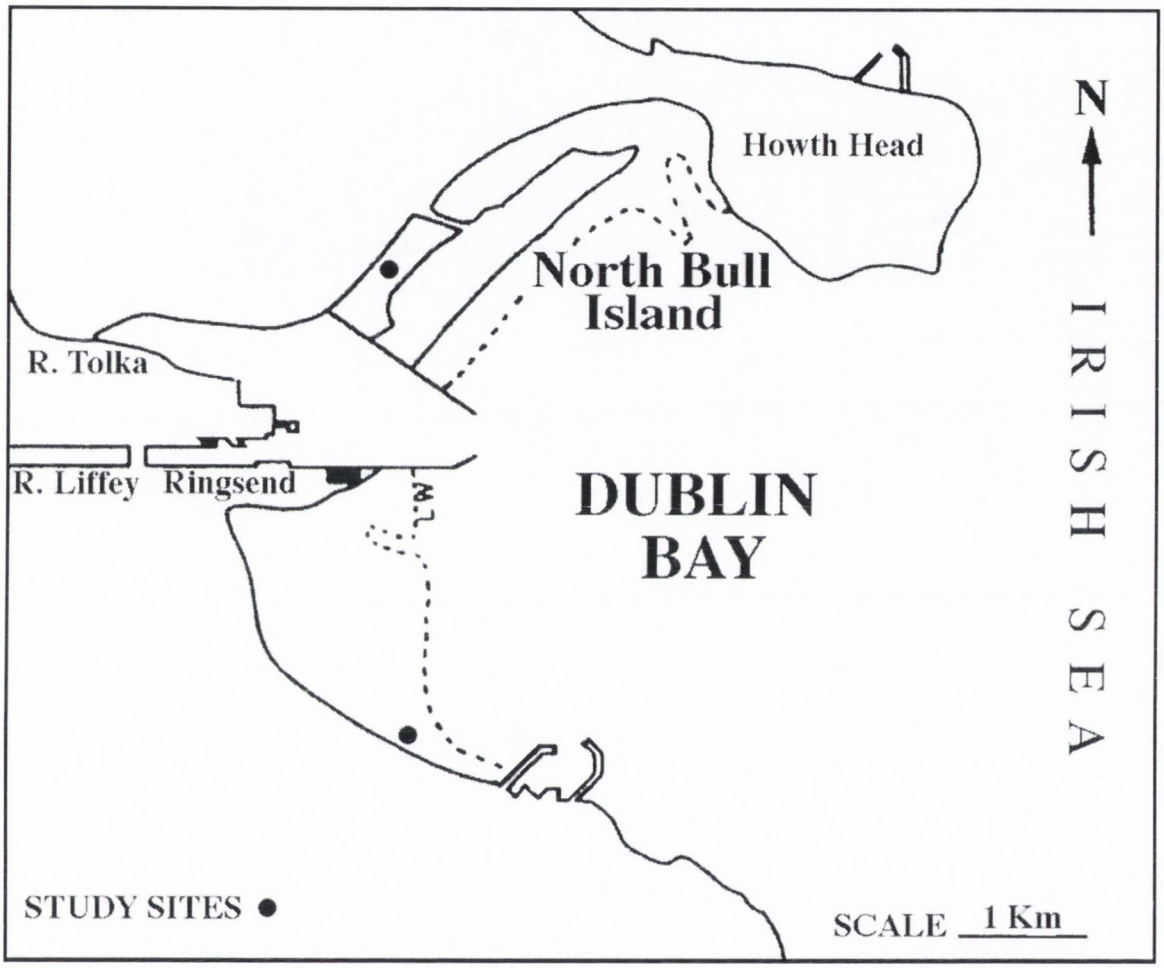


Figure 2.1. Location of study sites in Dublin Bay. The Bull Island sampling site is indicated by the black dot to the north side of the bay and Blackrock the dot to the south.

exposed in comparison with the South Bull lagoon. As the flat is part of a contiguous stretch of sandy substrates that run along the majority of the south of the bay, it is open ended and subjected to wind and wave exposure on a regular basis. The landward edge of the site consists of a defensive seawall, to which the high tide extends on most occasions. Due to the topography of the site, ebbing waters drain to the northeast, flooding *via* the same route. In contrast to the Bull Island site, this produces a single physical gradient from high to low shore. The infaunal bivalve *Tellina tenuis* is the dominant primary consumer at this site. There is some confusion as to the precise feeding strategy utilised by this species, with some authors claiming that they are capable of deposit and filter feeding behaviours (Trevellion, 1971).

2.2.2 Determining the appropriate scale of study

As no information describing the scale of spatial variability in the distribution and abundance/concentration of either the mollusc species or the food resource (microphytobenthos, measured as chlorophyll *a*) examined at the study sites was available, a number of preliminary studies were conducted to determine optimal sampling methodologies. Rational determination of sampling methodologies was considered preferable to randomly assigning effort without prior consideration of appropriateness. 'Best possible methodologies' were defined as those that gave the greatest resolution of the extent of spatial variability in distribution and abundance/concentration of the targeted species and their food sources within the whole study site, while remaining mindful of financial, logistical and time constraints associated with extensive field sampling and laboratory processing. The *a priori* design of statistically appropriate and achievable sampling methodologies and the repeatability of these methods are essential for the establishment of medium to long-term routine environmental monitoring programmes. There is disagreement about systematic versus random sampling with some field ecologists suggesting that estimates from systematic sampling are on average better than those from random sampling (Dytham, 1999). Simple random sampling is useful for homogeneous areas with stratified random sampling suited for heterogeneous sites. A stratified random approach improves the representation of the sample by reducing sample error but prior information about the area is necessary to create groups that are then sampled independently using a random process. It can be difficult to select revelent

stratification variables. Pooler & Smith (2005) found that systematic sampling designs with sampling units equally spaced more efficient than random sampling for estimating spatial distribution and abundance of a mussel population. A systematic approach to sampling was used in this study in order to obtain an even spread of data points over the population. The use of a randomly chosen starting point further increased sampling precision (Pooler & Smith, 2005).

Preliminary investigations into the spatial variability in distribution and abundance within the study sites aimed to provide information on:

1) The number of sampling stations required to adequately represent the site
2) The distribution of these stations within the study site (sampling grid)
3) The number of replicates required within station
4) The dimensions of the replicate samples taken at each station

During these first experiments, multiple cores (samples) were taken with replicates within station. This 'replication-within-replicate' aimed to more accurately identify the variation at different scales associated with the distribution and abundance of the target organisms so that an optimal sampling protocol could be defined.

2.2.3 Standard field sampling methodology for sediment pigments

As laboratory trials suggested that pigment concentration could be adequately gauged from relatively small (1-2cm^2) sediment samples, and variation in the spatial scale of concentration was unknown, relatively small samples were taken using modified plastic 5ml medical syringes. The base of each syringe, which would normally act as the securing point for the needle, was removed so that it could be pushed easily into the sediment to yield a circular core with a surface area of 2.01cm^2. Although each syringe was pushed into the sediment to a depth approximately 4cm, only the top 0.5cm layer was used to determine pigment concentration. The depth to which light penetrates sediment is typically <2mm (Paterson *et al.*, 1998). Microphytobenthos migrate into and out of this photic zone with diel and tidal cycles (Serôdio *et al.*, 2001; Paterson *et al.*, 1998; Smith & Underwood, 1998; Underwood & Kromkamp, 1999). A depth of 0.5cm was chosen to determine pigment concentration in order to

include microalgae actively photosynthesising at the time of sampling and also microalgae below the photic zone due to either migration or the effects of tidal disturbance. The taking of additional sediment below that necessary for analysis acted as a barrier to contamination of the surface layer which remained uppermost in the syringe cylinder. Gentle upward pressure was applied to the plunger of the syringe as it was removed from the sediment, the suction acting to retain the sample within the tube. The open end of each labelled syringe was covered with a strip of laboratory 'parafilm'. Sample cores were returned immediately to the laboratory and frozen within the syringes until processed in the laboratory. Freezing of samples does not cause degradation of chlorophyll *a* and can increase the extraction efficiency (Lenz & Fritsche, 1980; Marker *et al*, 1980; Nusch, 1980). All samples were frozen in order to ensure comparable results.

2.2.4 Laboratory and data analyses for sediment pigments

Sediment samples within syringes were allowed to defrost at room temperature before removal from syringes. The lower, unwanted portion of the core was slowly ejected from the tube and discarded until only the top 0.5cm surface sample remained. This portion was placed directly into labelled centrifuge tubes. Although large, visibly conspicuous pieces of filamentous algae were removed prior to analysis, it was not always possible to be 100% sure that pieces of macroalgae were absent from all samples. Replication reduced the influence of such contamination, and specific outliers associated with contamination were removed when identified (Zar, 1999). This procedure was rarely performed with <5 data points removed in total. Pigment analysis was determined following the method of Nusch (1980), Sartory and Grobbelaar (1984) and Brennan (1991). Sediment was placed in 10ml of 90% ethanol and heated for 5min at 78°C (boiling point of ethanol). Each sample was then left in the dark for 24h to extract pigments present. Samples were then centrifuged at 3000rpm for 10min, and the absorbance of chlorophyll *a* in the clear supernatant measured on a spectrophotometer at 665nm and then at 750nm to correct for turbidity. Samples were then acidified with 2N HCl and the absorbance read again at each wavelength to correct for phaeopigments, the degradation products of chlorophyll *a*. The concentration of chlorophyll *a* and phaeopigments were calculated using the following formulae:

Chl *a* $= 29.6 (E^{b}_{665} - E^{a}_{665}) v / A \times l$

Phaeopigment $= 20.8 \times E^{a}_{665} \times v / A \times l$ – Chl *a*

Where

Chl *a* = Concentration of chlorophyll *a* in μg cm^2

29.6 & 20.8 = Factors derived from the specific absorption coefficients for chlorophyll *a* in 90% ethanol (82) and the acid ratio E^{b}_{665}/E^{a}_{665} for pure chlorophyll *a* (1.7)

E^{b}_{665} = Extinction of extract at 665 nm before acidification

E^{a}_{665} = Extinction of extract at 665 nm after acidification (both values corrected for turbidity by subtraction of the 750 nm reading)

v = Volume of ethanol used to extract sample (ml)

A = Area of sediment used in sample (cm^2)

L = Path length of spectrophotometer cuvette (cm)

Results were converted to mg m^{-2}.

Comparisons among samples, replicates and stations were conducted using ANOVA and Kruskal-Wallis (non-normal data) tests where appropriate, with Scheffe or Dunns post-hoc tests applied where necessary.

The data from each sampling co-ordinate was entered in to the spatial analysis computer package 'Surfer'® (Golden Software, California) to produce variogram models. A variogram is a two-dimensional expression of a three-dimensional function within which the variability between samples with a known distance from each other is expressed. A variogram is calculated by averaging one half of the distance squared of the sample values over all pairs of observations with the specified separation distance and direction. The two dimensional interpretation displays the magnitude of the variation between the values of any combination of points as the spatial distance between them increases and uses all possible data pairs. It is based on the underlying principle that on average, two values closer together are more similar than two observations further apart. The nugget effect, the intercept value on the y-axis, is the theoretical inherent variability that would exist between points at zero distance when extrapolated back from input data. The units on the x-axis correspond to the measurement units of the grid (e.g. metres) and units on the y-axis represent the square of the value measured (e.g. concentration). Variogram models were used to

grid spatial data using a kriging algorithm for subsequent construction of contour plots.

2.2.5 Preliminary investigation of spatial variability in pigment concentration-sampling design

2.2.5a Initial determination of medium-scale variability (samples within station)

As no information was available on the spatial variability in sediment chlorophyll *a* concentration, an initial investigation into the level of variation was conducted with a view to determining 'best possible method' for sampling within station. A station at mid-shore level was selected randomly by dividing the site into $20m^2$ blocks and selecting randomly one of these blocks to sample prior to arriving at the sampling site. The mid-shore was selected as that most likely to be representative of both higher and lower shore levels. Differences between shore levels were to be investigated when within-station sampling protocol was defined. A $25cm^2$ quadrat was thrown backwards over the shoulder into the randomly selected sampling block at both the Blackrock and Bull Island study sites in January 2000. Four syringe corers were dropped from standing height into the quadrat, and cores removed from the sediment at the base of each. A further 5 quadrats, each containing 4 core samples, were taken randomly within the $1m^2$ area surrounding the initial quadrat position. The localised position of each of these subsequent quadrats in the 15 spaces available had been randomly assigned prior to arrival at the sample site. A further 4 replicates were taken in the adjacent $1m^2$ area to the left of the initial sampling area, again the position of each quadrat being dictated by prior random assignment. This process was repeated for a third time 4m from the first quadrat. The design of this sampling protocol aimed to provide data for a number of samples located varying distances from each other, but within an easily worked 'sampling station'. The use of cores with quadrats, and multiple quadrats within distinct areas of the station aimed to give some indication of the scale of spatial variability at that time.

2.2.5b Determination of small-scale variability (samples within replicates)

A second experiment was conducted in February 2000 to examine small-scale, within-quadrat variability. A $25cm^2$ Perspex quadrat was drilled to create 29 holes at known

distances into which syringe corers could be fitted (Figure 2.2). The closest distance between holes was 1cm, the greatest 28cm. The holes were spaced in this way to create a wide variety of 'between-sample distances', which is desirable when constructing models of spatial variability. The sampling location and the position of the first quadrat within it were determined as described above. Three additional quadrats were positioned to the left of the first quadrat parallel to the shore, with a 25cm gap between each. Sediment cores were analysed as above.

2.2.5c Determination of large scale variability (stations within site)

To investigate differences in sediment chlorophyll *a* at larger spatial scales it was necessary to reduce the numbers of cores taken per quadrat. The financial and logistical constraints of sampling large numbers of cores within each replicate would have required a massive sampling programme that would likely have been too large to repeat on anything less than an annual basis. To determine the minimum number of samples required in each replicate to obtain relatively robust estimates of pigment concentration, the data from the previous experiment was further sub-divided into smaller sections containing equal numbers of samples and compared using ANOVA. For samples from both Bull Island and Blackrock at least some of the mean values obtained from 'quarters' within a quadrat were significantly different from each other (Table 2.1), but at scales larger than quarters significant differences were not determined due to wide error associated with the means. Further sub-division into smaller areas containing fewer numbers of neighbouring samples often revealed significant differences between divisions existed, but these were often associated with relatively low mean error. With consideration of this small-scale variability and sampling constraints, it was decided that quadrats (replicates) intended for examining between station differences should contain 5 sampling cores. These were positioned in the centre of each quarter of the quadrat, and also one in the centre of the quadrat.

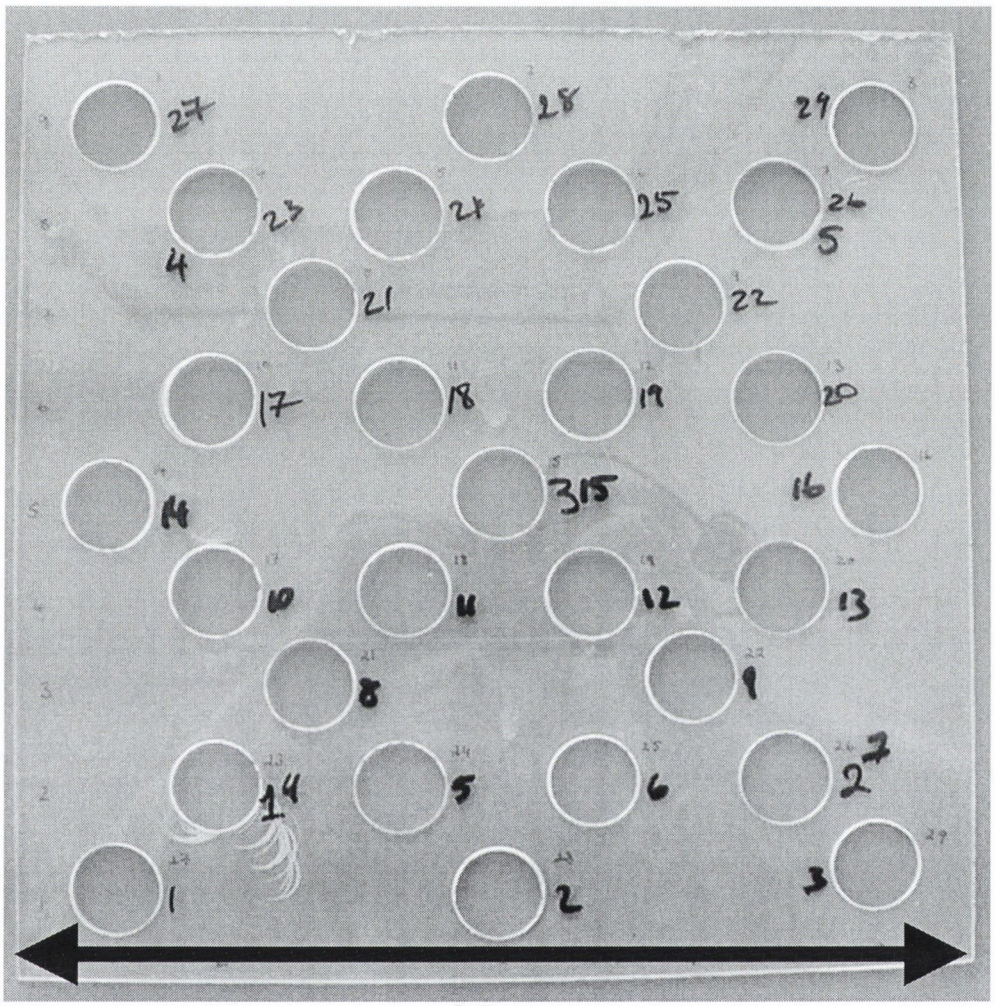


Figure 2.2. Quadrat ($625cm^2$) with holes to allow core samples of sediment photopigments to be taken for analysis of fine-scale spatial variability.

Table 2.1. Comparison of ANOVA of chlorophyll *a* data for quarters within each quadrat examined in section 2.3.1b.

Site	Quadrat	F-ratio	df	p-value
Bull Island	1	3.2	3	0.04*
	2	4.8	3	0.01*
	3	0.6	3	0.61
	4	0.2	3	0.87
Blackrock	1	0.2	3	0.86
	2	3.2	3	0.04*
	3	0.6	3	0.61
	4	2.8	3	0.04*

To determine large-scale variability in pigment concentration and ascertain the appropriate distance between sampling stations, the distance between quadrats was extended incrementally along a line at mid-shore level parallel to the shore. A total of 5 sampling cores were taken from pre-determined locations within each quadrat in March 2000. The distance between quadrats 1 and 2 was 25cm, and this distance was doubled for each subsequent quadrat with the exception of the final quadrat at each site (Table 2.2). Sediment cores were analysed as above. This geometric series was chosen to maximise the different scales at which variation could be examined across the flat and to investigate whether variation increased with increasing distance.

Table 2.2. Distances (m) between each pair of quadrats in transect lines parallel to shoreline at two intertidal sites in Dublin Bay.

Quadrat	Blackrock & Bull Island
1-2	0.25
2-3	0.50
3-4	1
4-5	2
5-6	4
6-7	8
7-8	16
8-9	32
9-10	50

2.2.6 Field sampling and laboratory methodologies for Hydrobia and Tellina

Spatial variation in the distribution and abundance of the primary consumer species to be studied had not been quantitatively described prior to this study. A number of sampling units (cores and quadrats) of varying sizes were tested to ascertain the most appropriate for obtaining sufficient numbers of individuals to determine the

population size composition within station, while not 'over-collecting' and causing excessive and unnecessary laboratory work. Sub-sampling macrofaunal species from core samples without bias is problematic. Sufficient numbers of specimens were deemed to have been collected when length-frequency distributions were not fragmented.

2.2.6a Hydrobia

The optimum sampling unit size for *Hydrobia* was determined to be between 40-80cm^2. The placing of quadrats and manual removal of sediment and animals within was not practical on the fluid mud of Bull Island, and so a core was used at this site. A section of standard household waste-pipe was fashioned as a corer with a sampling area of 70.9cm^2. Cores were taken to a depth of 10cm and placed directly into a labelled plastic bag.

Plastic bags containing these cores were stored in a cold room (5°C). Each sample was sieved through a 0.25mm sieve and the contents of the sieve placed in a field tray with seawater. Live *Hydrobia* were easily identified either by actual movement or movement tracks left on the tray. The shells of dead *Hydrobia*, lacking an operculum and often white in colouration due to bleaching, were discarded. Live *Hydrobia* were placed in 70% alcohol using forceps. Shell height (SH) was measured from posterior to anterior shell tips (Figure 2.3) using Mitutoyo Absolute Digimatic callipers to 0.1mm.

2.2.6b Tellina

The optimum sampling unit size for the larger bodied and less numerically abundant *Tellina* was determined to be >100cm^2. Using a sampling core was impractical in this instance due to the weight and volume of sediment that would have been removed to achieve the sampling depth of 15cm. This increased depth, compared with the Bull Island core, reflected the infaunal nature of *Tellina* and the increased depth to the anoxic layer at this site. A 25x25cm quadrat was placed on the surface of the sand, and the sediment removed to a depth of 15cm using a spade and placed into a 0.5mm

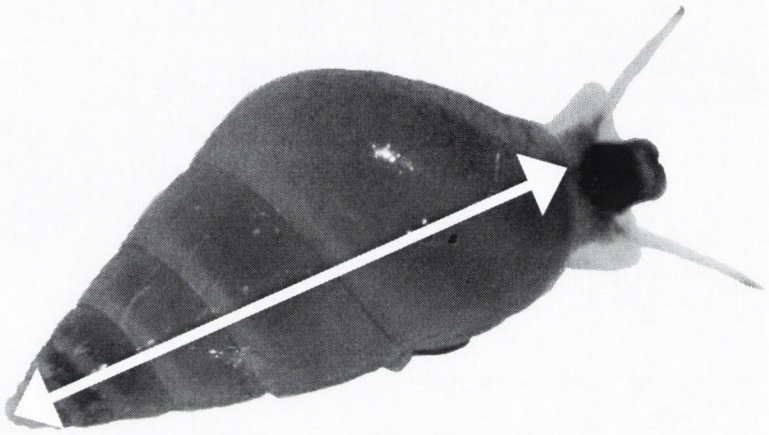


Figure 2.3. *Hydrobia ulvae* showing shell height (SH) measurement from posterior to anterior shell tips.

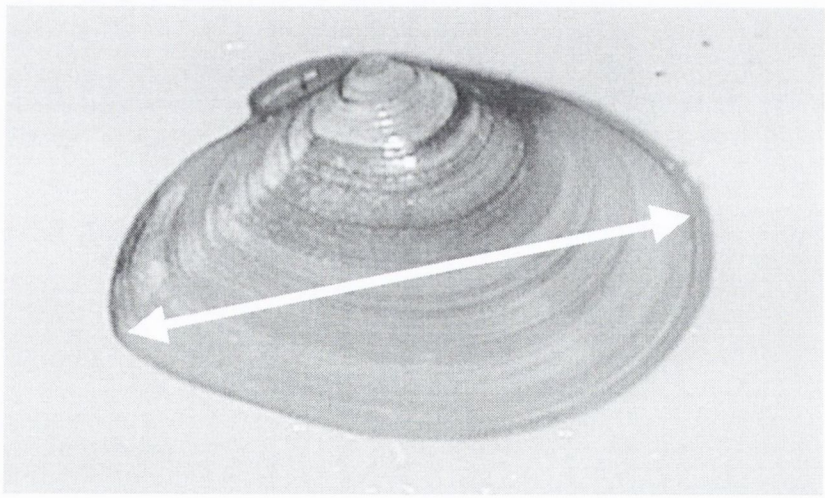


Figure 2.4. *Tellina tenuis* showing shell height (SH) measurement from posterior to anterior shell tips.

mesh sieve. Samples were sieved on site to remove the need to carry large quantities of sediment to the laboratory. The contents of the sieve were removed and placed into a labelled plastic bag.

Live *Tellina*, easily identified by the presence of flesh within the shell, were placed in 70% alcohol using forceps. Shell height was measured from posterior to anterior shell tips (Figure 2.4) using Mitutoyo Absolute Digimatic callipers to 0.1mm.

2.2.7 Site specific spatial variability in pigments, Hydrobia and Tellina distribution and abundance

The results of the previous investigations were combined to determine a sampling protocol to examine spatial variation in the target organisms within the entire sites at a given time.

2.2.7a Sampling at Bull Island and Blackrock

A synoptic survey of both sites was undertaken in April 2000, a surveyor's tape was used to mark out sampling stations across the Bull Island site at low tide (Figure 2.5). The four primary transects A, B, C and D began 20m from the seawall and extended across the flat with stations positioned every 20m for a total of 12 stations. Each of these transects were 50m apart in the down-shore direction. A shorter sampling distance between stations across the flat was chosen as this was likely to be the stronger of the 2 physical gradients at the site.

Two further transects consisting of 10 stations were sampled 100m to the east and west of the primary transects. Transect X was 100m to the east of transect D and began 40m from the sea wall with stations 20m apart. Transect Y was 100m to the west of transect A and began 20m from the sea wall. The difference in the location of the first sampling station for transects X and Y facilitated an even spread of stations on each side of the creek which meandered through the lagoon. These supplemental transects were positioned further from the other transects to determine how rapidly the

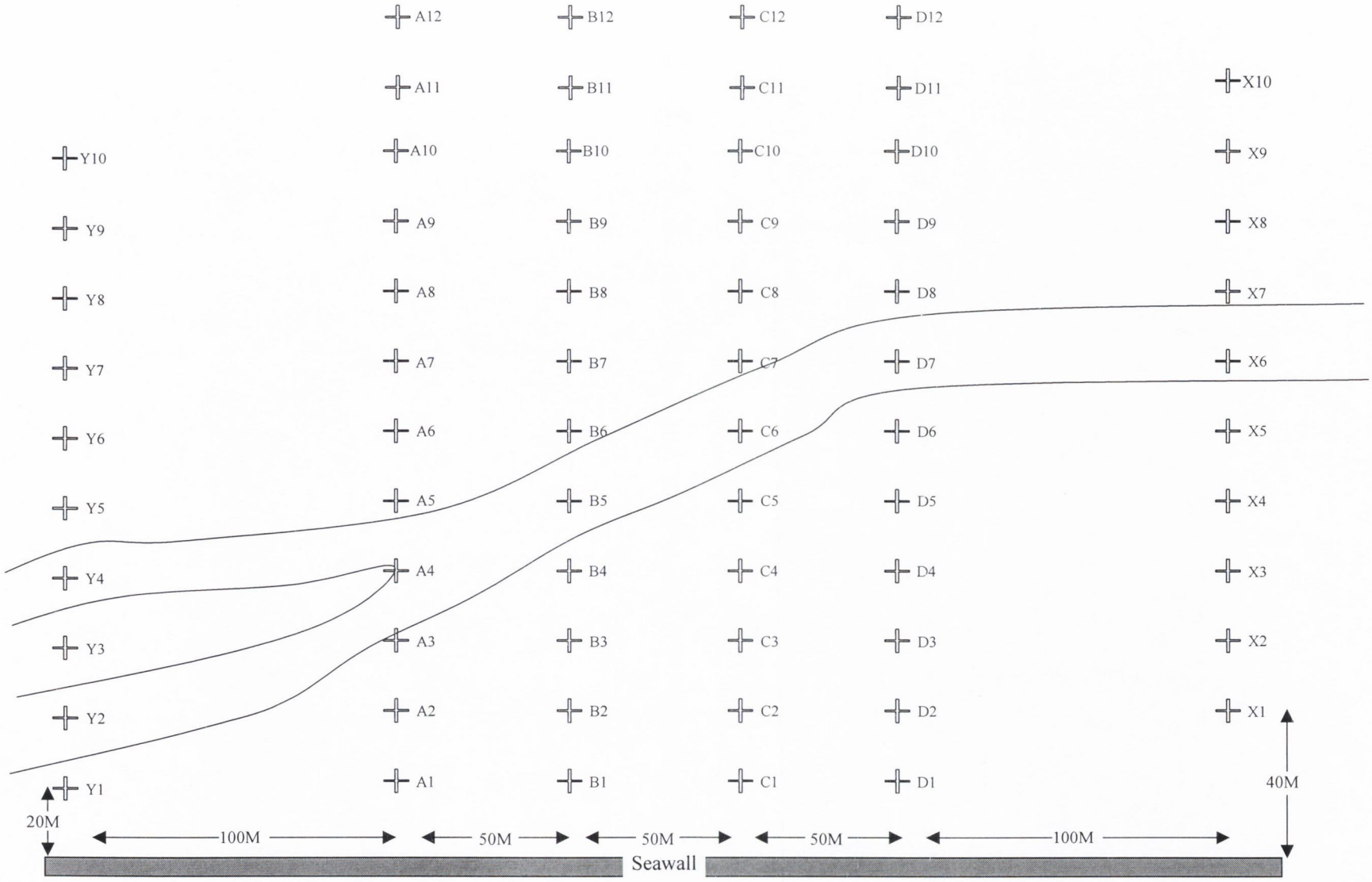


Figure 2.5. Sampling stations in each transect at Bull Island, April 2000.

down-shore physical gradient affected distribution and abundance, if at all. Three cores for pigment concentration and three for *Hydrobia* density were taken within $1m^2$ areas at each station following the methodologies described in 2.2.3 and 2.2.6a respectively. The sampling of 3 cores for *Hydrobia*, rather than a single larger core, removed the possibility that one unrepresentative sample could have been taken by chance.

Four transects A, B, C and D were sampled vertically at low tide across the sand flat at Blackrock (Figure 2.6). Transect A began 20m to the left of the wall of Blackrock Baths. The first station of each transect was located 20m from the seawall, with 20m between each subsequent station and 50m between transects. No additional transects were required (see Bull Island above) as the 4 transects covered the majority of the sand flat under examination. Due to the topography of the sand flat, water was retained along the lower shore at low tide restricting the length of transects. As a result transect A had 5 stations, transect B had 7, transect C had 9 and transect D had 10 stations. Three cores for pigment concentration and three for *Tellina* density were taken within $2m^2$ areas at each station following the methodologies described in 2.2.3 and 2.2.6b respectively. Pigment concentration was analysed as described in section 2.2.4.

2.2.7b Bull Island-data analysis

Shell height (SH) data from each station (3 replicates) were pooled and length-frequency distributions (LFD) of *Hydrobia* were constructed. Prior to pooling the data, the LFD from each replicate was compared with that of the other replicates and pooled data using Kolmogorov-Smirnov tests. The Kolmogorov-Smirnov test is a one sample test for assessment of goodness of fit of an observed to an expected cumulative frequency distribution (Zar, 1999). No significant differences were detected at any station. An example of a pooled station (A1) histogram along with each replicate is shown in Figure 2.7.

The pooled LFD data for each station was smoothed by a 3 point running average to account for natural biological variability in the population. Smoothing is used to

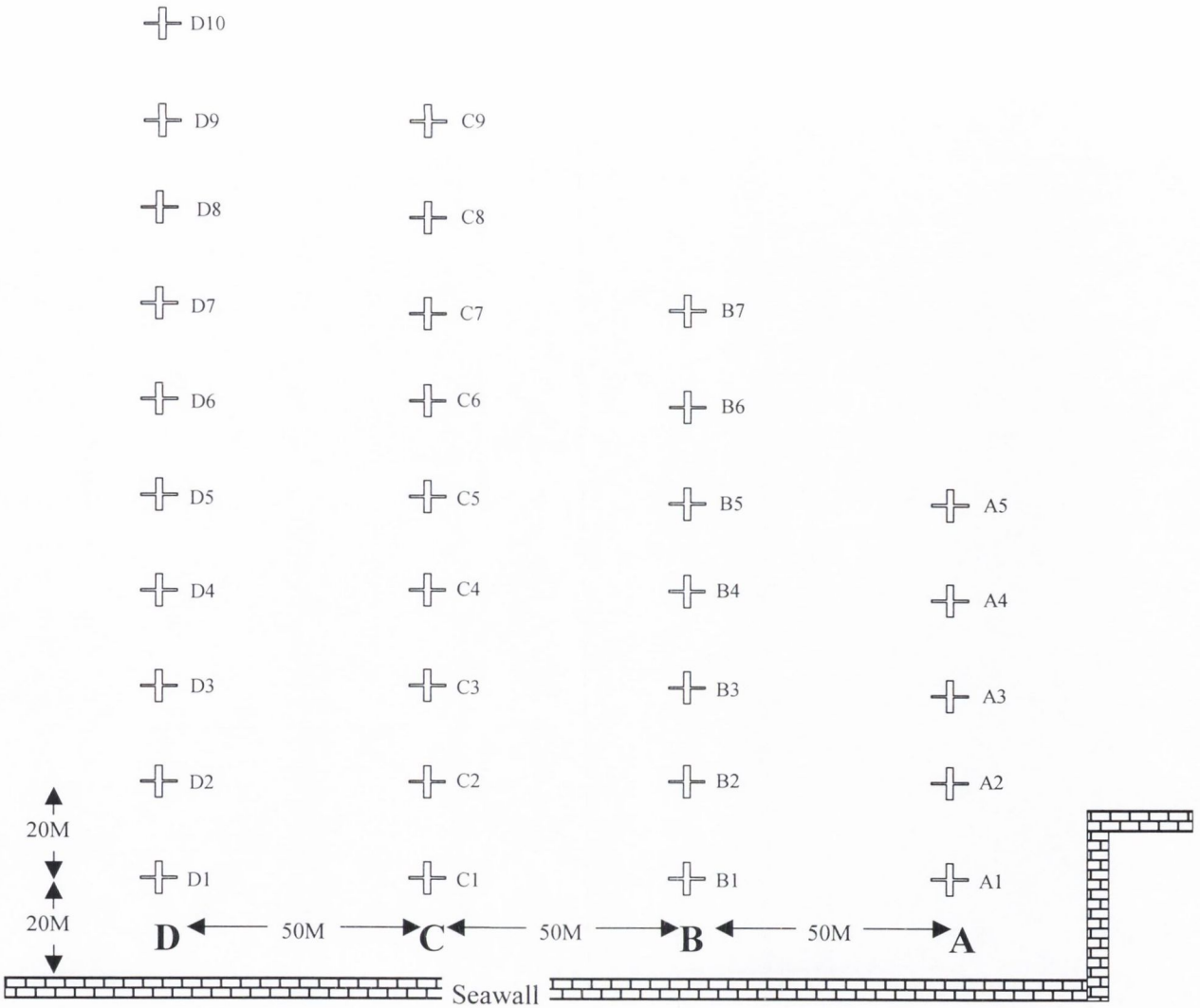


Figure 2.6. Sampling stations in each transect at Blackrock, April 2000.

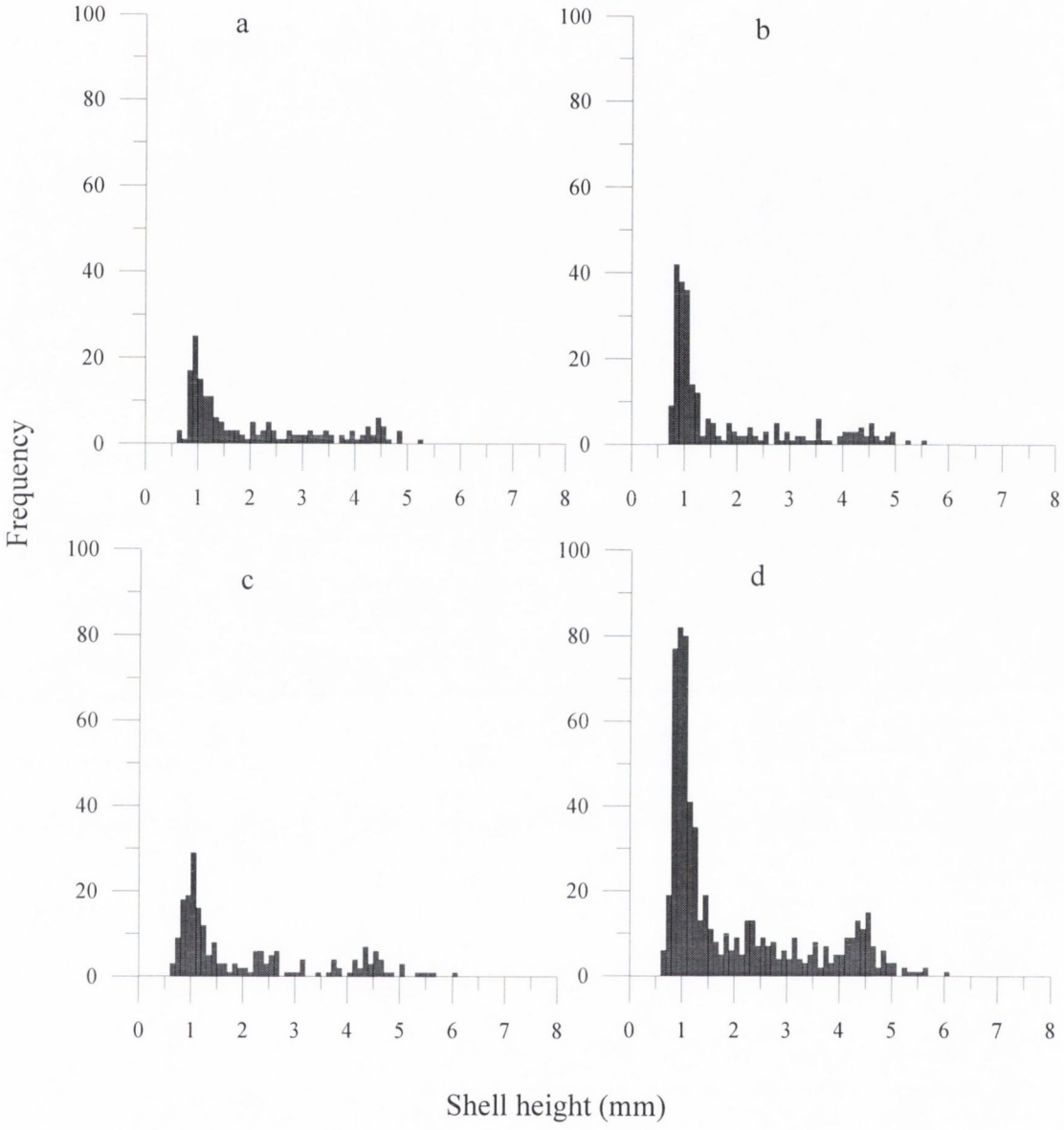


Figure 2.7. *Hydrobia* size-frequency histograms (unsmoothed) of a) replicate A1a (N=245), b) replicate A1b (N=177) and c) replicate A1c (N=212) and d) pooled A1 station (N=634), Bull Island, April 2000.

reduce short-term volatility among adjacent low population bins in histograms and as a result adjust for sharp discontinuities. Bar widths were set at 0.1mm (SH) as this interval was sufficiently fine to reveal growth intervals in laboratory experiments. Normal distributions, representing cohort/year groups, were separated from the composite LFD using the cohort analysis computer programme MIX 2.3 (MacDonald & Green, 1988). The programme separates normal distributions by chi-square maximum likelihood estimation.

All composite LFDs of *Hydrobia* at stations containing >250 individuals were examined for similarity (Kolmogorov-Smirnov test). This number was chosen as that required to prevent fragmentation of the data associated with the second and third cohorts. Half of the 52 stations with >250 individuals had identical composite LFD. The same normal distributions, representing cohorts, were separated from each of these composite LFDs using MIX. Separation of the first or third cohorts was also possible in the remaining LFDs. Due to low numbers and data fragmentation in the second cohort however, it was often not possible to separate this cohort from the first in the remaining LFDs, although these closely resembled the other LFDs in shape and appearance. For the purpose of constructing distribution maps all composite LFDs were divided based on the criteria of those that could be statistically separated into the three distinct settlement cohorts. For example see Figure 2.8. The right 'cut off points' or boundaries of each cohort were examined for all stations and the 3 most distinct cohorts/size class groups selected. Four groups were distinguished readily in most LFDs, <1.6mm, 1.6–3.1mm, 3.1–5mm and >5mm (SH). These size groups were used to represent the LFD at all stations.

Spatially referenced abundance data for each size class were entered in to the Surfer® Package to produce variogram models and contour plots as described in 2.2.4. Differences with shore level and between transects were examined using ANOVA or Kruskal-Wallis as appropriate, with post-hoc tests revealing sources of significant variation. Relationships between variables were examined using Pearson-product correlations.

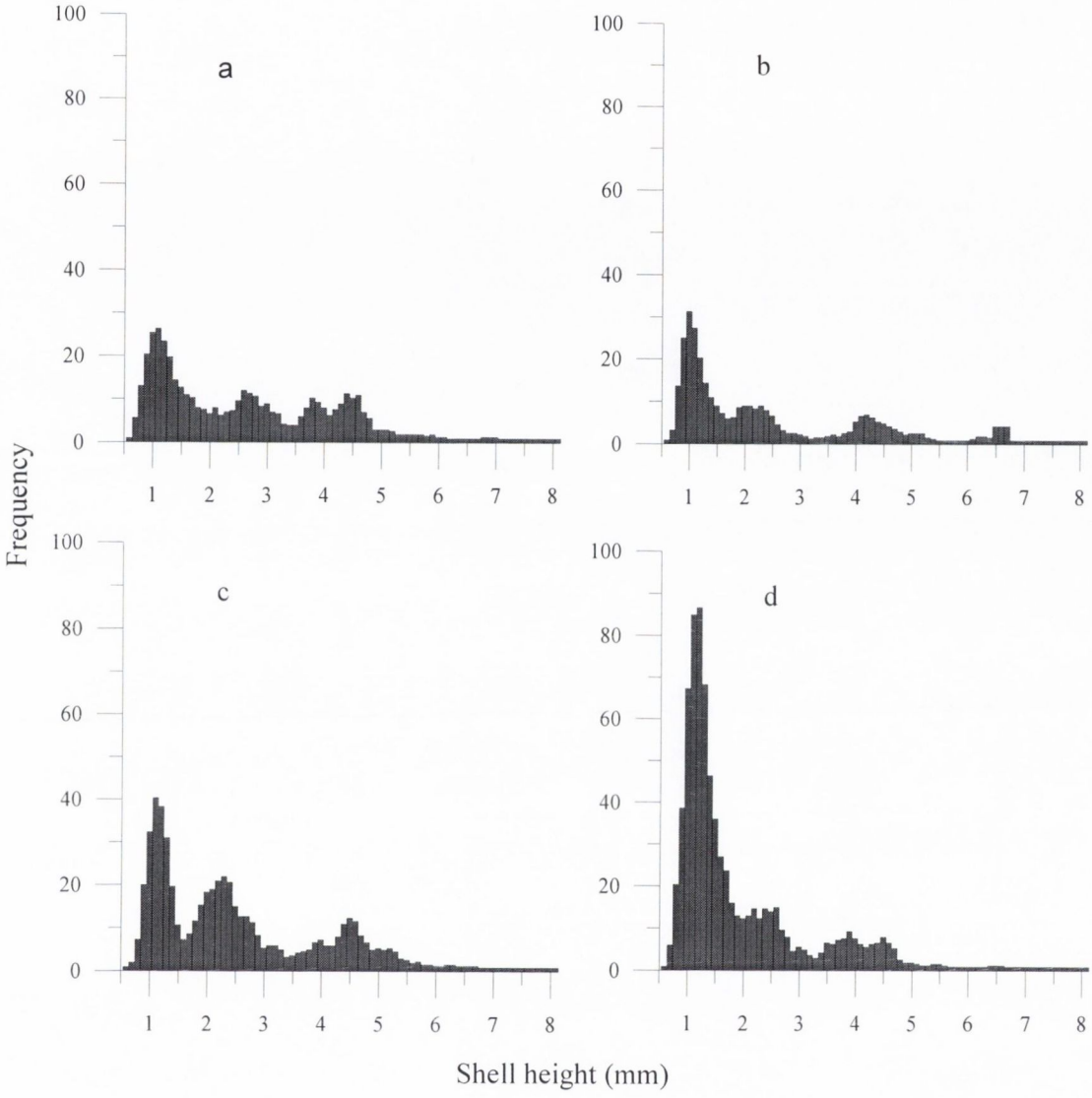


Figure 2.8. Smoothed *Hydrobia* size-frequency distributions from selected stations at Bull Island, April 2000. a) station Y1 (N=405) , b) station B3 (N=284), c) station A2 (N=533) and d) station C4 (N=732).

2.2.7c Blackrock-data analysis

Data from each station (3 replicates) were pooled and LFD of *Tellina* shell height (SH) were constructed. Prior to data pooling, each replicate was plotted along with the pooled data to verify that the station histogram was representative of the replicate data. At each station replicates showed the same distribution pattern as in the pooled histograms. An example of a pooled station (A1) histogram along with each replicate is shown in Figure 2.9.

The pooled station data was smoothed by a 3 point running average to account for natural biological variability in the population. Smoothed histograms were examined for distribution patterns in order to distinguish size classes/year groups within the population. Bar widths were set at 0.2mm (SH), as this gave the most detailed histograms without resulting in data fragmentation. Shapes of distributions were tested for significant differences using Kolmogorov-Smirnov tests.

Distinct cohort/year groups were identified from the LFDs using MIX 2.3 (MacDonald & Green, 1988). Although size class cohorts were successfully separated for all stations, none were applicable to the whole site. Clear differences in length-frequency were observed at different shore levels. Examples of cohort means and proportions isolated using MIX at certain stations emphasise the different LFDs observed (Table 2.3). Distributions did not clearly correspond to published data of *Tellina tenuis* year groups (Stephen, 1928; Barnett & Watson, 1986; Wilson, 1996), most likely due to blending of cohorts. As a result of the failure to adequately separate distinct cohorts that were representative of the whole site and the lack of correlation between the data at different stations and the published literature, an arbitrary size class scale was applied. A cohort bandwidth of 4mm was selected as most closely representing the approximate components of the distributions over all transects and shore levels. Some published literature and the arbitrary size classes chosen showed similarities. The intervals compared with published values of approximately 4mm for 1+ *Tellina*, and 8 mm for 2+ (Barnett & Watson, 1986; Wilson, 1996). Barnett & Watson (1986) also found that above 12mm SH year groups were indistinguishable and included only individuals >3+ years old.

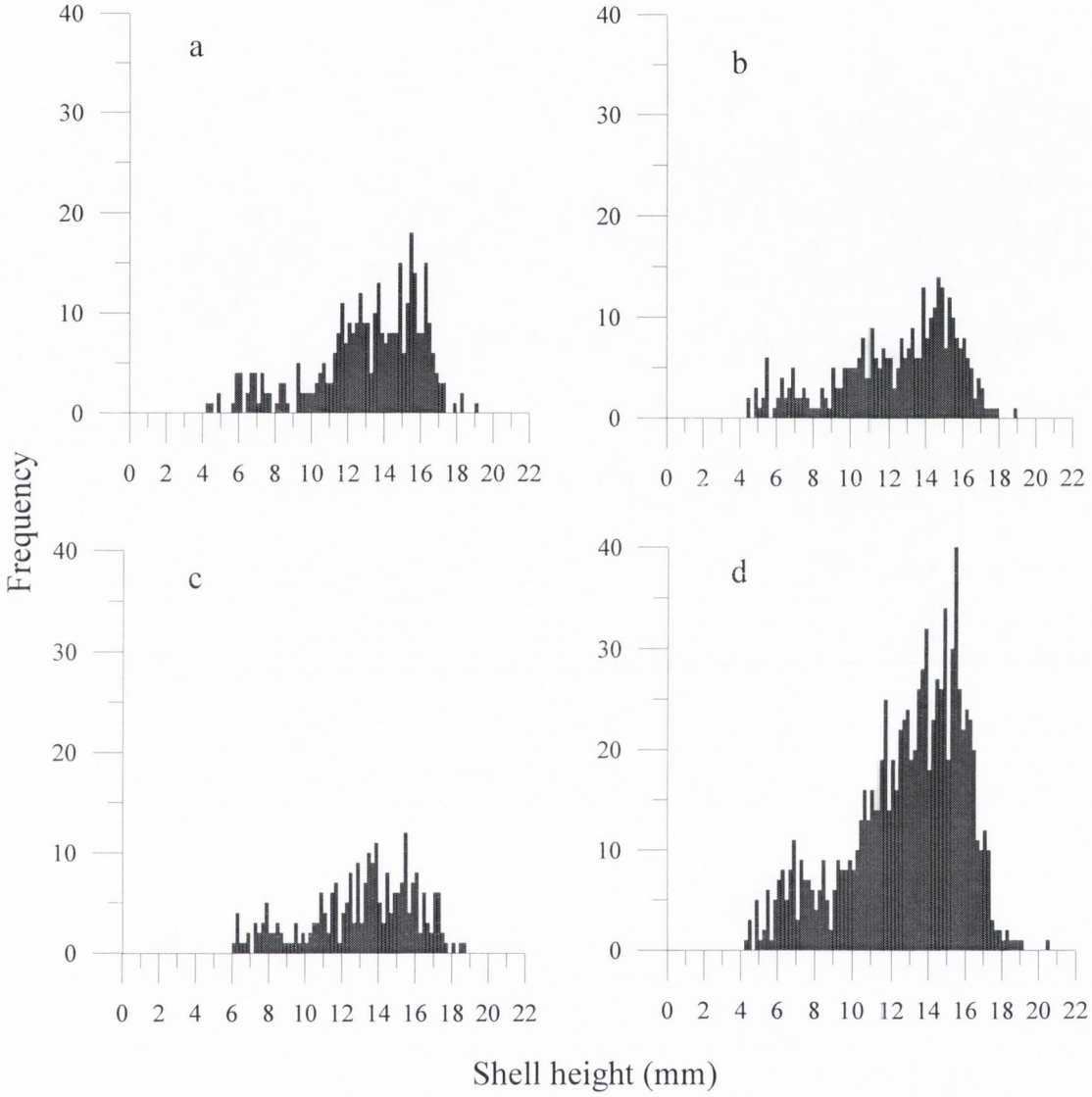


Figure 2.9. Size-frequency distribution of *Tellina* at Blackrock, April 2000, for a) replicate A1a (N=349), b) replicate A1b (N=334), c) replicate A1c (N=245) and d) pooled A1 station (N=928).

Table 2.3. Mean (± S.D.) SH (mm) and proportion (± S.D.) of individuals within *Tellina* cohorts separated using MIX on selected stations to highlight the variability in size-frequency distribution at different shore levels at Blackrock.

Station	N	No. cohorts isolated	Mean ± SD	Proportion ± SD
A1	928	3	7.0 ± 1.4	0.16 ± 1.4
			11.8 ± 1.4	0.36 ± 1.4
			15.3 ± 1.2	0.48 ± 1.2
D1	712	3	3.6 ± 0.7	0.05 ± 0.7
			6.5 ± 1.3	0.15 ± 1.3
			12.9 ± 2.2	0.79 ± 2.2
B4	768	5	4.4 ± 1.0	0.17 ± 1.0
			6.9 ± 1.0	0.16 ± 1.0
			9.9 ± 1.3	0.22 ± 1.3
			12.5 ± 1.1	0.37 ± 1.1
			15.0 ± 1.5	0.08 ± 1.5
C8	838	2	5.6 ± 1.8	0.44 ± 1.8
			11.1 ± 2.1	0.56 ± 2.1
D8	891	2	5.5 ± 1.8	0.43 ± 1.8
			10.9 ± 2.0	0.57 ± 2.0

Spatially referenced abundance data for each size class were entered in to the Surfer® package to produce variogram models and contour plots as described in 2.2.4. Differences with shore level and between transects were examined using ANOVA or Kruskal-Wallis as appropriate, with post-hoc tests revealing sources of significant variation. Relationships between variables were examined using Pearson-product correlations.

2.3 Results

2.3.1 Preliminary investigation of spatial variation in pigment concentration

2.3.1a Medium-scale variability (samples within station)

Levels of chlorophyll *a* differed significantly among quadrats at Bull Island (Figure 2.10) (ANOVA, F=3.84, df =13, $p \leq 0.01$). Mean (±S.E.) sediment chlorophyll *a* was 137.5±7.5mg m^{-2}, ranging from 55.5 to 273.4mg m^{-2} (N=56). The mean chlorophyll *a* concentration of quadrats within the first $1m^2$ grouping was 144.7±9.2 mg m^{-2} compared with 177.1±15.1mg m^{-2} and 87.1±4.6mg m^{-2} for those 2 and 4m away respectively. The concentration in the latter group was significant lower than in the two former (ANOVA, F= 16.63, df =2, $p \leq 0.01$). Mean sediment chlorophyll *a* was greater than average phaeopigment in all quadrats (Figure 2.10). Levels of phaeopigment at Bull Island differed significantly among quadrats within grouping

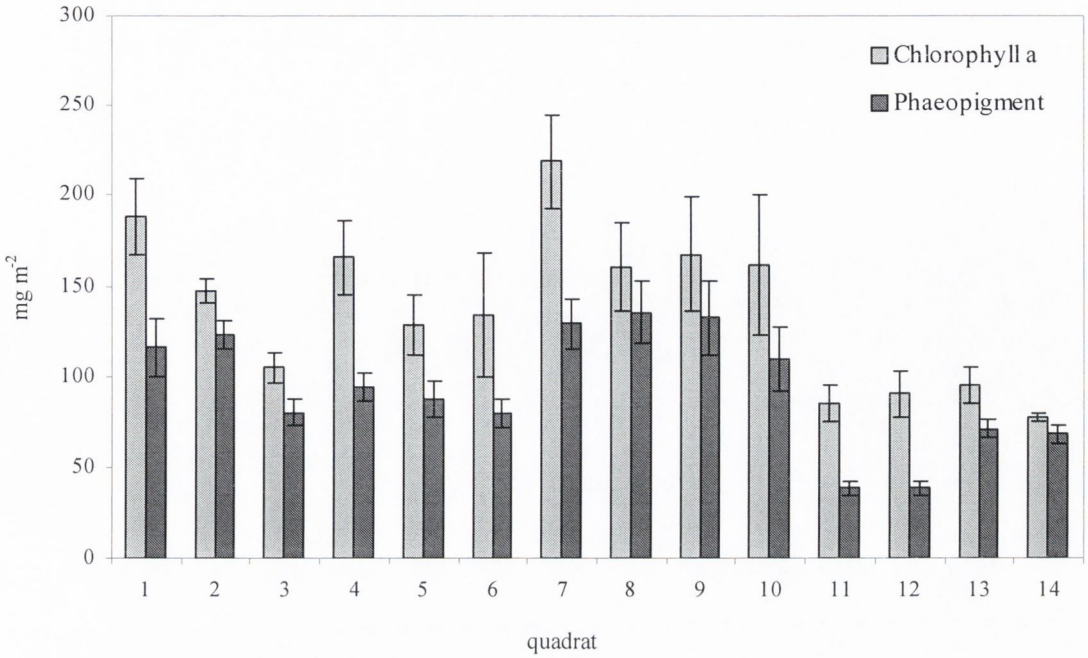


Figure 2.10. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) within quadrats at varying sampling distances across the Bull Island mudflat, January 2000 (N=56). See methods for sampling distances (samples within station).

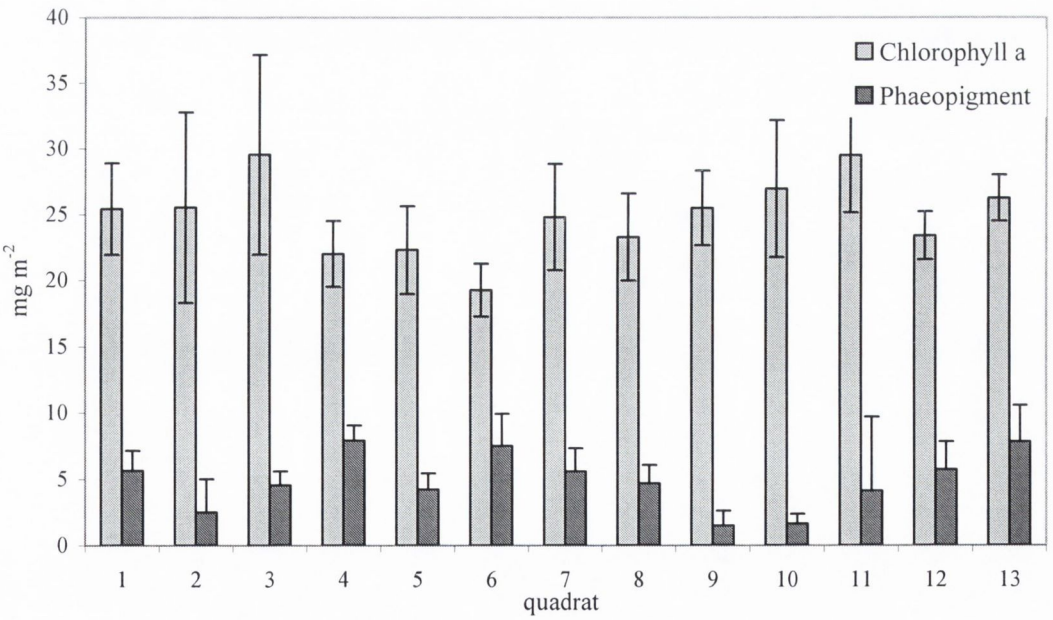


Figure 2.11. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) within quadrats at varying sampling distances across the Blackrock sandflat, January 2000 (N=55). See methods for sampling distances (samples within station).

(ANOVA, F=8.02, df =13, $p \leq 0.01$). Mean phaeopigment concentration within the first m^2 sampled was 96.7±5.0mg m^{-2} compared with 126.7±8.1mg m^{-2} and 54.1±4.5mg m^{-2} for the areas 2 and 4 m away respectively. Significant differences were observed among each of these sets of quadrats (ANOVA, F=32.62, df =2, $p \leq 0.01$).

At Blackrock chlorophyll *a* did not differ significantly between quadrats (ANOVA, F=1.53, df=13, p=0.15) (Figure 2.11). Average sediment chlorophyll *a* was 24.9±1.1mg m^{-2}, and ranged from 13.8 to 50.3mg m^{-2} (N=55). The average chlorophyll *a* of all the quadrats within the first m^2 sampled was 23.9±1.8mg m^{-2} compared with 25.2±1.8mg m^{-2} and 26.4±1.7mg m^{-2} for the areas located 2 and 4m, respectively. Mean sediment chlorophyll *a* was greater than average phaeopigment in all quadrats at this site. Levels of phaeopigment also showed no significant differences (ANOVA, F=1.40, df=13, p=0.24) among quadrats (Figure 2.11). The average phaeopigment concentration within the quadrats within the first m^2 grouping sampled was 5.5 ± 0.7mg m^{-2} compared with 3.4 ± 0.7mg m^{-2} and 5.9 ± 2.0mg m^{-2} for the areas located 2 and 4m away, respectively. These differences were not significant at the 5% level (ANOVA, F=1.03, df=2, p=0.36). Lower concentrations of both chlorophyll *a* and phaeopigment were observed at Blackrock when compared with Bull Island.

2.3.1b Small-scale variability (samples within replicates)

Average chlorophyll *a* levels at Bull Island were 147.0±9.0mg m^{-2}, 124.2±11.5mg m^{-2}, 217.9±15.4mg m^{-2} (S.E.) and 156.2±15.9mg m^{-2} for quadrats 1, 2, 3 and 4 respectively (Figure 2.12). Quadrat 3 had significantly higher concentrations of chlorophyll *a* than all other quadrats (ANOVA, F=9.21, df=3, $p \leq 0.01$). Mean phaeopigment levels were higher than chlorophyll *a* in all quadrats (Figure 2.12). Average phaeopigment concentrations ranged from 235.2±19.7mg m^{-2} in quadrat 2 to 422.6±29.4mg m^{-2} in quadrat 4. Significant differences in phaeopigment were observed among quadrats (ANOVA, F=8.60, df=3, $p \leq 0.01$) with lower concentrations in quadrat 2 than in 1 and 4 and quadrat 4 higher than quadrat 3.

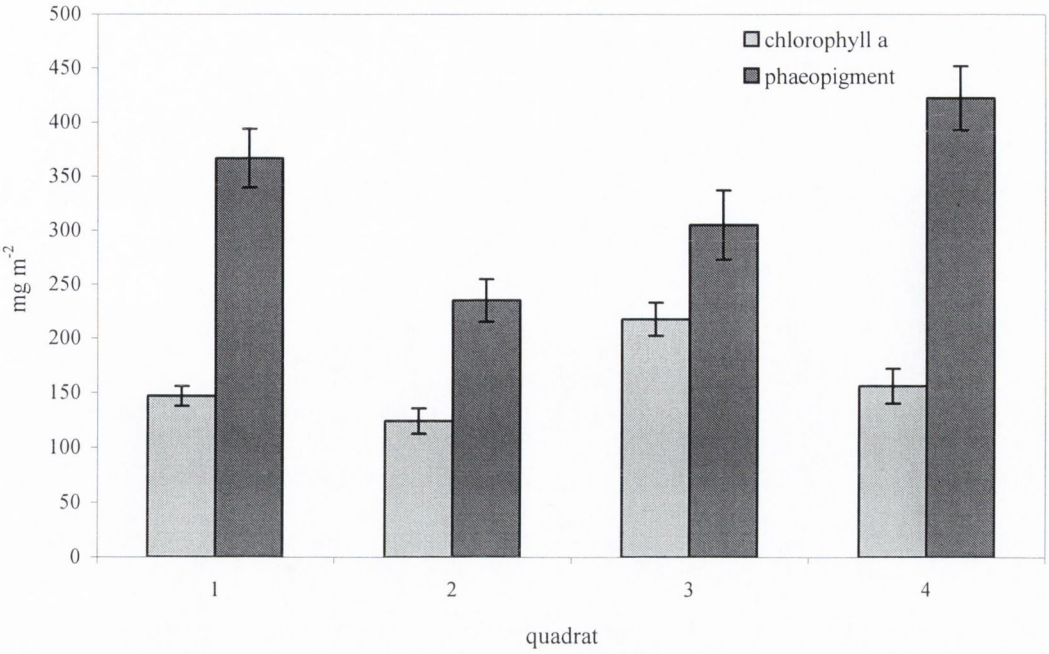


Figure 2.12. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) within quadrats at Bull Island, February 2000 (N=115). See methods for sampling distances (samples within replicates).

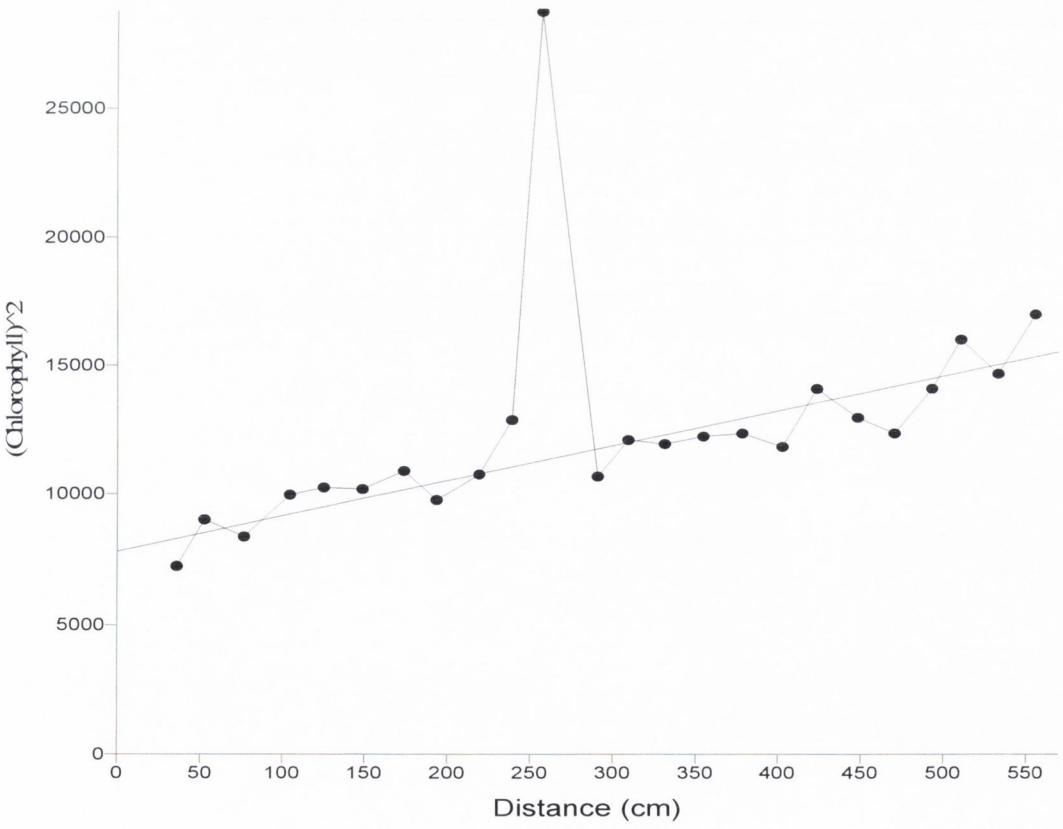


Figure 2.13. Variogram of small-scale spatial variation (samples within replicates) in chlorophyll *a* concentration at Bull Island, February 2000.

At Bull Island variability in sediment chlorophyll *a* concentration increased with distance between sampling points, but the fitted model did not level off to reach an asymptote at the spatial scales studied (Figure 2.13). The nugget effect (variability at minimal distance, y-axis intercept) and the slope of the variogram fitted line was high (slope=7.6), increasing 50% before the maximum distance examined was reached. This implied that there was a high inherent variability in chlorophyll *a* concentration over small distances and that a concentration gradient also occurred across the sampling area as a whole, at least at the time of sampling and shore level studied. The large 'spike' that was observed in the variogram coincided with the first distance that expressed variability between quadrats. Contour plots revealed a high degree of variability within quadrat 1, which also contained the largest range in chlorophyll *a* values (Figure 2.14). Quadrat 2 had an increasing concentration gradient from left to right, while quadrat 3 showed a decreasing gradient from left to right. Quadrat 4 contained few contour lines when plotted on the same scale as the other quadrats indicating that chlorophyll *a* concentration was relatively uniform in comparison. Variograms of phaeopigment concentrations could not be constructed due to excessively high variability in concentration at all spatial scales. Variograms cannot be correctly employed when variation is so great as to result in a linear slope = 0.

Mean chlorophyll *a* concentrations at the Blackrock site were 58.6±0.7mg m^{-2}, 56.0±0.5mg m^{-2}, 58.6±0.9mg m^{-2} and 58.8±1.0mg m^{-2} for quadrats 1, 2, 3 and 4, respectively (Figure 2.15). No significant differences were observed among quadrats (ANOVA, F=2.6, df=3, p=0.06). Mean phaeopigment levels were lower than chlorophyll *a* in all quadrats (Figure 2.15). Average phaeopigment concentrations ranged from 2.8±1.8mg m^{-2} in quadrat 4 to 7.4±0.5mg m^{-2} in quadrat 2. Significant differences in phaeopigment were observed among quadrats (ANOVA, F=8.3, df=3, $p \leq 0.01$). Quadrat 2 was significantly higher in phaeopigment content than quadrats 1 and 4, and quadrat 3 was higher than quadrat 4. The Blackrock site had lower concentrations of chlorophyll *a* and phaeopigment when compared with Bull Island.

The variogram of chlorophyll *a* concentration for Blackrock showed spatial variability increasing with distance between sampling points (Figure 2.16). The nugget effect (variability at minimal distance) was very much lower than that recorded at Bull Island. The slope of the fitted line was shallow (slope = 0.03), did not reach an

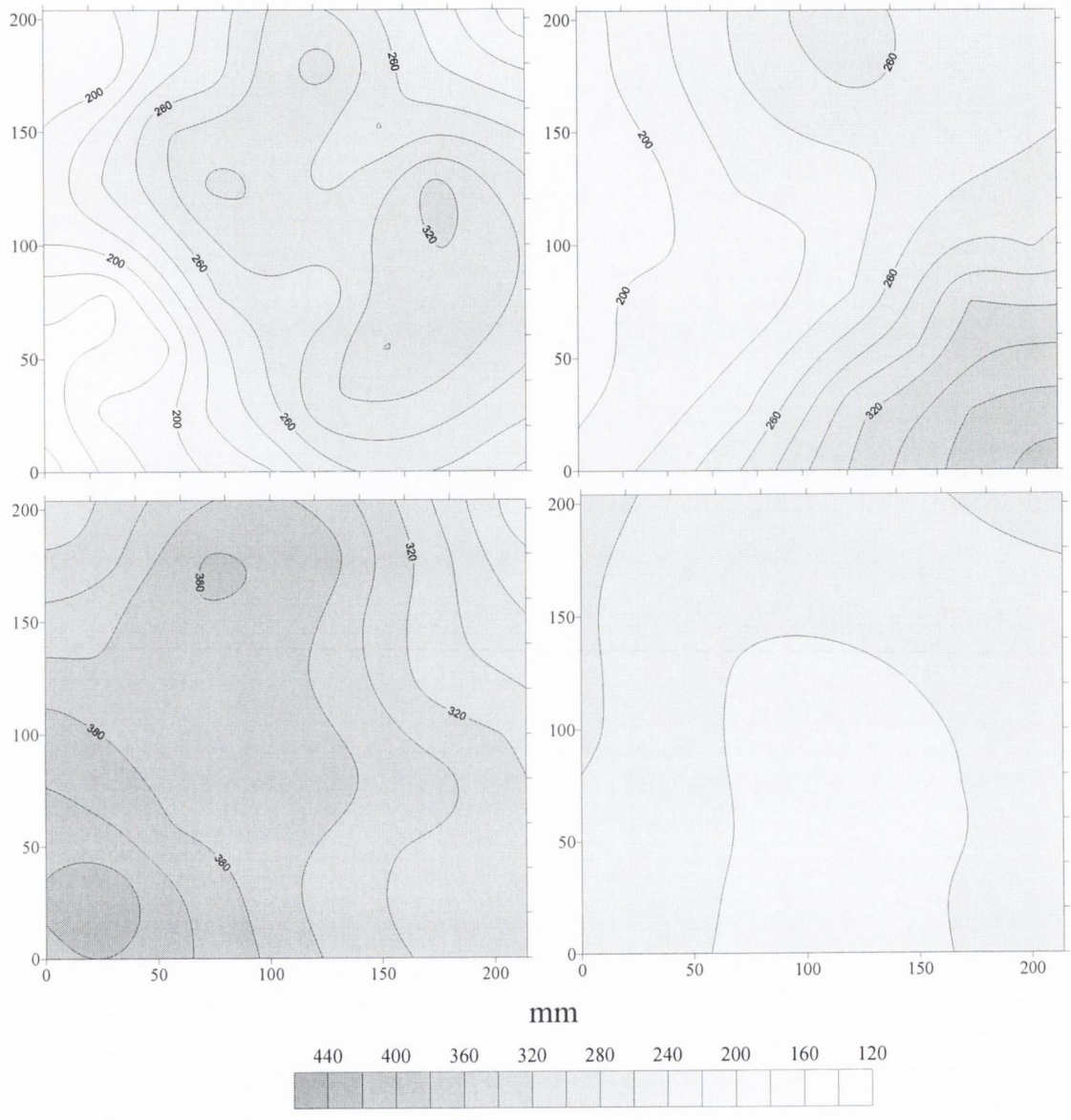


Figure 2.14. Contour plots of chlorophyll *a* (mg m^{-2}) concentration between quadrats at Bull Island, February 2000. These quadrats were positioned in sequence along the shore, and not in the orientation shown. See methods for details (replicates within samples).

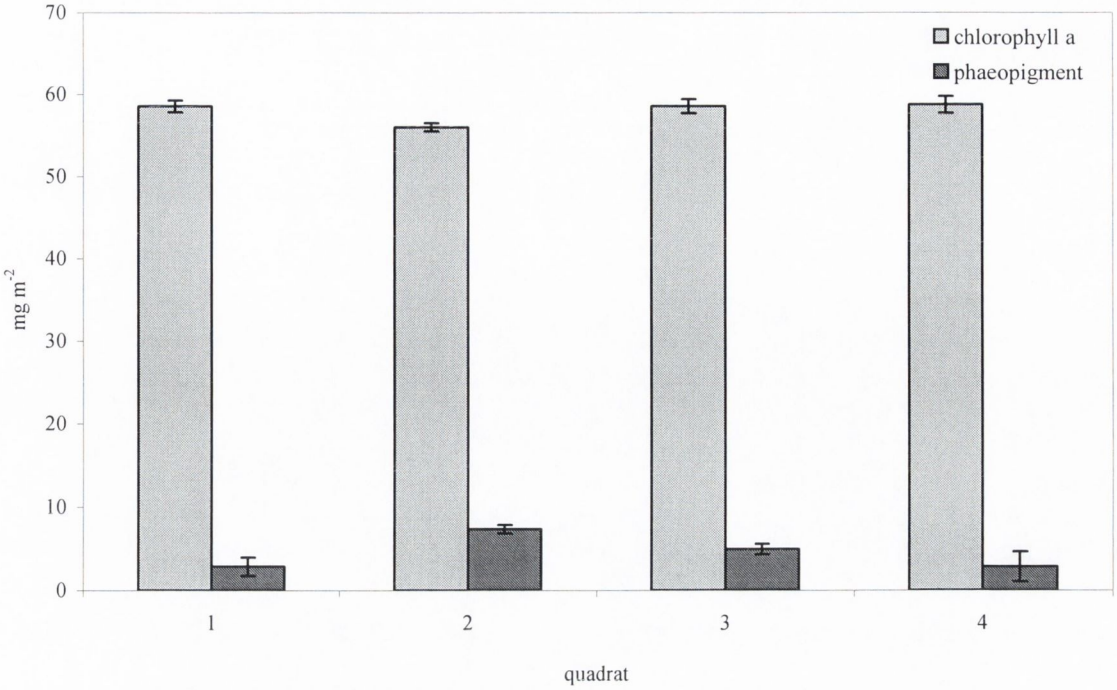


Figure 2.15. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) within quadrats at Blackrock, February 2000 (N=116). See methods for sampling distances (samples within replicates).

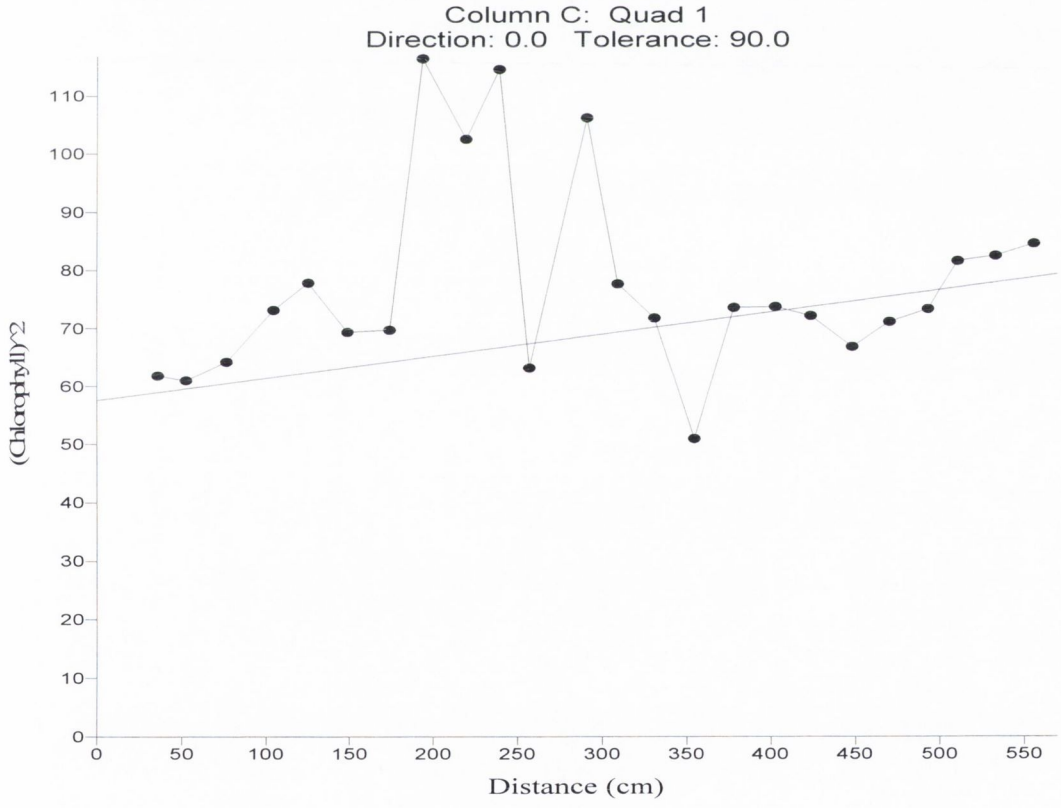


Figure 2.16. Variogram of small-scale spatial variation (samples within replicates) in chlorophyll *a* concentration at Blackrock, February 2000.

asymptote and increased only 30% within the same distances examined at Bull Island (50%). Chlorophyll *a* was relatively variable (although less so than at Bull Island), and although spatial variability increased slightly with distance suggesting a concentration gradient, it was only very slight at the scales/shore level examined. Variograms of phaeopigment concentrations could not be constructed due to excessively high variability in concentration at all spatial scales.

As chlorophyll *a* values were much lower at Blackrock than Bull Island the colour scale was modified to show smaller changes in chlorophyll *a* concentration. Quadrat 1 showed the largest range of concentration with highest values in the centre of the quadrat (Figure 2.17). Quadrat 2 showed values increasing from left to right. Quadrats 3 and 4 did not have a wide range and were fairly uniform in chlorophyll *a* content.

There was a significant positive correlation (Pearson-product = +0.52, $p \leq 0.01$) between chlorophyll *a* and phaeopigment at Bull Island (Figure 2.18). Contrastingly, a significant negative correlation (Pearson-product = -0.79, $p \leq 0.01$) was observed between these parameters at Blackrock (Figure 2.19). .

2.3.1c Large scale variability (stations within site)

Mean chlorophyll *a* content at Bull Island increased with distance from the first quadrat sampled (Figure 2.20). The mean (±S.E.) chlorophyll *a* content ranged from 74.2±5.1mg m^{-2} in quadrat 7 to 111.1±7.4mg m^{-2} in quadrat 9. Significant differences among quadrats were observed (ANOVA, F=3.83, df=9, $p \leq 0.01$). Mean phaeopigment levels corresponded closely to chlorophyll *a* in most quadrats (Figure 2.20). The average phaeopigment concentration was 87.6±8.0mg m^{-2}. Significant differences in phaeopigment levels were observed among quadrats (ANOVA, F=3.0, df=9, $p \leq 0.01$). The resulting variogram of chlorophyll *a* concentration had a high nugget value, a linear relationship that increased only slightly with distance from the point of origin (slope = <0.01), was exceptionally noisy (peaks) and failed to reach asymptote (Figure 2.21). This indicated that there was no uniform gradient in chlorophyll *a* distribution at the shore level examined during the sampling period and variability was high even at small spatial scales. The peaks indicated that at greater

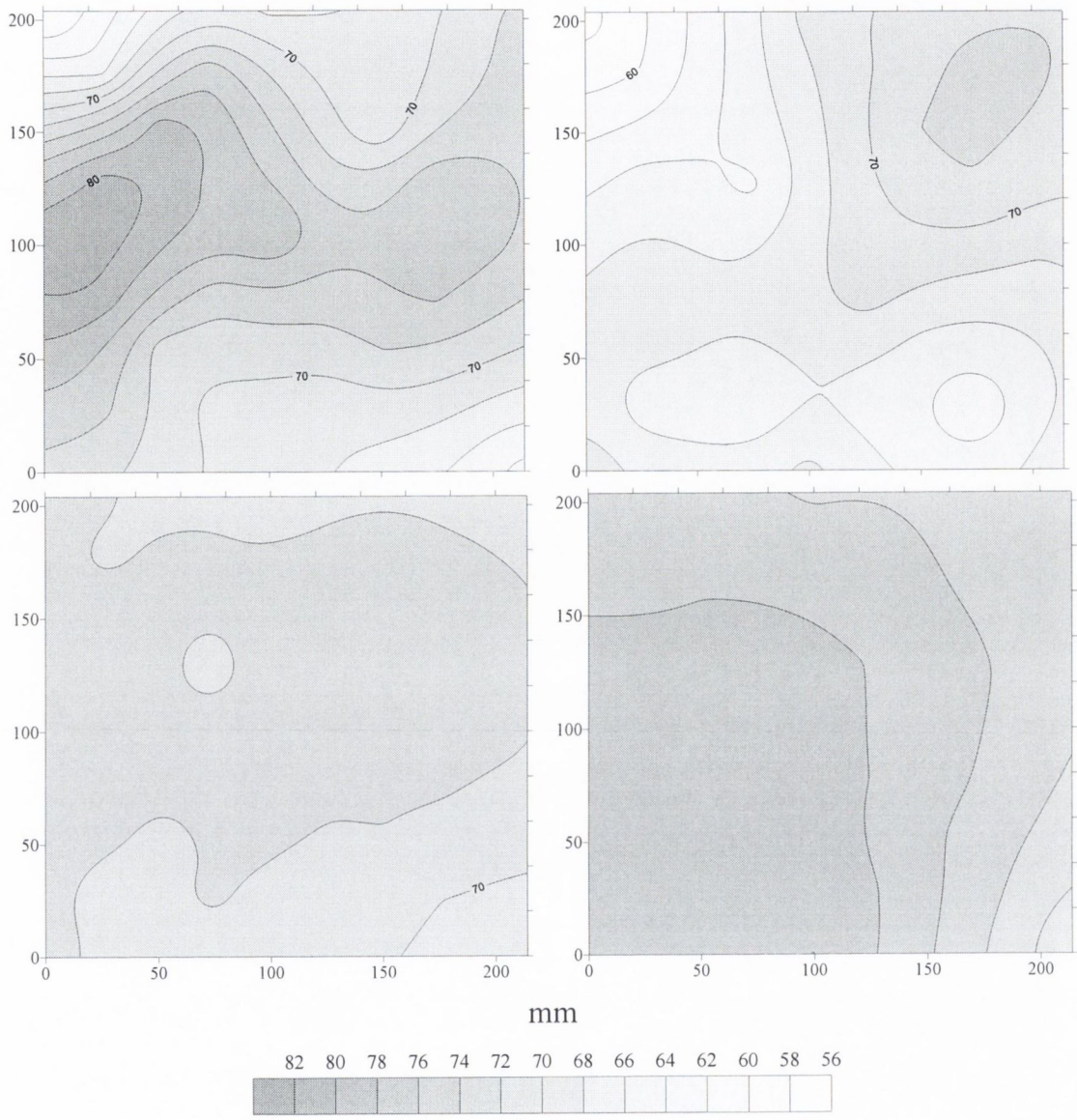


Figure 2.17. Contour plots of chlorophyll *a* (mg m^{-2}) concentration between quadrats at Blackrock, February 2000. These quadrats were positioned in sequence along the shore, and not in the orientation shown. See methods for details (replicates within samples).

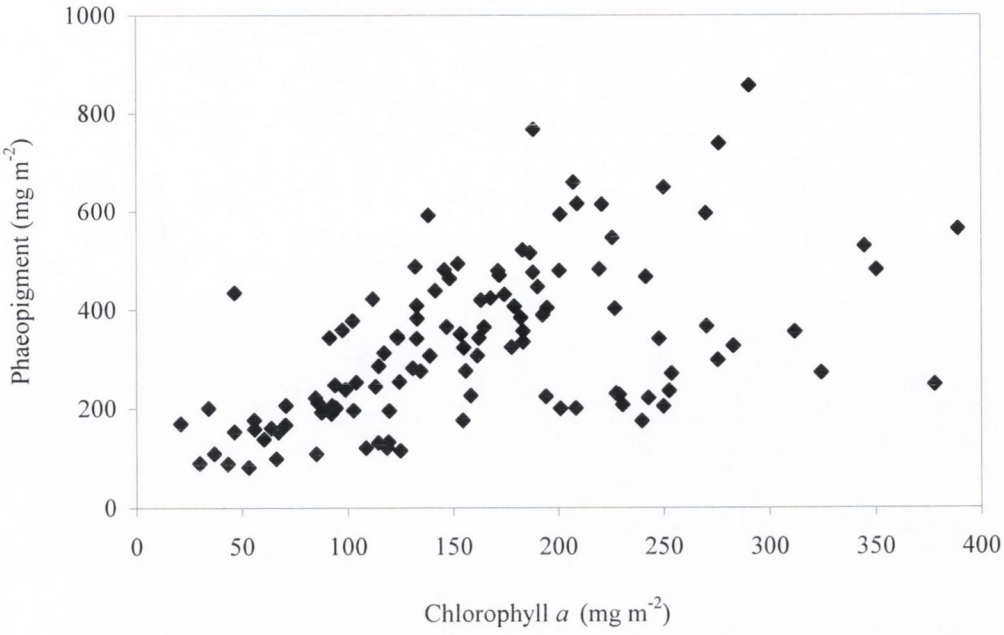


Figure 2.18. The relationship between mean chlorophyll *a* and phaeopigment levels at Bull Island, February 2000 (N=115). Pearson-product = +0.52, $p \leq 0.01$.

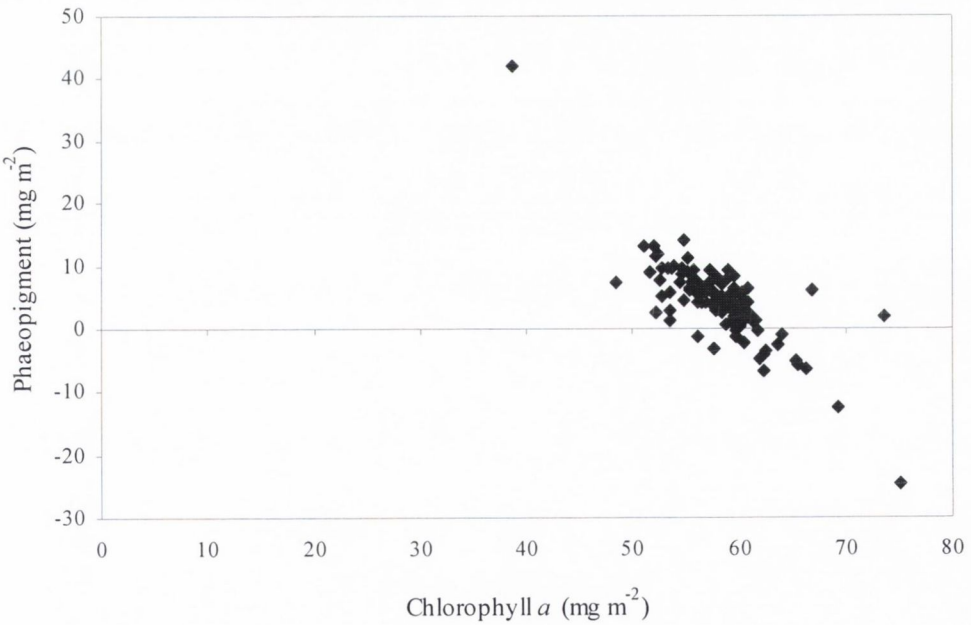


Figure 2.19. The relationship between mean chlorophyll *a* and phaeopigment levels at Blackrock, February 2000 (N=116). Pearson-product = -0.79, $p \leq 0.01$.

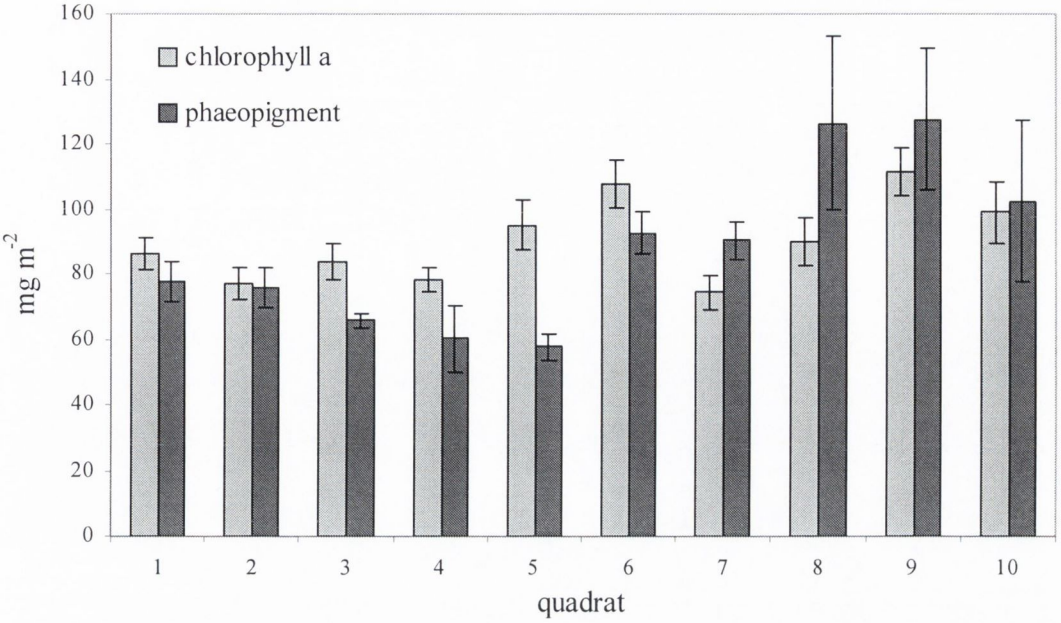


Figure 2.20. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) at Bull Island, March 2000 (N=65). The distance between quadrats 1 and 2 was 25cm, and this distance was doubled for each subsequent quadrat with the exception of the final quadrat which was 50m from quadrat 9.

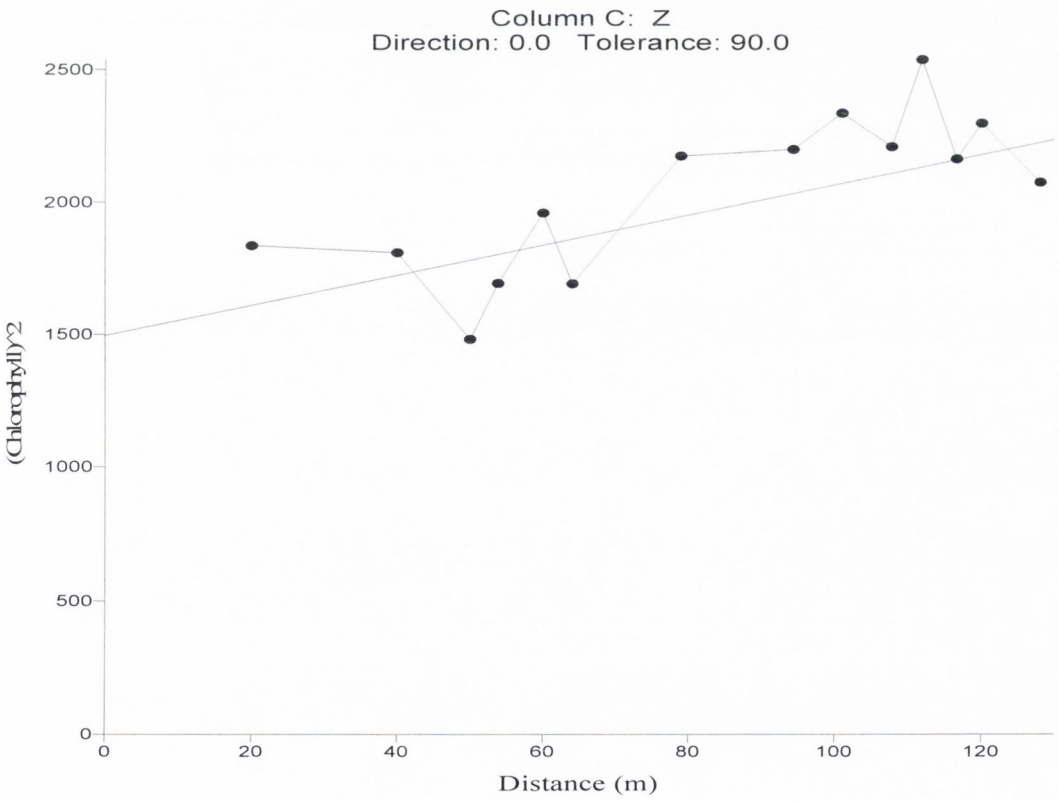


Figure 2.21. Variogram of large scale variability (stations within site) in chlorophyll *a* concentration at Bull Island, March 2000.

distances very irregular disparities in distribution occurred, highlighting a high degree of patchiness.

Mean chlorophyll *a* concentration at Blackrock also increased with distance from the first quadrat (Figure 2.22). Mean chlorophyll *a* per quadrat ranged from 55.2±0.9 to 78.9±2.5mg m^{-2}. Significant differences among quadrats were observed (ANOVA, F=18.26, df=9, $p \leq 0.01$). Mean phaeopigment levels were low compared with chlorophyll *a* (Figure 2.22). The average phaeopigment concentration at Blackrock was 3.8±0.7mg m^{-2}. No significant differences in phaeopigment concentrations among quadrats were observed (ANOVA, F=1.92, df=9, p=0.08) even though a negative average value was recorded in quadrat 10. Lower concentrations of chlorophyll *a* and phaeopigment were observed at Blackrock compared with Bull Island. The variogram constructed for the Blackrock site increased only very slightly with distance from the point of origin (slope = <0.01) (Figure 2.23). This indicated that a significant gradient did not exist at the site at this time, and chlorophyll *a* distribution was relatively uniform on a site-wide basis. The low nugget effect relative to that modelled for Bull Island indicated that the spatial variability in this parameter was reduced at the site, but some inherent variability still existed.

The concentration of chlorophyll *a* and phaeopigment were positively correlated (Pearson-product = +0.47, $p \leq 0.01$) at the Bull Island site (Figure 2.24). A negative correlation was observed (Pearson-product = -0.53, $p \leq 0.01$) between these variables at Blackrock (Figure 2.25).

2.3.2 Site specific spatial variability in pigments, Hydrobia and Tellina distribution and abundance

2.3.2a Bull Island

Mean chlorophyll *a* concentration at Bull Island was lowest along the centre of the study area and corresponded very closely to the location of the creek (Figure 2.26a). All transects showed high concentrations along the higher shore levels either side of the site and a decrease toward the creek. There were no statistically significant differences among transects (ANOVA, F=0.89, df=5, p=0.48) and few differences

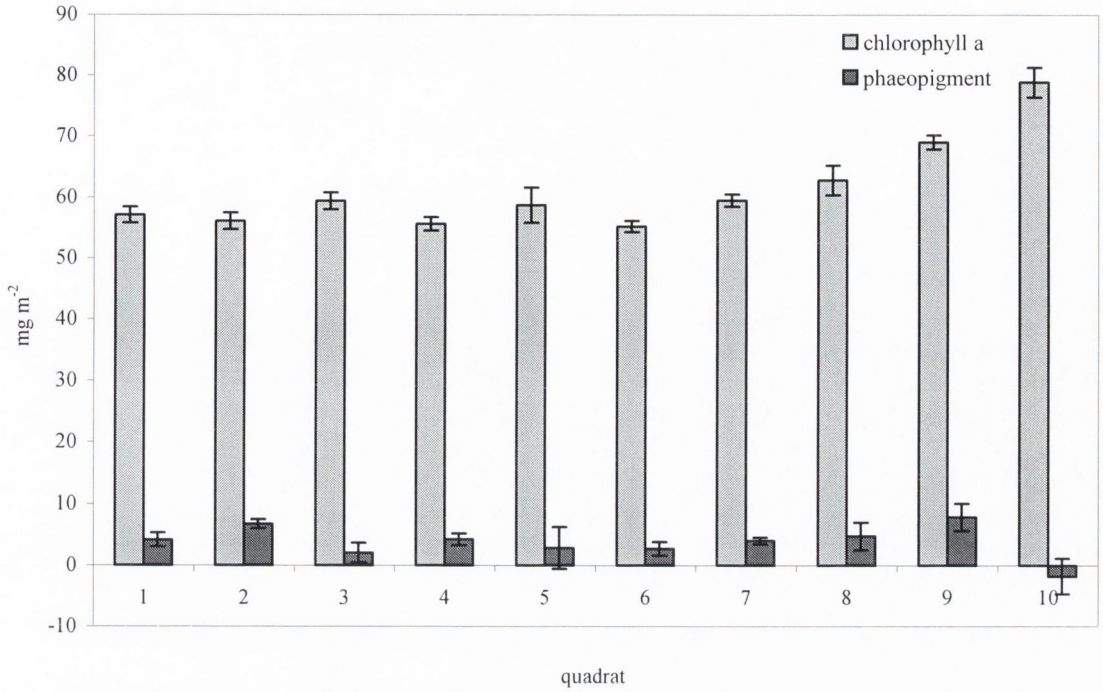


Figure 2.22. Mean chlorophyll *a* and phaeopigment levels (mg m^{-2} ± S.E.) at Blackrock, March 2000 (N=50). The distance between quadrats 1 and 2 was 25cm, and this distance was doubled for each subsequent quadrat with the exception of the final quadrat which was 50m from quadrat 9.

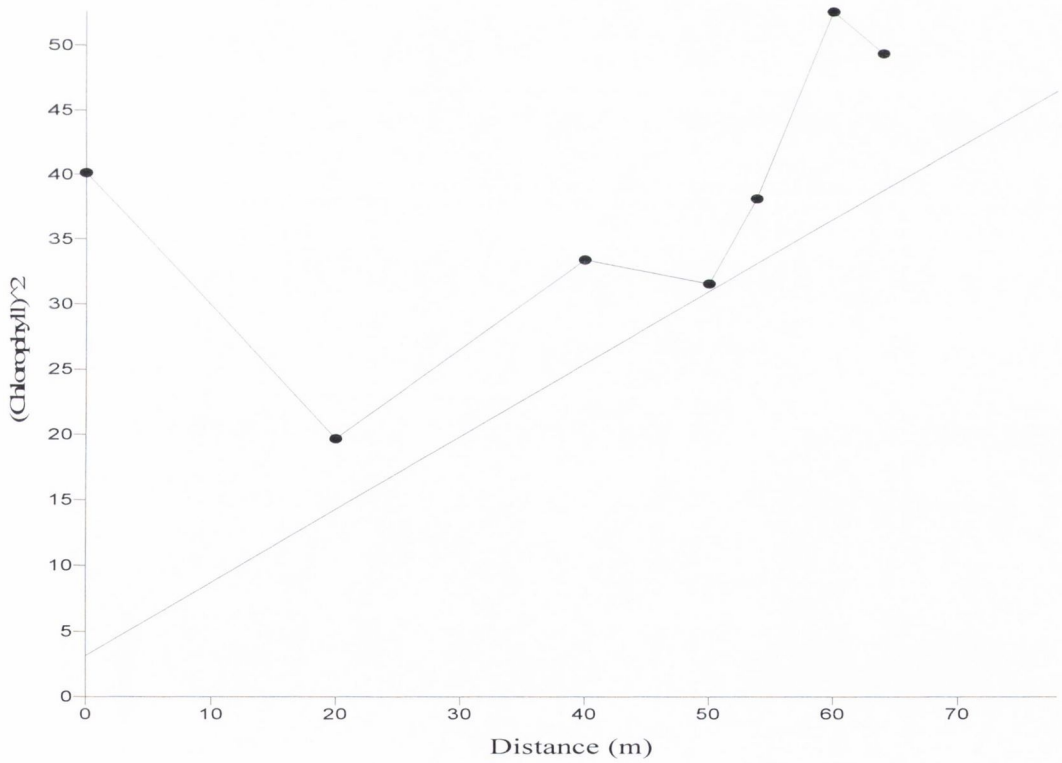


Figure 2.23. Variogram of large scale variability (stations within site) in chlorophyll *a* concentration at Blackrock, March 2000.

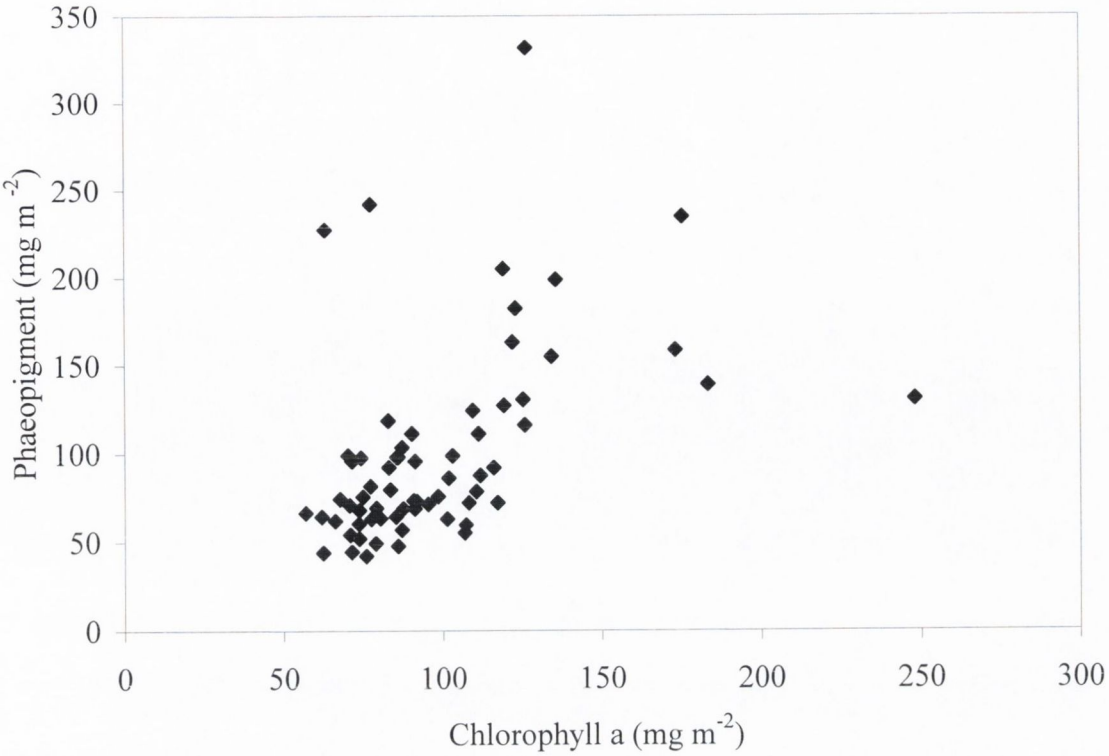


Figure 2.24. The relationship between mean chlorophyll *a* and phaeopigment levels at Bull Island, March 2000 (N=65). Pearson-product = +0.47, $p \leq 0.01$.

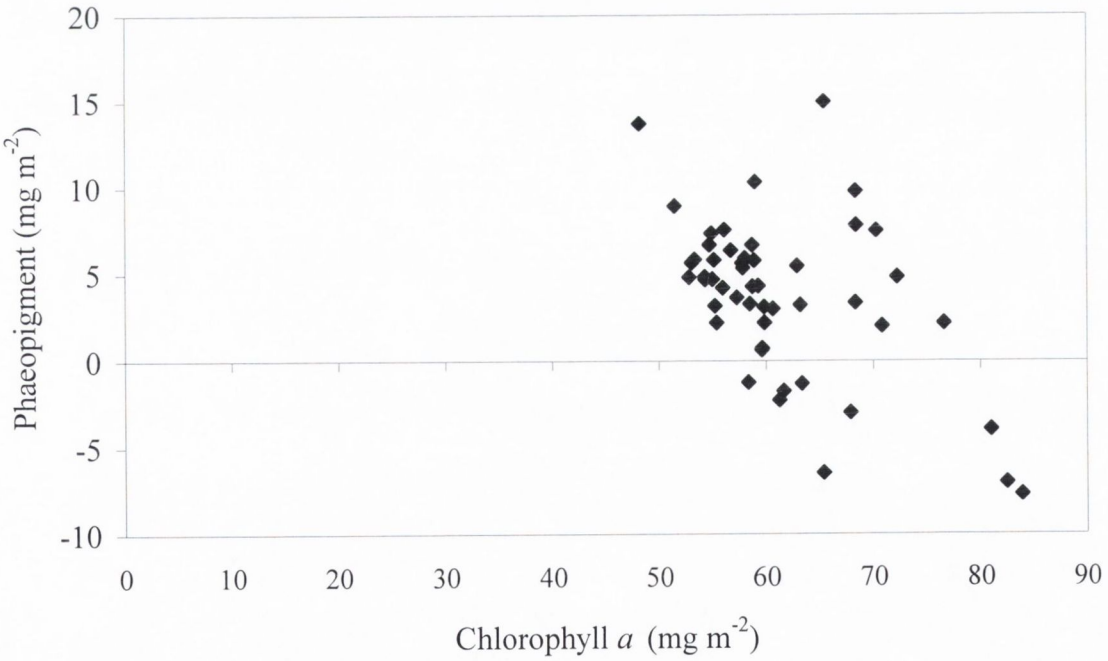


Figure 2.25. The relationship between mean chlorophyll *a* and phaeopigment levels at Blackrock, March 2000 (N=50). Pearson-product = -0.53, $p \leq 0.01$.

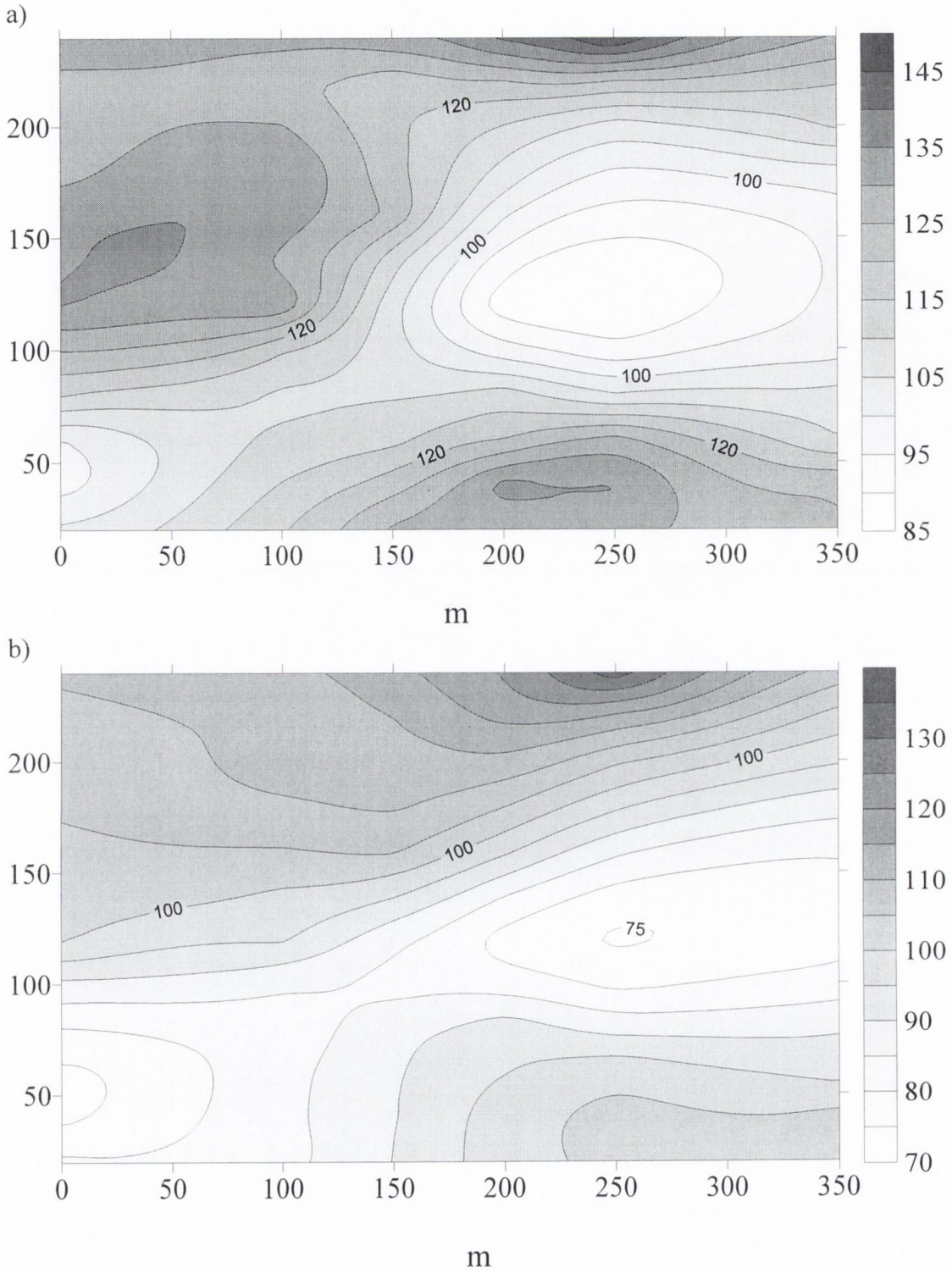


Figure 2.26. a) Mean sediment chlorophyll *a* (mg m^{-2} ± S.E.) at Bull Island, April 2000. b) Mean sediment phaeopigment (mg m^{-2} ± S.E.). N=207.

with shore level. The latter was due mainly to the variation in the shore level at which the meandering creek crossed each transect. Shore level 12 had significantly higher mean (±S.E.) chlorophyll *a* concentrations, 178.1±17.9mg m^{-2}, than shore levels 5 and 7, 96.2±7.3 and 95.4±4.9 mg m^{-2}, respectively (Kruskal-Wallis, χ^2=27.3, df=11, p≤0.01).

Mean phaeopigment distribution at Bull Island showed a similar pattern to chlorophyll *a* distribution (Figure 2.26b). There was a general trend of lower values along the creek increasing toward the upper shore levels. There were no statistically significant differences among transects (ANOVA, F=0.94, df=5, p=0.46). Shore level 5 had a mean value of 61.2±5.1mg m^{-2} which was significantly less than the recorded average values of 106.6±9.6, 109.6±8.4 and 162.5±32.7mg m^{-2} for shore levels 1, 9 and 12, respectively (Kruskal-Wallis, χ^2=33.5, df=11, p≤0.01). No other significant difference occurred among shore levels, this was probably again due to the varying position of the creek.

The total abundance of *Hydrobia ulvae* at Bull Island showed an increase from transect Y toward transect X (Figure 2.27a). Densities ranged from 423 individuals m^{-2} at station Y2 to 42,324m^{-2} at station X8. This low abundance at station Y2, which was located in the creek, was the only station with <2,000 individuals m^{-2} recorded. There were 7 recorded values >30,000m^{-2}. However, the majority of the stations (25) had values in the range 10,000m^{-2} to 20,000 individuals m^{-2}. Lowest densities in each transect were always recorded in areas that corresponded to the location of the creek. There were no significant differences in *Hydrobia* abundance among any transects (ANOVA, F=1.27, df=5, p=0.62). There were also no differences with shore level mostly due to the fact that gradients from high to low shore differed horizontally across the flat (ANOVA, F=0.71, df=11, p=0.72). The overall average shell height (SH) of *Hydrobia* decreased in a gradient from transect Y to transect X (Figure 2.27b), although there were no statistically significant differences recorded among-transects (ANOVA, F=1.49, df=5, p=0.80). A trend of decreasing size was also observed from station Y1 to station X10, although again there were no statistically significant differences in mean size between shore level observed (ANOVA, F=0.99, df=11, p=0.07). Areas with higher abundance generally contained numerous smaller

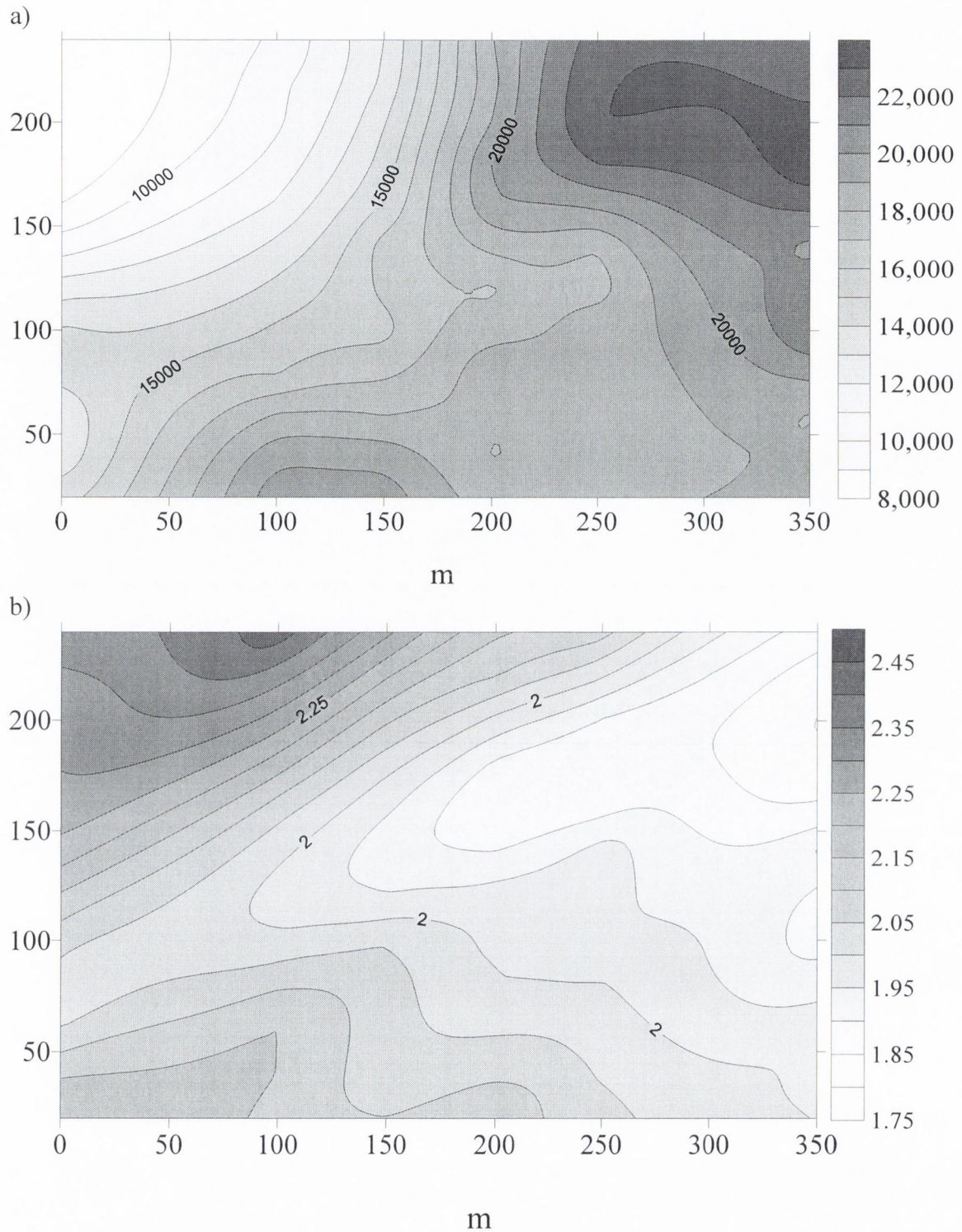


Figure 2.27. a) Density of *Hydrobia ulvae* m^{-2}, Bull Island, April 2000. b) Overall mean SH (mm) of *Hydrobia*. N=25,105.

sized individuals, and so corresponded to lower mean size (Figure 2.27). The majority of smaller individuals were located closer the mouth of the lagoon, extending down but not into the creek. Mean size of *Hydrobia* was greater at higher shore levels away from the mouth of the lagoon, often coinciding with lower overall mean abundance, but no significant differences in the distribution of different sized animals were recorded.

The smallest size class consisting of individuals of <1.6mm SH, representing recently settled cohorts, showed an increasing abundance from the far high shore (top left in plot) at transect Y toward the entrance of the lagoon at transect X (Figure 2.28a). Abundance in this cohort ranged from 188 individuals m^{-2} at station Y2 to 37,433m^{-2} at X8. The overall mean SH in this size class was 1.1mm±0.01 (S.E.). Slightly larger individuals appeared to be located in the areas of lowest density at the far high shore in transects Y and A (Figure 2.28b). No significant differences in either abundance or mean SH were observed with shore level or among transects for this size class however. The distribution, of particularly, abundance, and to some extent, mean size, was very similar to those for the entire population shown in Figure 2.27. This suggested that the numerical dominance of this smallest size class strongly influenced the overall mean population size and abundance patterns observed.

Lowest abundance in the size class 1.6–3.1mm SH occurred at the mouth of the lagoon, on the far high shore (top left in plot) and along the creek (Figure 2.29a). Mean (±S.E.) density in this class was 2,992±269 individuals m^{-2} reaching a maximum of 11,145 m^{-2} at station A3. Lowest densities within each transect always corresponded to stations in or on the edges of the creek, but no significant differences existed (ANOVA, F=0.95, df=11, p=0.45). Mean SH in this size class was slightly larger with distance from the mouth of the lagoon and along the upper shore (Figure 2.29b), but no significant differences existed among transect (ANOVA, F=1.31, df=5, p=0.07) or shore level (ANOVA, F=0.2.40, df=11, p=0.22). Overall mean SH was 2.18±0.02mm, with a maximum of 2.87mm SH recorded at station Y2. Unlike the <1.6mm SH class, the size and abundance distributions for this size class, and all larger groups, did not resemble closely that of the overall population distribution.

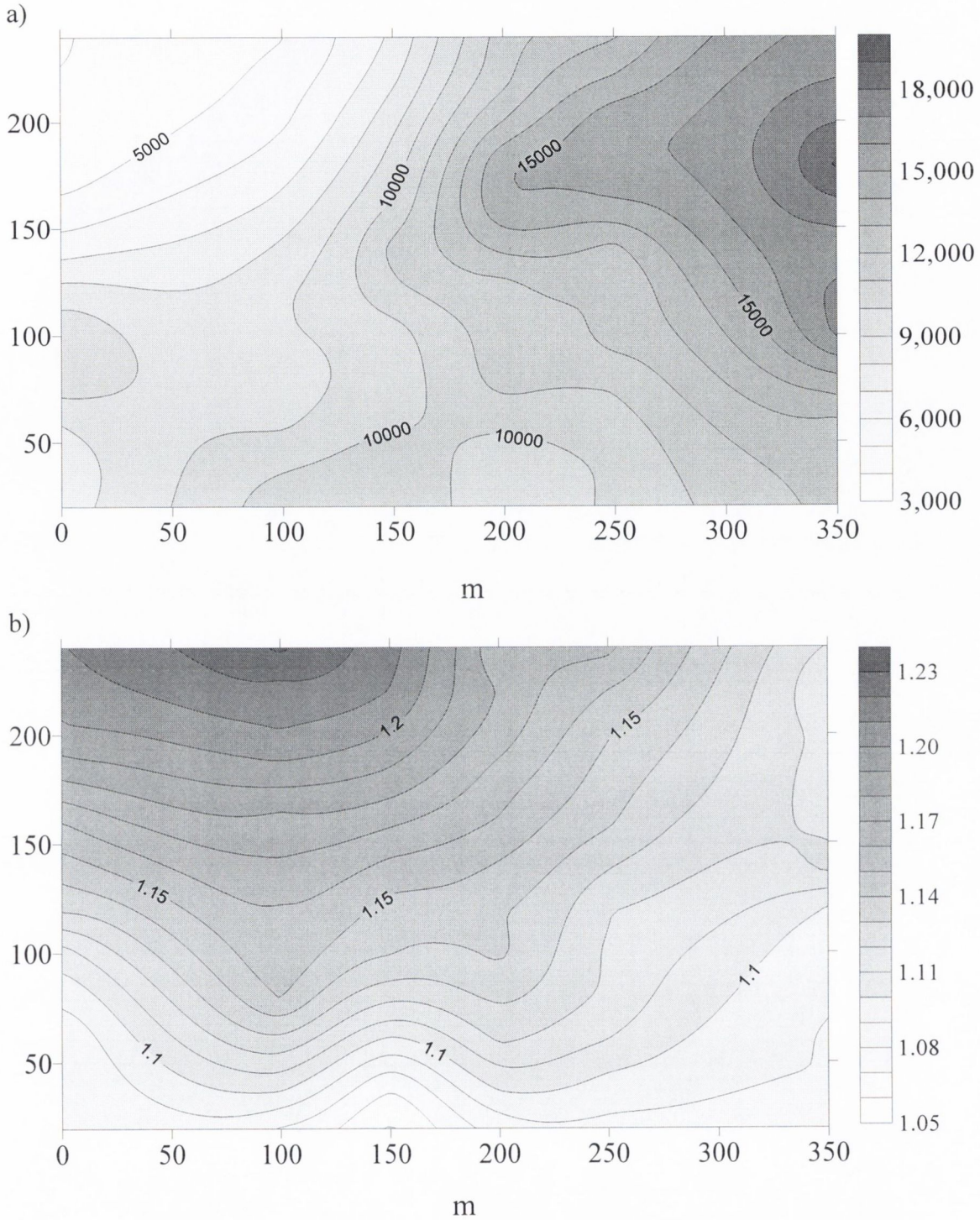


Figure 2.28. a) Density of *Hydrobia* m^{-2} in the cohort <1.6mm SH at Bull Island. b) Mean size of *Hydrobia* (SH) in this cohort, Bull Island, April 2000. N=15,921.

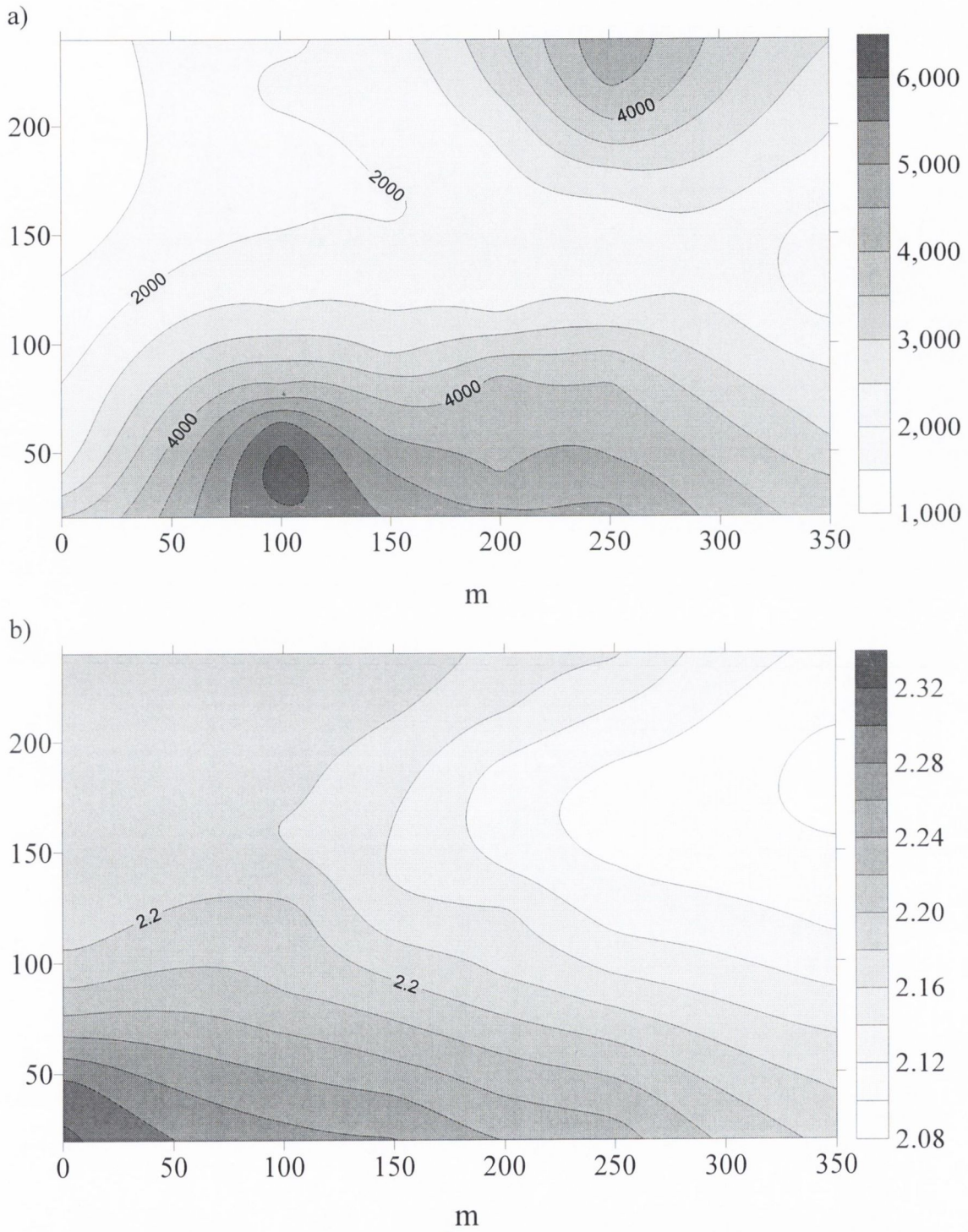


Figure 2.29. a) Density of *Hydrobia* m^{-2} in the cohort 1.6–3.1mm SH, at Bull Island. b) Mean SH of *Hydrobia* in this cohort, Bull Island, April 2000. N=4,470.

Individuals within the size range 3.1–5mm SH occurred in lowest abundance along the creek and furthest from the lagoon entrance (Figure 2.30a). The highest overall density in this size class of 11,474 individuals m^{-2} was observed at station D12. Average density overall was 2,962±2.2 individuals m^{-2}, which was almost identical to the average density determined for the size class 1.6-3.1mm SH. Mean size and abundance distributions of these 2 size classes were also extremely similar. Mean SH decreased only slightly from inner to outer lagoon (Figure 2.30b). The average size in this size class was 4.0±0.01mm SH. No significant differences were observed with shore level or among transects in this size class for either abundance or mean SH.

The abundance contour plot of the size class containing individuals >5mm SH revealed similar distributions to the 2 previous size classes (Figure 2.31a), although densities observed for this size class were much lower with a mean of 222±39m^{-2}. Fourteen stations had no individuals of >5mm SH, while 19 stations had abundance <100m^{-2}. The maximum density of 2,116m^{-2} was observed at station D12, at the centre of the high abundance on the far high shore. The mean SH and abundance in this size class did not differ significantly. The highest mean SH's were recorded along the far high shore at transects A and B (Figure 2.31.b). Overall mean SH in this size class was 5.49±0.05mm. The maximum station SH of 6.88mm was observed at station C7, this was based on only a single individual however.

Statistically significant positive correlations were observed between the spatial distribution of chlorophyll *a* and *Hydrobia* for the two larger cohorts (Table 2.4). Overall abundance and the distribution of individuals in size classes <3.1mm SH were not significantly correlated with chlorophyll *a*. There were no significant correlations observed between the distribution of phaeopigment and overall abundance, or with that of the smallest size class. The abundance of the three cohorts above 1.6 mm SH were positively correlated with distribution of phaeopigment. Chlorophyll *a* was not significantly correlated with mean SH in any size class except the 3.1-5mm SH cohort (Table 2.5). Phaeopigment concentration was positively correlated with mean SH in the <1.6mm cohort but not for any other size classes.

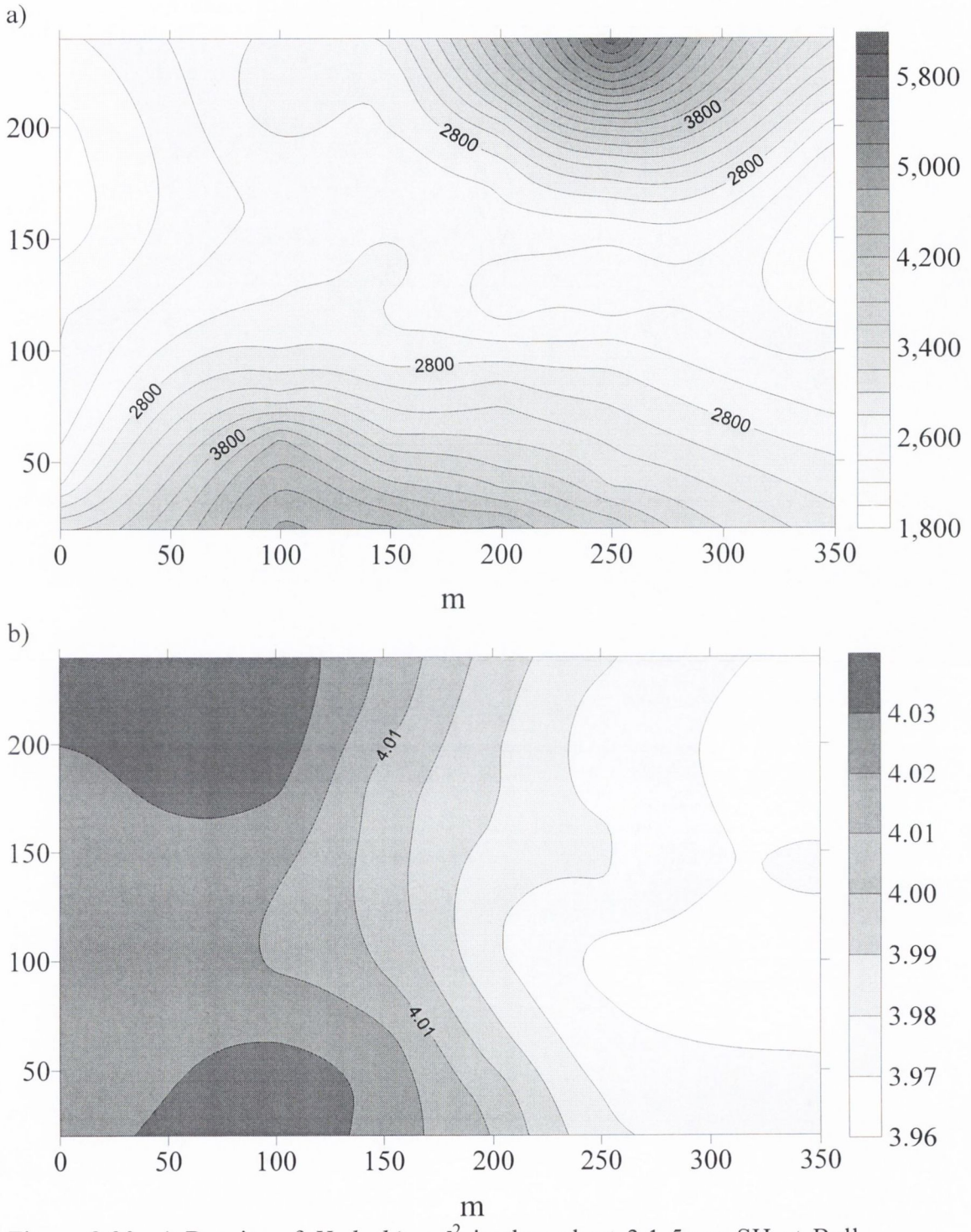


Figure 2.30. a) Density of *Hydrobia* m^{-2} in the cohort 3.1-5mm SH at Bull Island. b) Mean SH of *Hydrobia* in this cohort, Bull Island, April 2000. N=4,391.

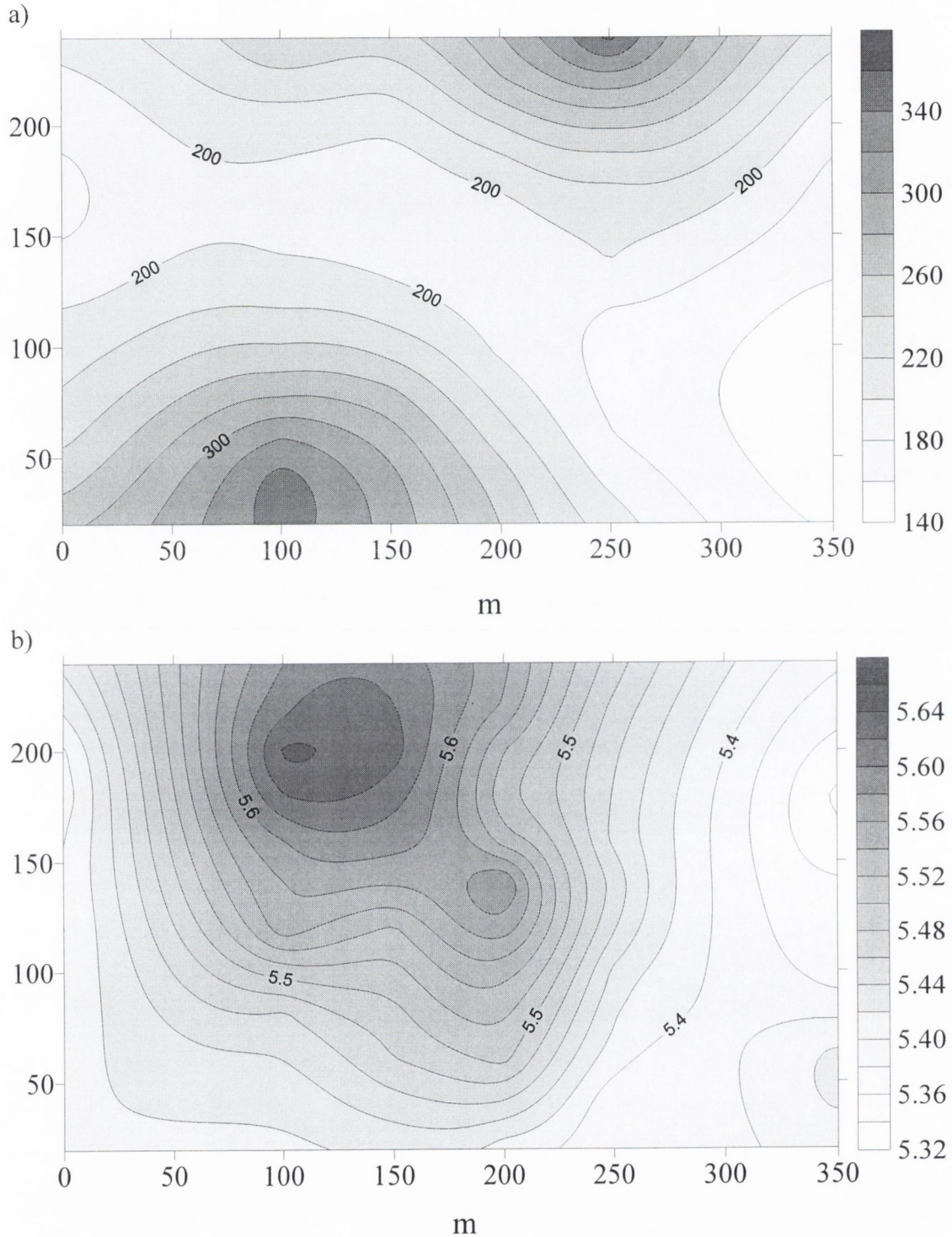


Figure 2.31. a) Density of *Hydrobia* m^{-2} in the cohort >5mm SH at Bull Island.
b) Mean size of *Hydrobia* in this cohort, Bull Island, April 2000.

Table 2.4 Pearson's correlation between mean chlorophyll *a* and phaeopigment concentration (mg m^{-2}) and abundance of *Hydrobia* in various size classes (mm).

* indicates differences significant at <0.05 level and includes Bonferroni adjustment.

SH	Chlorophyll *a*	P-value	Phaeopigment	P-value
All	+ 0.05	0.70	+ 0.15	0.23
< 1.6	- 0.06	0.62	- 0.03	0.83
1.6–3.1	+ 0.17	0.17	+ 0.30	0.01*
3.1-5	+ 0.30	0.01*	+ 0.49	≤ 0.01*
>5	+ 0.35	≤ 0.01*	+ 0.48	≤ 0.01*

Table 2.5 Pearson's correlation between mean chlorophyll *a* and phaeopigment concentration (mg m^{-2}) and mean shell height of *Hydrobia* in various size classes (mm). * indicates differences significant at < 0.05 level and includes Bonferroni adjustment.

SH	Chlorophyll *a*	P-value	Phaeopigment	P-value
All	+ 0.14	0.24	+ 0.14	0.27
< 1.6	+ 0.16	0.19	+ 0.33	≤ 0.01*
1.6 – 3.1	+ 0.19	0.12	+ 0.12	0.34
3.1 - 5	+ 0.28	0.02*	+ 0.17	0.17
> 5	0.15	0.28	+ 0.10	0.46

Overall mean (±S.E.) chlorophyll *a* concentration in the current study was 115.8±3.5mg m^{-2}. This compared with a mean value of 137.5±75mg m^{-2} recorded during the investigation described in 2.3.1a (medium-scale variability) above. The mean value determined in experiment 2.3.1b (small scale variability) was 161.4±7.3mg m^{-2}, and 98.0±4.1mg m^{-2} in investigation 2.3.1c (large scale variability). The location of the three preliminary studies corresponded to mid-shore level at stations 8 in the current study. Mean chlorophyll *a* at this shore level was 123.7±10.9mg m^{-2}.

Average (±S.E.) phaeopigment concentration for the whole Bull Island site during this investigation was 95.5±3.7mg m^{-2}. A corresponding value of 93.1±5.0mg m^{-2} was recorded for the medium scale investigation described in 2.3.1a above. Mean concentrations of 331.8±15.0 and 100.7±6.9mg m^{-2} were recorded in trials 2.3.1b and c, respectively. Mean phaeopigment concentration of 101.6±10.3mg m^{-2} was

recorded at the shore level (stations 8) corresponding to the location of the preliminary investigations in this study.

The overall mean abundance of *Hydrobia* at Bull Island during this experiment was 17,110±1443 individuals m^{-2} (95% C.I.) (N=25,105). The total area of the study site was 69,000m^{-2}. Extrapolation suggested a rough total population estimate of 1,180,590,000 ($1.2x10^9$) *Hydrobia* within the study area at the time of the study.

2.2.3b Blackrock

Mean chlorophyll *a* concentration increased with distance down the shore at the Blackrock site (Figure 2.32a). Lowest concentrations (20mg m^{-2}) occurred in the corner of the site where the seawall and bath wall met (station A1), with values twice as high being recorded at the low shore level. Statistically significant differences occurred among shore levels (ANOVA, F=11.61, df=9, $p \leq 0.01$) and Scheffe post-hoc tests showed levels 1-3 varying from many lower shore levels (Table 2.6). Differences among transects were significant (ANOVA, F=9.49, df=3, $p \leq 0.01$) and Scheffe post-hoc showed transect A contained the lowest mean chlorophyll *a* concentrations compared with all other transects, these differences being significant from transects C and D (Table 2.7). Chlorophyll *a* in transect B was significantly lower than transect C. No significant differences were observed between transects C and D.

Table 2.6. Scheffe post-hoc of between-shore level comparison of distribution of mean chlorophyll *a*. * indicates differences significant at ≤ 0.05 level.

Shore Level	2	3	4	5	6	7	8	9	10
1	0.99	0.81	0.04*	0.01*	0.01*	≤ 0.01*	≤ 0.01*	≤ 0.01*	0.33
2		0.99	0.33	0.15	0.12	≤ 0.01*	≤ 0.01*	0.02*	0.68
3			0.90	0.70	0.65	≤ 0.01*	0.03*	0.18	0.95
4				0.99	0.99	0.24	0.50	0.91	0.99
5					1.00	0.45	0.76	0.98	1.00
6						0.72	0.91	0.99	1.00
7							1.00	0.99	0.95
8								0.99	0.99
9									0.99

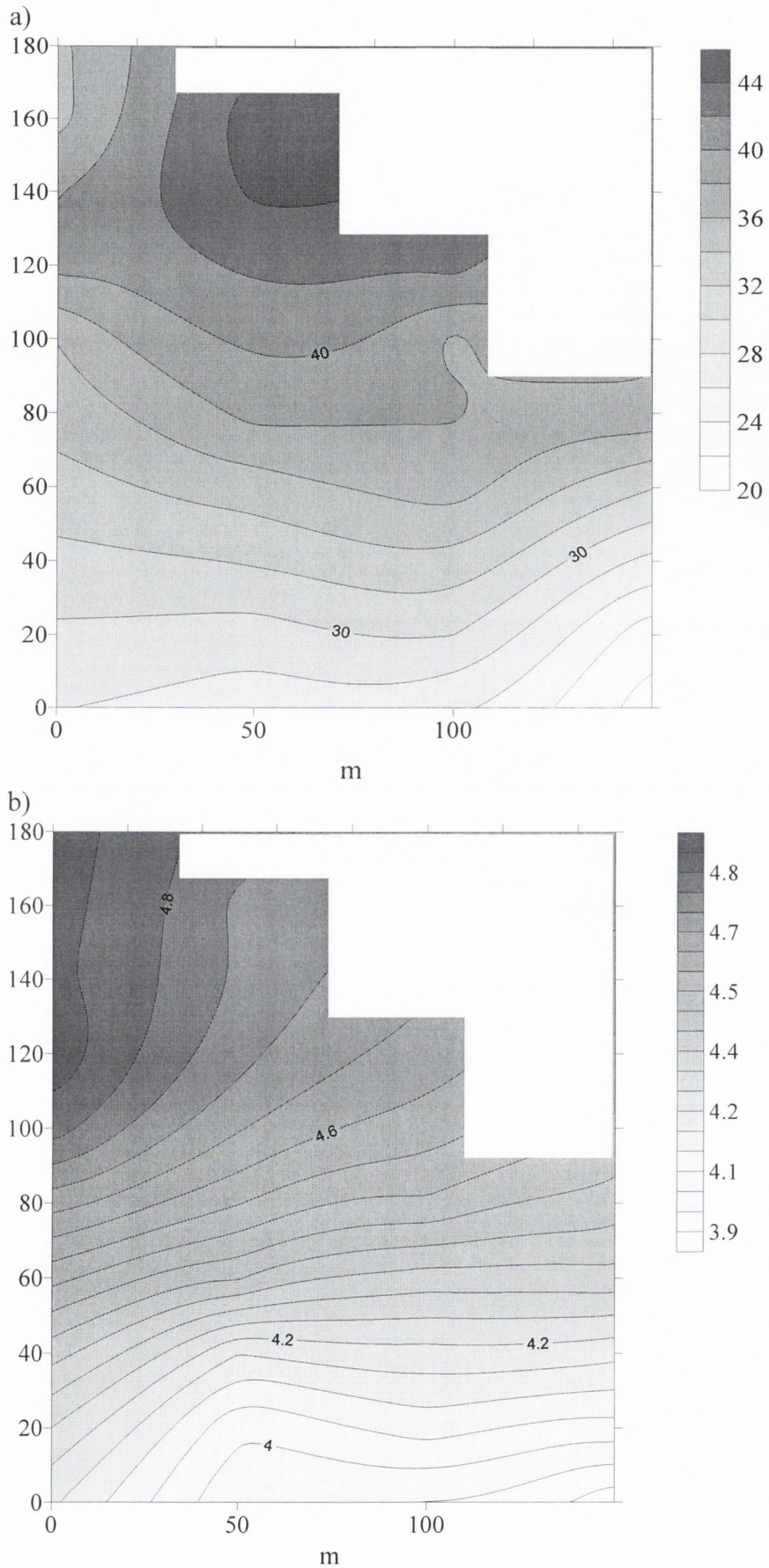


Figure 2.32. a) Mean sediment chlorophyll *a* (mg m^{-2} ± S.E.) at Blackrock. b) Mean sediment phaeopigment *a* (mg m^{-2} ± S.E.), April 2000. N=93.

Table 2.7. Scheffe post-hoc of between-transect comparison of distribution of mean chlorophyll *a*. * indicates differences significant at ≤ 0.05 level.

Transect	B	C	D
A	0.98	≤ 0.01*	0.04*
B		≤ 0.01*	0.06
C			0.38

Phaeopigment concentration displayed a similar pattern of distribution to chlorophyll *a* Figure 2.32b, with an increasing gradient occurring from the upper to lower shore, from station A1 to D10, although the concentration gradient was far less pronounced than for chlorophyll *a* levels. Mean values ranged between 3.9 and 4.9mg m^{-2}, illustrating a more uniform distribution at the time of the study when compared with chlorophyll *a*. The subtle change in phaeopigment concentration across the site resulted in no significant differences among transects A, B, C and D occurring, either overall (Anova, F=1.43, df=3, p=0.24) or among shore levels (ANOVA, F=1.06, df=9, p=0.51.

Overall population abundance of *Tellina tenuis* increased slightly toward the lower shore (Figure 2.33a). Densities were lowest along the upper shore at station C1 with total densities <1,000m^{-2} observed. Although abundance increased with distance from the seawall and upper shore level to reach >1,120 individuals m^{-2} at some stations, differences among shore levels were not significant (ANOVA, F=1.03, df=9, p=0.11). There were also no statistically significant differences in abundance among transects (ANOVA, F=0.99, df=3, p=0.07). The average SH of *Tellina* decreased rapidly in a gradient extending from station A1 to D10 (Figure 2.33b). Statistically significant differences (ANOVA, F=34.4, df=9, $p \leq 0.01$) in mean SH were observed among shore levels and Scheffe post-hoc tests showed that SH were significantly different at higher shore levels 1-3 and many other lower levels (Table 2.8). As abundance over the site did not change significantly, variation in mean SH was caused by increasing numerical dominance of smaller individuals with distance from the high shore. Larger individuals were more abundant at higher shore levels.

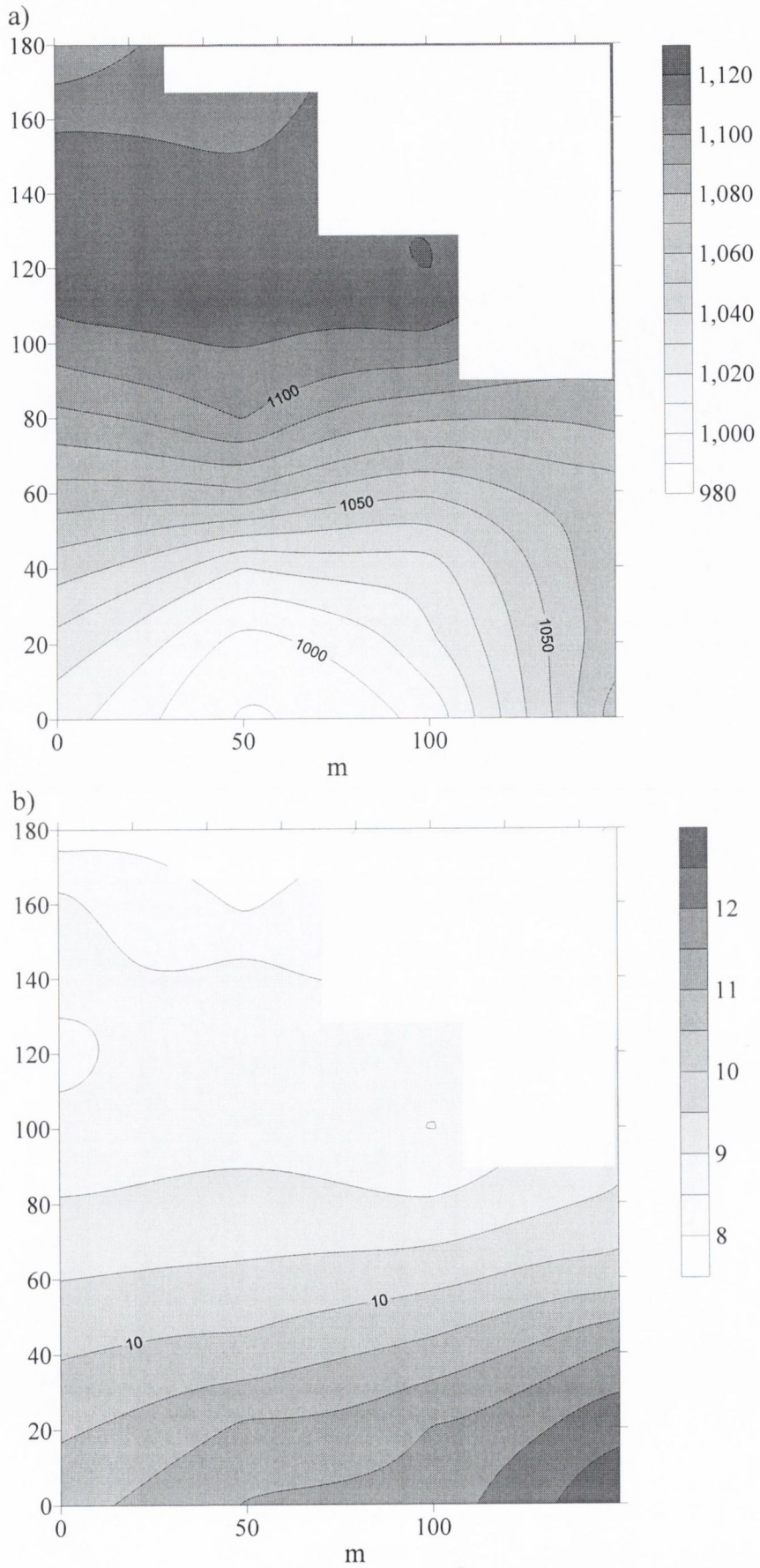


Figure 2.33. a) Density of *Tellina tenuis* m^{-2}. b) Overall mean shell height (mm) of *Tellina*, Blackrock, April 2000. N=25,104.

Table 2.8. Scheffe post-hoc of between-shore level comparison of distribution of mean SH of *Tellina*. * indicates differences significant at ≤ 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.95	0.12	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
2		0.79	0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
3			0.39	0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
4				0.78	0.21	0.19	0.37	0.09	0.08
5					0.98	0.97	0.99	0.78	0.53
6						1.00	1.00	0.99	0.95
7							1.00	0.99	0.95
8								0.99	0.96
9									0.99

A strong gradient in abundance of individuals <4mm SH existed from the upper to lower shore (Figure 2.34) from station A1 across the study area to D10. No *Tellina* < 4 mm SH were encountered at station A1. This size group represented only a small proportion of the overall population at the site. The lowest abundance in each transect occurred at the highest shore level with values of 0, 8, 27 and 35 individuals m^{-2} recorded for A, B, C and D, respectively. Statistically significant differences (ANOVA, F=9.6, df=9, p≤0.01) were observed among shore levels and Scheffe post-hoc tests showed levels 1-3 all varying from level 6 (Table 2.9). There was a significant difference between abundance in transect A and D (ANOVA, F=3.15, df=3, p=0.04), with higher abundances observed in the former. The overall mean SH in this size class was 3.3±0.01mm. There was insufficient spatial variation in mean SH in this size class to construct a variogram, suggesting a relatively uniform size distribution over the area.

Table 2.9. Scheffe post-hoc tests of between-shore level comparison of distribution of abundance of *Tellina* in the size class <4mm SH. * indicates differences significant at ≤ 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.99	0.93	0.15	0.01*	≤0.01*	0.01*	0.09	0.12	0.11
2		0.99	0.52	0.08	≤0.01*	0.07	0.29	0.37	0.28
3			0.87	0.25	0.04*	0.21	0.58	0.67	0.49
4				0.98	0.52	0.95	0.99	0.99	0.97
5					0.98	1.00	1.00	1.00	0.99
6						0.99	0.98	0.97	1.00
7							0.99	0.99	0.99
8								1.00	0.99
9									0.99

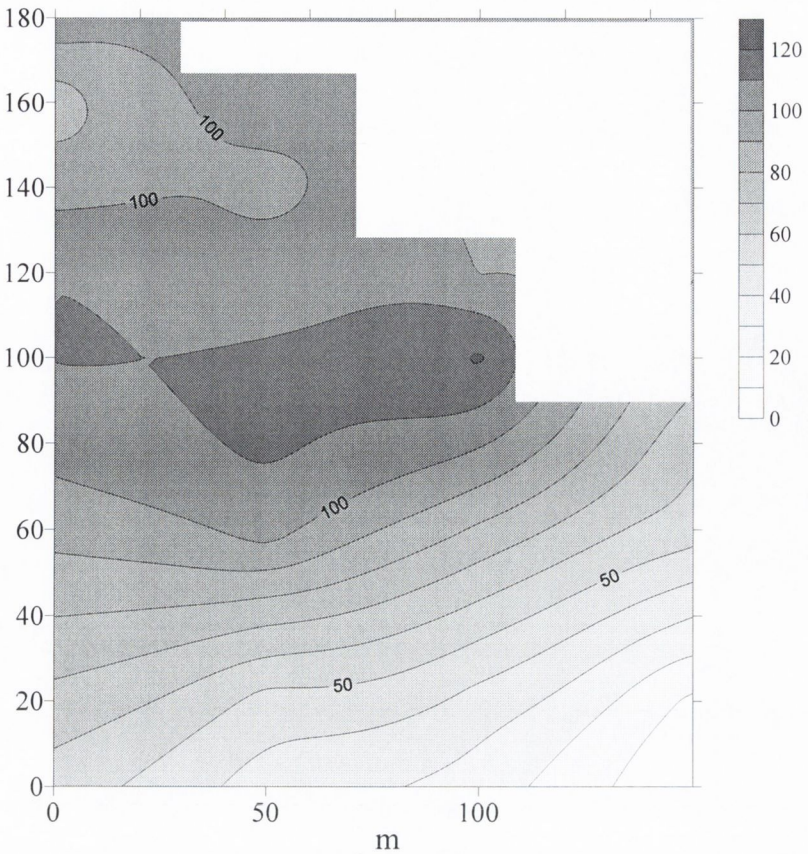


Figure 2.34. Density of *Tellina* m^{-2} in the size class < 4 mm SH , Blackrock, April 2000. N=1,663.

The distribution of abundance of the 4-8mm SH size class was similar to that of the <4mm SH size class, decreasing with increasing height on the shore (Figure 2.35a). Densities in this size class were greater than observed in the <4mm size class however, ranging between 93 and 428 individuals m^{-2}. Highest abundances of individuals within the 4-8mm SH size class were recorded in the lowest stations of transects A, C and D. Statistically significant differences (ANOVA, F=45.3, df=9, $p \leq 0.01$) were observed among shore levels and Scheffe post-hoc tests (Table 2.10) illustrating differences were mainly between shore levels 1-4 and those further down the shore. There were no statistically significant differences in abundance among transects in this size class (ANOVA, F=2.62, df=3, p=0.14). Although mean SH was highest at station A1 and lowest towards the midshore around transects C and D (Figure 2.35b), there were no significant differences among the shore levels (ANOVA, F=1.24, df=9, p=0.17). Comparison of the distributions of abundance and mean SH revealed that larger individuals were distributed in areas of lower density for this size class.

Table 2.10. Scheffe post-hoc test of between-shore level comparison of distribution of abundance of *Tellina* in the size class 4-8mm SH. * indicates differences significant at ≤ 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.99	0.41	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
2		0.76	0.02*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
3			0.57	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
4				0.06	≤0.01*	≤0.01*	0.03*	≤0.01*	0.02*
5					0.97	0.92	0.99	0.75	0.75
6						1.00	1.00	0.99	0.99
7							1.00	0.99	0.99
8								0.99	0.99
9									0.99

Although differing slightly in distribution to the two smaller size classes, individuals within the 8-12mm size class showed a similar trend of increasing abundance from the upper to lower shore (Figure 2.36a). Again, abundances at shore levels 1-3 were significantly different (ANOVA, F=13.4, df=9, $p \leq 0.01$) from several lower shore levels (Table 2.11). Densities were greater than those observed in the two smaller size classes with an average density of $362 \pm 11 m^{-2}$. There were no statistically significant differences in abundance among transects for this size class (ANOVA,

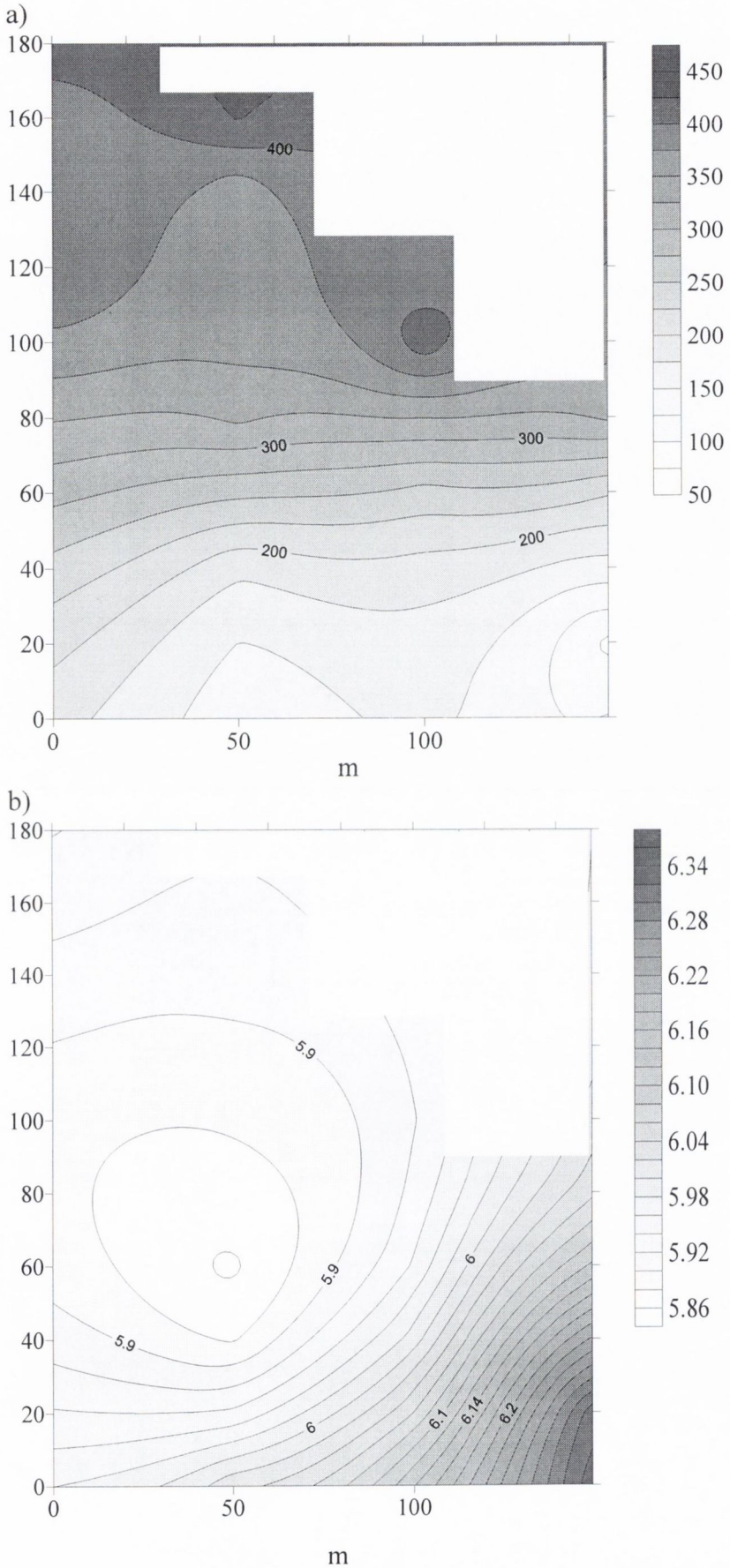


Figure 2.35. a) Density of *Tellina* m^{-2} in the size class 4-8mm SH. b) Mean SH of *Tellina* in the size class 4-8mm, Blackrock, April 2000. N=6,381.

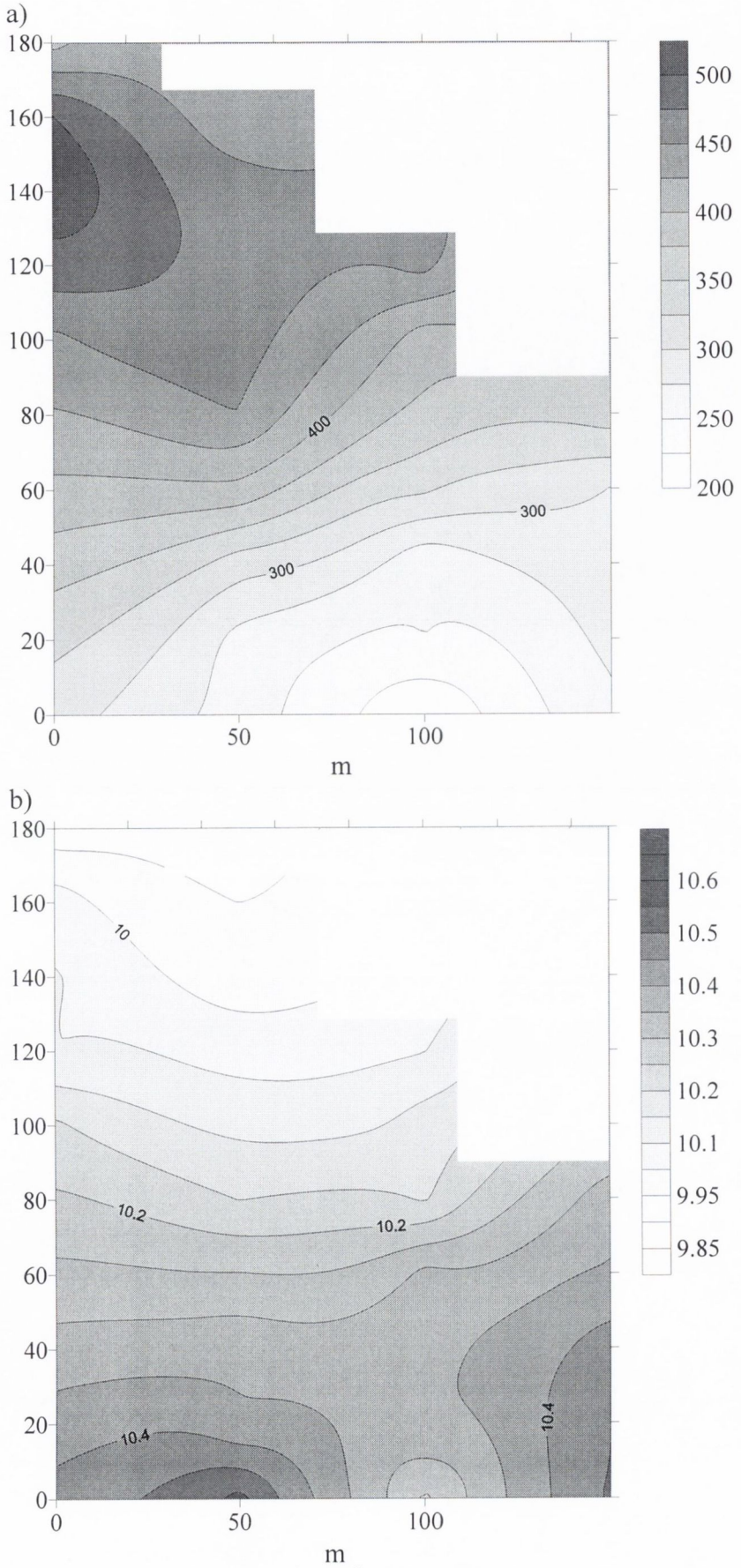


Figure 2.36. a) Density of *Tellina* m^{-2} in the size class 8-12mm SH. b) Mean SH of *Tellina* in the size class 8-12mm, Blackrock, April 2000. N=8,417.

F=2.7, df=3, p=0.41). On the upper shore the mean SH was 10.5 mm, compared with 9.8 mm at the lowest shore levels (Figure 2.36b). This slight increase in size between the lower and upper shore levels was not sufficient to result in any statistically significant differences occurring however. Comparison of the spatial distribution of density and mean size in this size class again revealed that smaller animals generally occurred in areas of higher abundance.

Table 2.11. Scheffe post-hoc test of between-shore level comparison of distribution of abundance of *Tellina* in the size class 8-12 mm SH. * indicates differences significant at ≤ 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.99	0.97	0.26	≤ 0.01*	0.03*	≤ 0.01*	≤ 0.01*	≤ 0.01*	0.52
2		1.00	0.83	0.06	0.19	≤ 0.01*	≤ 0.01*	0.03*	0.88
3			0.91	0.09	0.27	≤ 0.01*	0.01*	0.04*	0.93
4				0.78	0.95	0.10	0.18	0.38	0.99
5					0.99	0.89	0.93	0.99	0.99
6						0.79	0.86	0.97	0.99
7							1.00	0.99	0.86
8								0.99	0.88
9									0.97

In contrast to the 3 smaller size classes, individuals between 12-16mm SH were less numerous with distance from the high shore level (Figure 2.37a). Highest abundance occurred at the station A1 (663 individuals m^{-2}), decreasing toward D10 ($83m^{-2}$). This was the most numerically abundant size class at Blackrock, with greater variability in abundance than all other classes. Abundance at shore levels 1 and 2 were significantly higher (ANOVA, F=15.8, df=9, $p \leq 0.01$) than at station 5-10 and at level 3 when compared with 6-10 (Table 2.12). Transect A had significantly higher abundance than transect D (ANOVA, F=3.79, df=3, p=0.02). Mean SH in this size class also decreased significantly from upper (mean 14.1mm) to the lowest shore level (12.8mm) (Figure 2.37b). Differences were significant (ANOVA, F=38.7, df=9, $p \leq 0.01$) among shore levels 1-2 and all below level 4 and between level 3 and all below 5, Table 2.13. Mean size was smaller with decreasing abundance in this size class, the converse pattern of that observed for the smaller size classes.

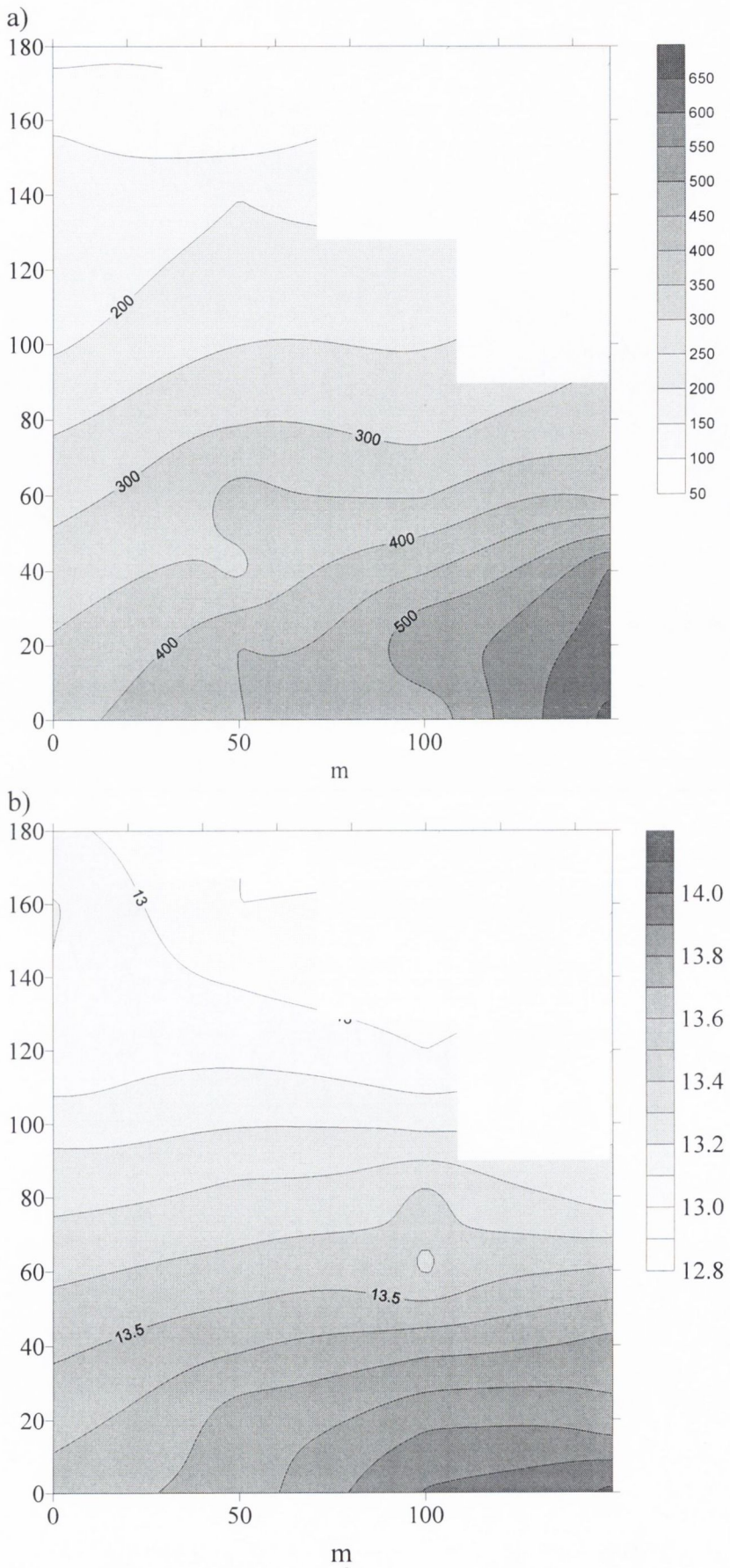


Figure 2.37. a) Density of *Tellina* m^{-2} in the size class 12-16mm SH. b) Mean SH of *Tellina* in the size class 12-16mm, Blackrock, April 2000. N=8,159.

Table 2.12. Scheffe post-hoc tests of between-shore level comparison of distribution of abundance of *Tellina* in the size class 12-16mm SH. * indicates differences significant at ≤ 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.99	0.99	0.33	0.05*	0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
2		0.99	0.19	0.03*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
3			0.54	0.11	0.03*	≤0.01*	0.02*	≤0.01*	0.01*
4				0.99	0.78	0.51	0.55	0.18	0.27
5					0.99	0.95	0.94	0.58	0.62
6						0.99	0.99	0.96	0.93
7							1.00	0.99	0.99
8								0.99	0.99
9									0.99

Table 2.13. Scheffe post-hoc tests of between-shore level comparison of distribution of mean SH of *Tellina* in the size class 12-16mm. * indicates differences significant at < 0.05 level.

Shore level	2	3	4	5	6	7	8	9	10
1	0.94	0.09	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
2		0.71	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
3			0.21	0.03*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
4				0.99	0.42	0.07	0.12	0.06	0.32
5					0.91	0.38	0.47	0.28	0.69
6						0.99	0.99	0.96	0.99
7							1.00	0.99	1.00
8								1.00	1.00
9									1.00

Similarly to the 12-16mm SH size class, the abundance of individuals >16mm SH decreased from the high to low shore (Figure 2.38). Although station A1 contained densities of 168 individuals m^{-2}, density decreased rapidly to <70 individuals m^{-2} in all other stations. Due to overall low abundance within this size class in all areas with the exception of the immediate area around station A1, no significant differences in abundance occurred among shore levels (ANOVA, F=0.87, df=9, p=0.21) or transects (ANOVA, F=1.35, df=3, p=0.22). Individuals within this size range were absent from the lowest shore levels of transects C and D. Mean (±S.E.) SH in this size class was 16.7±0.05mm, and no significant differences occurred among shore levels (ANOVA, F=2.72, df=9, p=0.08) or transects (ANOVA, F=1.22, df=3, p=0.15). Uniformity of SH within this size class resulted in the absence of sufficient spatial variation to construct a variogram for this parameter.

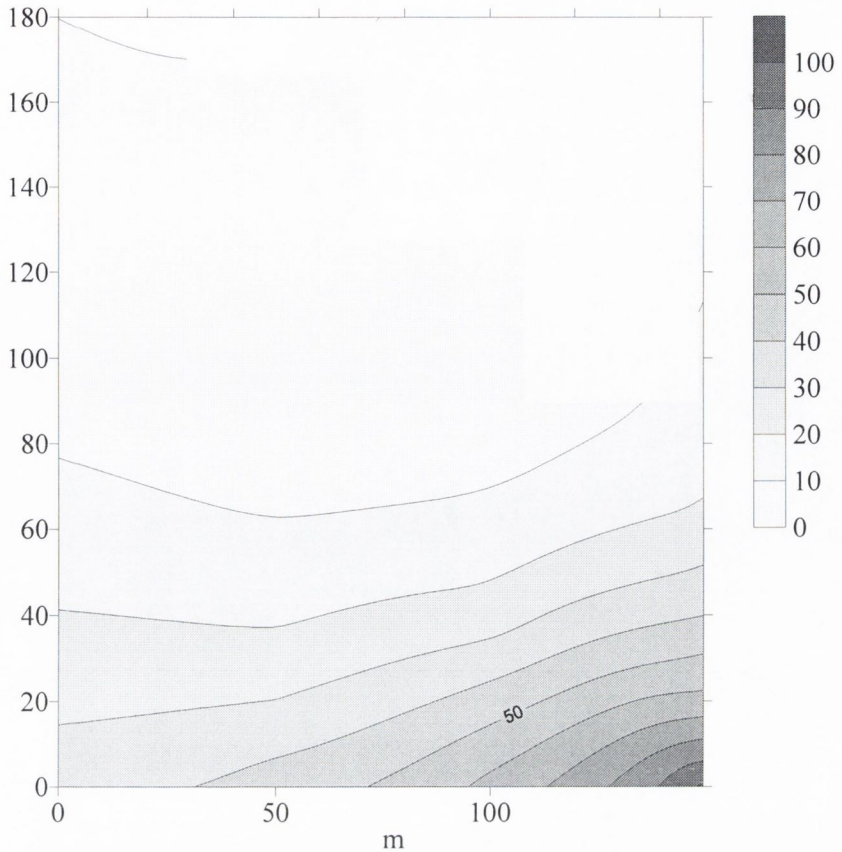


Figure 2.38. Density of *Tellina* m^{-2} in the size class >16 mm SH, Blackrock, April 2000. N=499.

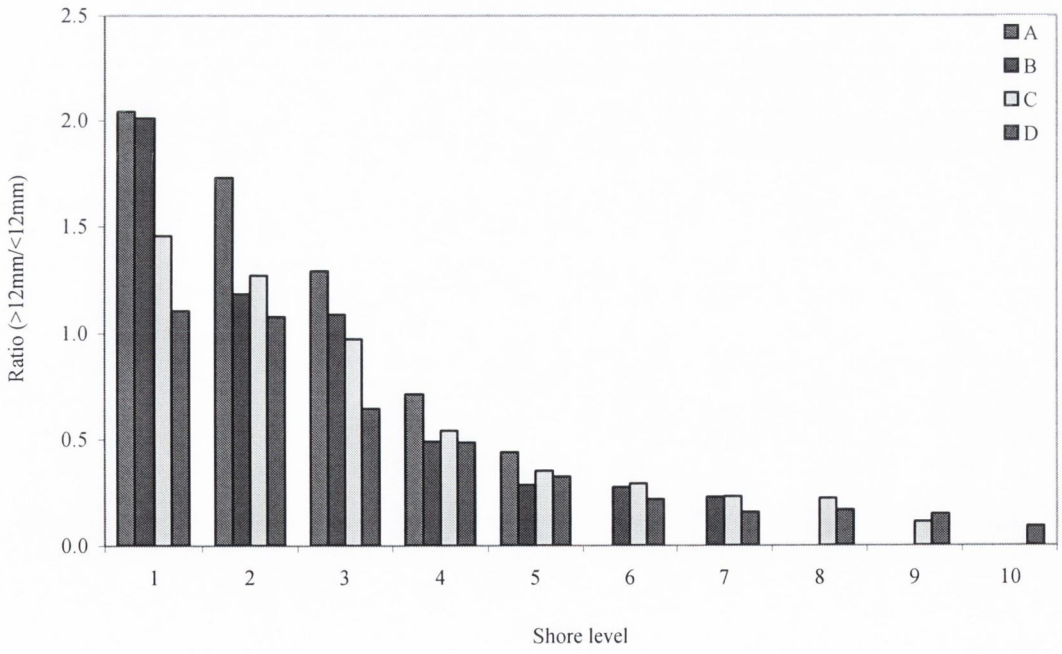


Figure 2.39. Ratio of *Tellina* sized > 12 mm (N=8,658) to < 12 mm SH (N=16,461) in each transect A-D, Blackrock, April 2000.

Similar patterns of distribution for both abundance and size were evident for the 3 smaller sizes classes, these differing from the similar patterns shared by the 2 larger size classes. The distribution patterns of the three small size classes, which likely correspond to individuals aged <3+ were combined and compared to that of the 2 largest size classes. The ratio of *Tellina* >12 mm SH compared to <12mm SH showed a clear decreasing trend down the sandflat, and also horizontally at most shore level from A to D (Figure 2.39). This was less clear for lower stations where transects A and B could not be sampled.

Statistically significant positive correlations were observed between chlorophyll *a* concentration and *Tellina* abundance in the three smaller size classes (Table 2.14). Conversely, abundance within the two size classes above 12mm SH were significantly negatively correlated with chlorophyll *a* concentration. Overall abundance was not significantly correlated with chlorophyll *a* concentration however. A negative correlation between density in the >16mm size class and phaeopigment concentration existed.

Table 2.14. Pearson's correlation between mean chlorophyll *a* (mg m^{-2}) and phaeopigment (mg m^{-2}) concentration and abundance in size classes (mm) of *Tellina* at each station. * indicates differences significant at < 0.05 level and includes Bonferroni adjustment.

SH	Chlorophyll a	P-value	Phaeopigment	P-value
All	+ 0.26	0.16	+ 0.22	0.23
< 4	+ 0.71	≤ 0.01*	+ 0.26	0.16
4 – 8	+ 0.73	≤ 0.01*	+ 0.33	0.07
8 – 12	+ 0.68	≤ 0.01*	+ 0.25	0.18
12 – 16	- 0.78	≤ 0.01*	- 0.20	0.29
> 16	- 0.61	≤ 0.01*	- 0.46	≤ 0.01*

Mean chlorophyll *a* was negatively correlated to the population size distribution of *Tellina* (Table 2.15). Statistically significant negative correlations between mean SH in each size class and chlorophyll *a* concentration occurred for all size classes with the exception of the <4 mm and >16 mm classes. Only the <4mm SH size class showed any significant correlation with phaeopigment concentration when mean size was considered.

Table 2.15 Pearson's correlation between mean chlorophyll *a* and phaeopigment concentration (mg m^{-2}) and mean SH in size classes (mm) of *Tellina* at each station. * indicates differences significant at < 0.05 level and includes Bonferroni adjustment.

SH	Chlorophyll *a*	P-value	Phaeopigment	P-value
All	- 0.79	≤ 0.01*	- 0.30	0.09
< 4	+ 0.28	0.13	+ 0.47	≤ 0.01*
4 – 8	- 0.56	≤ 0.01*	- 0.22	0.24
8 – 12	- 0.71	≤ 0.01*	- 0.18	0.34
12 – 16	- 0.80	≤ 0.01*	- 0.31	0.09
> 16	- 0.25	0.17	+ 0.01	0.95

Mean chlorophyll *a* concentration in this investigation was 33.5±0.9mg m^{-2}. The preliminary studies at Blackrock 2.3.1a, b and c (above) yielded mean values of 27.8±1.9, 58.0±0.4 and 61.2±1.1mg m^{-2}, respectively. The location of preliminary studies corresponded to shore level 3 in this trial, where a mean concentration of 29.0±1.4mg m^{-2} was recorded.

The three preliminary studies yielded mean (±S.E.) phaeopigment concentration values of 4.5±0.7, 4.5±0.6 and 3.8±0.7mg m^{-2} respectively (see 2.3.1a-c above). A comparable mean concentration of 4.5±0.2mg m^{-2} was recorded during the present study. The average phaeopigment value for shore level 3, which corresponded to the location of the early investigations, was 4.1±0.8mg m^{-2} in this study.

The mean abundance of *Tellina* over the whole area sampled was 1,080±31m^{-2} (95% C.I.). The total area of the study site was 18,500 m^{-2}. A total population estimate of 19,980,000 *Tellina* was suggested for the study area during the period of study.

2.4 Discussion

Considerable spatial variability in the abundance and size distribution of both primary consumer species and photopigments was observed at both sites. Although the magnitude of variability in abundance was much greater at the Bull Island site, size-distribution of the primary consumer varied dramatically at Blackrock despite the site

appearing to be visibly more uniform in sediment characteristics. Down-shore gradients were common, but considerable along shore variability also occurred. This suggested that the biological and physical parameters regulating spatial distribution at the sites affected conspecifics of varying size in different ways, although the causative agents were not examined in detail during the current study. The inequalities in the abundance and size distribution of the primary consumers, and of photopigment concentration suggested that careful consideration of the spatial hierarchy of sampling methodology for medium- to long-term monitoring programmes was warranted. Although particulate sediments often appear relatively uniform in nature, environmentally driven variation in species distribution patterns can occur over numerous scales (Hughes, 1999).

The results of the preliminary investigations were essential in designing an appropriate sampling protocol to examine spatial variability in the distribution of the primary producer and consumer species at the sites, particularly in the absence of any prior knowledge regarding these parameters. Randomly sampling in areas of very high heterogeneity can result in very high sampling errors (Bowman & Lewis, 1977; Hassell, 1987). Although only the photopigments were examined during these trials, previous studies have shown their distribution to be far more transient and heterogeneous than those of benthic consumers. Regular tidal suspension and deposition of photopigments can result in changes in concentration over relatively short durations (Baillie & Welsh, 1980; Varela & Penas, 1985; Shaffer & Sullivan, 1988; de Jonge & van Beusekom, 1992; Brotas & Catarino, 1995; Conde *et al.*, 1999; Lucas *et al.*, 2000). A sampling protocol that had been designed to take account of this was assumed to be appropriate for the less transient and fluctuating distribution of primary consumers. The preliminary investigations showed that there was a high degree of inherent variability in the distribution of photopigments over relatively short sampling distances at the Bull Island site, and that this increased with distance between points. This suggested that even very significant sampling replication within small areas would not necessarily result in a decrease in sampling error to provide more accurate estimates (Hassell, 1987). As dissimilarity between stations increased with distance between them due to overall concentration gradients, photopigment concentration recorded at one station was less likely to be representative of another

location, even if a high degree of accuracy had been achieved by very high within-station replication. As variograms failed to reach asymptote, it was not possible to define a threshold or maximum sampling distance required to account for spatial variability for all areas of the flats. This would have been possible for a habitat with more uniform photopigment concentration, and the sampling area could have been significantly reduced to the threshold sampling distance. The use of a clumping index such as Lloyd's Index of Patchiness could also have been used to investigate aggregation of photopigment at these sites. Examination of the chlorophyll data revealed that the variance was greater than the mean at both sites indicating a clumped distribution (Sokal & Rohlf, 1995). Although there was much higher inherent variability between samples at the Bull Island site when compared with Blackrock during the preliminary studies, the variograms revealed similar trends in the magnitude of variability with increasing sampling distance at the latter site. In both cases, the number of sampling stations was increased over the flat as a whole and replication within station decreased. This was considered likely to provide the most detailed overview of within-site spatial variability in the organisms studied when compared with greater distance between stations and higher within-station replication. Although replicates within-station were taken randomly within a relatively small area, stations were dispersed in a uniform manner (Hassell, 1987).

Although the preliminary investigations were useful in defining a sampling methodology for the large-scale site-wide trial, the different times of year at which they were conducted invalidated any direct comparison of results. Photopigment concentrations, and the ratio between them, varied considerably between the sampling dates. This suggested that intra-annual, and probably inter-annual variation in environmental parameters significantly affected microphytobenthic biomass and production, and probably the spatial distribution of the autotrophs and their degradation products. Spatial distribution of both *Hydrobia* and *Tellina* did not follow a Poisson distribution, where the variance approximately equals zero, but had clumped distributions with coefficients of dispersion >1. Seasonal and annual variations in the spatial distribution of indicator species should, therefore, be considered when determining sampling programmes with effort directed at various periods during the year depending on the reason for sampling.

Previous studies have recorded low spatial variability in chlorophyll *a* distribution between meters and 10s of meters (Shaffer & Onuf, 1983;Delgado, 1989), while others have recorded no significant differences (MacIntyre *et al.*, 1996; Guarini *et al.*, 2002). Spatial variability in photopigment concentration was relatively high at very small (cms) sampling distances during the present study, particularly at the Bull Island site. The mechanisms causing this fine-scale heterogeneity are not clear, as the area sampled within quadrats appeared uniform in sediment grain size, topography, aspect and drainage. Nutrients and detritus are known to be relatively patchy in the water column due to small-scale variations in hydrodynamics, and it is possible that disparities in deposition could account for some of the variability observed in the benthos. Fine-scale variation in hydrodynamic effects at the sediment/water column interface may result in slight variations in sediment mobility that decreases chlorophyll content as it increases. Bioturbation of the sediment by locomotory activity, deposit feeding and differential grazing pressure may also result in disparities in the distribution of photopigments (Page *et al.*, 1992; Galois *et al.*, 2000; Josefson *et al.*, 2002; Orvain *et al.*, 2004). This is probably common at the Bull Island site as the two most dominant primary consumers, *Hydrobia* and the amphipod *Corophium*, are both high mobile deposit feeders that occur in very high abundance. In contrast to the current study, where small-scale spatial variability in photopigment concentration was greater at the mudflat site, Shaffer & Onuf (1983) recorded higher variability with increasing sediment grain size.

More broad scale variations in the distribution of chlorophyll *a* in sediments has been previously shown to be influenced by a number of factors. Austen *et al.*, (1999) reported that sediment chlorophyll *a* content increased with exposure time during each tidal period, this being defined by the elevation of the flat. Chlorophyll *a* has also been shown to decrease in reduced salinity environments and increase with tidal height (Brotas & Catarino, 1995; Santos *et al.*, 1996). Underwood & Paterson (1993) recorded significantly higher chlorophyll *a* content on the high shore during all periods of the year. This pattern is generally repeated in sub-tidal areas, with higher chlorophyll *a* in decreasing water depth, presumably due to increasing light penetration to the benthos (Hummel, 1985). Although increasing chlorophyll *a* content was observed at higher tide levels at the Bull Island site, the opposite was true

at Blackrock. It seems likely that the increased physical activity in shallow parts of the flat, and possibly the scour produced by waves rebounding off of the hard sea defences, may result in increasing sediment mobility and reduced microphytobenthic biomass higher up the shore (Riedl, 1971). Aspect and nutrient supply will also govern medium- to large-scale differences in chlorophyll *a* distribution (Heip *et al.*, 1995). Point sources of anthropogenic nutrient enrichment can cause particularly strong productivity gradients.

The increase in phaeopigment concentration observed at Bull Island between investigations suggested that degradation of chlorophyll *a* increased as spring progressed at this site. This may have been due to an increase in grazing activity by primary consumers associated with increasing water temperature and/or species abundance, a more rapid turnover of chlorophyll *a* due to increasing irradiance (Baillie & Welsh, 1980; Bianchi *et al.*, 1988) or from deposition of phaeopigment derived from the degradation of phytoplankton, which was likely to have been negligible during the winter (Admiraal, 1984; Soetaert & Herman, 1995; Goto *et al.*, 1998). A similar increase in phaeopigment concentration was not observed at Blackrock. It is possible that the lower mobility of primary consumers, higher sediment mobility or more efficient physical or biological removal of phaeopigment occurred at this site (Riedl, 1971). It was not clear why the correlation between chlorophyll *a* and phaeopigment concentration was negative at Blackrock and positive at Bull Island during the preliminary experiments, and positive at both sites during the final large-scale experiment. It may have suggested that phaeopigment was transported by physical wave action during the former periods at Blackrock or that higher degradation of chlorophyll *a* had resulted in its build-up in certain areas without subsequent regeneration of microphytobenthos. The positive correlations were simply the result of continuous higher production in certain areas resulting in greater degradation. The lack of negative correlation during the early months of the year at Bull Island may suggest that microphytobenthic production at this site remains active during the winter period. Remineralisation within marine sediments is known to reduce nutrient limitation of primary production over most of the year in many types of sediment, particularly when they are relatively immobile (Epstein, 1997a,b; Sullivan, 1999; Decho, 2000).

Although the size distribution and abundance of *Hydrobia* varied across the Bull Island site, few significant differences were apparent between areas not at the extreme high shore or within the influence of the creek. This suggested that sampling on any other areas on the mudflat would probably yield samples that were representative of the majority of the rest of the habitat for most cohorts. The higher abundance of the smallest cohort toward the mouth of the flat may indicate that sampling should be conducted toward this end of the site if settler/early benthic phase abundance information is required. The lack of significant variation between mean cohort-specific shell height across the site suggested that sampling at most mid shore levels would probably yield a size-frequency distribution representative of the whole site, although very large individuals may not be well represented. Conversely, the high variability in group-specific abundance and mean shell height recorded at the Blackrock site indicated the potential difficulty in defining one particular sampling area to yield samples that were representative of the whole site. The distribution of some cohorts was extremely non-uniform, being confined to relatively restricted areas of the shore. The opposing directional gradients observed between the 3 smaller and 2 larger size groupings would make it extremely difficult to sample all sizes effectively in a single restricted area located on the low or high shore of the flat. Additional confusion was caused by the significant variability in the size-frequency distributions recorded at each sampling station, which suggested considerable spatial variability in growth rates occurred at relatively short distances across the flat. Overall abundance did not different significantly with the exception of the extreme high shore however. Although samples taken in one location were therefore unlikely to represent the size-specific abundance occurring at stations located even relatively short distances away, they would give a fairly good indication of population abundance.

A combination of pre- and post-settlement process processes created the heterogeneity observed in the distribution of the two primary consumer species studied. Patchiness in larval supply and settlement patterns due to hydrodynamic influences can contribute to heterogeneity in subsequent benthic distribution (Gaines & Roughgarden, 1985; Gaines *et al.*, 1985; Roughgarden *et al.*, 1988). Inequalities in spatial distribution at settlement can be masked rapidly when early benthic stages are

particularly mobile, as is the case for *Hydrobia ulvae* (Robinson & Tully, 2000b). Mortality is generally quite high during the early benthic life history stages, and spatial disparity in its rate can be significantly large to affect distribution and abundance over relatively small scales, particularly for sedentary species (Gosselin & Qian, 1997; Gosling, 2003). Variation in physiological tolerances may have accounted for the differences in cohort/group specific distribution patterns, with older individuals more capable of existing on the high shore where predation pressure from marine predators is extremely limited. Sediment grain size can also influence the distribution of benthic species (Robinson & Tully, 2000a), particularly deposit feeders that must feed as well as live in/on the substrate. As the substrate type at Blackrock consisted of a relatively uniform sediment type it seems unlikely that this affected *Tellina* size and abundance distribution however. Some degree of patchiness in sediment type was observed at the Bull Island site. Some areas appeared to contain more viscous mud, while others appeared to have a higher sand content. Some isolated strips and patches on the high shore were covered with small to medium sized stones and pebbles, although these were underlain with mud. Although sediment type did not appear to influence the size-specific distribution patterns of *Hydrobia*, no formal analysis was conducted as mixing of sediments from all levels sampled using the corer to sample *Hydrobia* at this site prevented isolation of the surface layer.

The variation in size-frequency distributions recorded among sampling stations for *Tellina*, and to a much lesser degree *Hydrobia*, may suggest that differential growth and/or mortality rates occurred across the study sites. This may have also been evidenced by the spatial variability in cohort/group-specific mean size. Stephen (1928) stated that *Tellina* growth rate increased with distance up the shore, and that growth rates in proximity to and below the low water mark were much reduced. High variability in individual growth rate within cohort commonly leads to the merging of cohorts in fast growing species, those that have more than one settlement event during a season or those that persist for several years beyond maturity (Barnett & Watson, 1985; Robinson & Tully 2000a). Variability in growth rate can become even more pronounced after maturity, when a varying proportion of energy budget is directed between somatic and reproductive growth. Multiple brooding is a common trait to many marine invertebrate species, and is generally considered to be an adaptation for maximising the potential for at least some recruitment success. Variation in

environmental conditions, in particular temperature and food, are also known to influence growth and mortality rates (Sandulli & Pinckney, 1999; Aljetlawi *et al.*, 2000; Rossi, 2002). It is possible that relatively small-scale variation in these parameters resulted in the apparent disparity in growth rates at the study sites.

Studies of a population of *Hydrobia ulvae* in France (Haubois *et al.,* 2004), recorded a pattern of increasing abundance at higher shore levels similar to those recorded in the current study but the authors made no attempts to link this distribution pattern to other environmental parameters. Although the abundance of larger sized *Hydrobia* were correlated with photopigment concentration in the current study, with greater densities occurring on the high shore, no causative relationship was apparent. Although it is possible that the patterns of larger sized *Hydrobia* and chlorophyll *a* distribution were similar due to the deposition patterns over the previous tidal cycles, only a small proportion of *Hydrobia* were seen to display floating dispersive behaviour on any given tide. Additionally, the spatial distribution of microphytobenthic organisms (chlorophyll *a*) were unlikely to change significantly on a site-wide basis with each tidal event considering the low physical energy status at the site and the relatively high cohesive link that can occur between the phytobenthos and sediment surface (Sullivan, 1999). As larger specimens were found over most areas of the mudflat, with the exception of the creek, but were located in greater densities at the high shore, this may have suggested that this was the preferred shore level for these older cohorts. Larger specimens were also recorded in greater numbers on the high shore by Fish & Fish (1974) in the Dovey estuary in Wales. Physical stress and aerial exposure would be much greater at these higher shore levels. The higher concentrations of chlorophyll *a* may permit larger *Hydrobia* to graze sufficiently during the relatively short periods of immersion however, reducing the need for locomotory activity and reducing the potential for food limitation. A number of studies have shown chlorophyll *a* concentration to be greater at higher levels of the shore (Heip *et al.*, 1995; Serodio & Catarino, 2000). The correlation between some sizes of consumer species and chlorophyll *a* distribution may suggest that some degree of bottom-up regulation of the primary consumers occurs at the sites and further investigation is warranted.

The lower abundance of small-sized *Hydrobia* on the extreme high shore relative to other levels may have been due to the occurrence of numerous larger individuals at this tidal elevation, lower physiological tolerance of the higher exposure levels occurring there or the differential feeding activity of predatory species. Patchiness in recruitment seems an unlikely reason considering the relatively high mobility of this species (Hassell, 1987). As at least some individuals from the smallest cohort were located in all areas of the flat, a lack of physiological tolerance also seems an unlikely reason for their low abundance on the high shore. Competitive interaction between conspecifics is thought to significantly reduce feeding time, and some level of avoidance behaviour may occur if smaller individuals are more severely affected (Blanchard *et al.*, 2000). The fact that individuals in the smallest cohort seemed to be less restricted in their distribution across the mudflat may support the notion that they disperse across a wider area to mitigate the effect of intra-specific interactions. Food concentration may be sufficient only to support smaller individuals in some low shore areas as although more numerically abundant, the much lower individual body size and biomass of small specimens, and presumably their impact on the environment, may be less combined than the affect of less abundant, but significantly larger specimens (Robinson & Tully, 2000b). Byers (2000) stated that smaller mud snails dispersed in response to high density, and larger individuals in response to food limitation. Although the smallest cohort occurred in relatively high abundance over much of the site, overall their numbers did not significantly exceed that of individuals in larger size classes, which were generally just contained within a smaller area. This may suggest greater habitat specificity develops with increasing size/age (Robinson & Tully, 2000b).

The absence of *Hydrobia* in the creek at Bull Island may be an indication that current speed made this area unsuitable for colonisation by this species or that the access afforded to marine predators was sufficient to remove all specimens as the creek never fully drains. Alternatively, *Hydrobia*, particularly of a larger size may have been absent due to the lack the chlorophyll *a*, and presumably microphytobenthic food sources in this area. It is probable that current speeds significantly reduced phytobenthos by continually reworking sediments in the creek (Lucas *et al.*, 2000, 2001), and therefore limited *Hydrobia* distribution due to food limitation.

It was not clear why such clear and opposing trends in size-specific distribution of *Tellina* were observed at the Blackrock site. Stephen (1928; 1932) stated that the density of *Tellina* decreased with increasing tidal height on a sandflat studied in Scotland, but larger individuals were encountered more frequently on the higher shore levels. He concluded by stating that *Tellina* was adapted to grow and survive better in the intertidal zone, and was not a successful sub-tidal species. The occurrence of larger individuals on the high shore at Blackrock, and the apparent spatial variation in growth rate, agreed with these findings. It is not clear why greater sizes are attained at the high shore, or whether this is achieved by faster growth and/or greater longevity however. It would seem logical to assume that physiological stress and disturbance were greater on the high shore (Boaden & Seed, 1993), and that these would reduce the growth and abundance of *Tellina*. It is possible that the concentrated suspended food sources that occur at the water's edge may significantly increase overall food availability despite being only available for a limited period. Higher sediment chlorophyll *a* concentrations were observed at the high shore levels in the current study. Alternatively, some other form of physical or biological stress may be imposed down the shore. For example, flatfish, which are common in Dublin Bay, have been observed feeding on the extended inhalant siphons of *Tellina* when submerged, this significantly reducing the feeding efficiency of the species (Trevallion, 1971). Many predatory bird species feed at the water's edge, which results in higher grazing pressure on the low to mid shore.

Although the exact timing of the reproductive phase of *Hydrobia* varies geographically (Bachelet *et al.*, 1987; Cardoso *et al.*, 2002) and that of *Tellina* is poorly understood, the large-scale April investigation was conducted with the assumption that the spring settlement of new recruits from the plankton would be well established for both species. Cursory examination of the contents of several sieved sediment samples at both sites 2 weeks prior to the main sampling event had revealed the presence of at least some individuals within the smallest cohort/size grouping of both species at their respective sites. Although large quantities of newly settled *Hydrobia* were subsequently encountered in most areas of Bull Island, the smallest *Tellina* size group was very poorly represented at the Blackrock site. Although the former species is known to settle in very large numbers directly from the plankton at intervals through the year (Cardoso *et al.*, 2002), little is known of the early benthic

phase of the latter. The lack of very small *Tellina* at the Blackrock site may suggest that planktonic phase post-larvae settled away from the adult habitat and subsequently recruited sporadically at later dates, that the main recruitment phase of this species had yet to occur or that very high post-settlement mortality of juvenile cohorts occurs. Recruitment failures that are caused by unfavourable environmental conditions are not uncommon for marine invertebrate species, and these can account for an apparent deficit in early benthic life history stages (Connell, 1961 & 1985; Robinson & Tully, 2000a). Small-scale, patchy recruitment failure is especially common in the intertidal zone, resulting in considerable variation in the abundance and size distribution of sedentary species in particular (Gaines *et al.*, 1985). Other authors have recorded recruitment failure of *Tellina* (McIntyre, 1970, Dekker & Beukema, 1999). Secondary benthic recruitment to the adult stocks has been documented for mussels and scallop (Boaden & Seed, 1993), and is postulated to occur for *Tellina*. This may be an adaptation to avoidance of the physical stresses imposed in the intertidal zone, as larger individuals are generally considered to be more tolerant than smaller conspecifics. Other species use this tactic to avoid being ingested by larger juvenile and adult species, including conspecifics, and digested or incorporated into pseudo-faeces. This is particularly useful when high densities of filter feeding species occur, such as is common on sandflats such as Blackrock.

Although the laboratory methods used in the current study are considered relatively accurate for determining relative photopigment concentration (MacIntyre *et al.*, 1996), they are probably inappropriate for use when particularly fine-scale variation is to be examined. Although fluorometric methods yield results of a similar accuracy to the methods employed in the current study, high performance liquid chromatography (HPLC) provides greater accuracy and partitions chlorophyll into its base components and derivatives (Pinckney *et al.*, 1994). The method is more time consuming and expensive than spectrophotometric analysis however. The latter method was considered to be adequate for the determination of the relative distribution of photopigments in the current study as description of fine-scaled variation in chlorophyll biomass was not among the study aims.

If a monitoring programme were undertaken a systematic approach from a random start point would be recommended for chlorophyll *a* analysis at both Bull Island and

Blackrock. At Bull Island a sample size of 15 would detect differences in the population mean of 20 mg m^{-2} while at Blackrock which was more homogeneous a sample size of 7 would detect 10 mg m^{-2} differences (probability=0.05, power=0.8). This would be the minimum sample number recommended. Spatially, the coefficient of dispersion suggested a clumped distribution (Sokal & Rohlf, 1995) at both sites. Although increasing variation was observed with increasing distance, no minimum distance between samples was determined. However, chlorophyll samples within $1m^2$ could be considered replicates of that station and stations should be positioned >1m apart. Gradients in sediment chlorophyll *a* concentrations were observed between different shore levels at both Bull Island and Blackrock and any sampling programme would require sampling stations at different shore zones. Alternatively a sampling programme that consistently sampled one shore zone only (e.g. middle shore) could also be recommended if trends over time rather than absolute values were the aim of the monitoring programme.

A systematic approach for abundance records of the primary consumer species would also be recommended. *Hydrobia* abundance was high and fluctuated greatly over the sampling site. As a result a large number of samples (N=164) would be required to detect a change in mean population abundance of 500 individuals m^{-2} (probability=0.05, power=0.8). Higher differences of 1000 and 2000 m^{-2} would require a sample number of 43 and 12, respectively. If sampling were restricted to one zone or one time of year these sample number requirements would reduce. A difference of $500m^{-2}$ in *Tellina* abundance could be determined using just 4 samples, however, a more meaningful difference, for this species, of 250 or 100 m^{-2} would require sample sizes of 12 and 64, respectively.

The results of this study highlight the importance of considering spatial variability in population parameters when planning a medium- to long-term sampling programme where the numbers of samples that can be taken in any one sampling event are constrained. Samples must yield results that are representative of the whole population, or of the population under examination, and this cannot be achieved without knowledge of the spatial and size distribution patterns of the target species. Even when a single variable such as chlorophyll *a* is examined, high spatial

heterogeneity in distribution can result in unrepresentative results if this patchiness is not determined *a prior*. Initial indications also suggested that the nature of this heterogeneity is probably highly dynamic over temporal scales due to variability in the biological and physical factors regulating abundance and size distribution, the results of this study provide a guideline for the further development of sampling protocols for intertidal sites in Dublin Bay. Although the purpose of any monitoring programme should determine the scale of sampling required to meet the programme goals. Results presented in this chapter will probide a valuable baseline for any future studies of chlorophyll *a* or of primary consumers *Hydrobia* or *Tellina* in the Dublin Bay system.

Chapter 3. Temporal variability in the abundance of two intertidal primary consumers and in the concentration of chlorophyll *a*.

3.1 Introduction

Wide variations in biological and physical processes generally occur with season in temperate marine environments. In many cases these seasonal fluctuations will result in relatively predictable changes in species population size, either directly or through their affect on required resources and\or interacting species (Rossi, 2002). Depending on the nature of the environmental variation, the result may be a positive, negative or non-significant change in population size. In natural circumstances, a complex suite of regulatory processes will affect a population at any one time. When interaction between processes occurs their combined influence may serve to reduce, negate or magnify resultant effects on the population. In addition to seasonal variation, stochastic fluctuations in environmental parameters can cause temporal perturbations in population size (Cadee, 1980; Shaffer & Onuf, 1983; Brotas & Catarino, 1995). Their affects can be particularly strong in comparison with seasonal changes as they are by nature less predictable, often of greater magnitude and, therefore, harder for species to tolerate and adapt to. Changes in the temporal and spatial influence of both seasonal and stochastic fluctuations in physical and biological parameters can result in significant intra- and inter-annual variability in population size and distribution.

The distribution and abundance of a species will depend in part on spatial and temporal variations in resource availability. Resource requirements vary with species, and often between conspecifics of varying life history stage within the same population (Forbes, 1989; Levinton, 1989). Ontogenetic shifts in resource utilisation are common to most species, generally being associated with changes in physiology or morphology, and may occur a number of times during the lifecycle (Lopez & Levinton, 1987). Although the availability of a wide range of resources can affect the survival and growth of various species, food is often considered to be the most important in regulating population size (Pinckney & Sandulli, 1990; Aljetlawi *et al.*, 2000). Despite the ability of some species to tolerate varying periods of food limitation, the condition of many others will deteriorate rapidly during shortage. Although mobile species may be able to relocate to areas were food availability is

increased, sedentary species and those that are obliged to remain in the habitat due other resource requirements, for instance shelter, must effectively wait for food to be delivered to them. In common with many other resources, regulation of population size by food limitation can be density-dependent or independent (Montagna *et al.*, 1983; Montagna *et al.*, 1995). For example, the former would occur if a particular species occurred in such high densities that insufficient food was available to sustain all individuals, the latter if a factor unrelated to the feeding activity of the consumer had caused a decline in the abundance of or access to the food resource. As a result changes in the population dynamics or size of one species, and so the magnitude of direct or indirect competition for resources, can positively or negatively affect those of another.

The main food sources available for primary consumers in the intertidal environment consist of the phytobenthos and phytoplankton. Due to deposition and resuspension by tidal currents a constant flux of these two food sources occurs between the sediment surface and water column (Conde *at al.*, 1999; Lucas *et al.*, 2001). This can make quantification of the relative productivity of each of these components difficult, particularly in the intertidal zone where the potential for flux is greatest. Due to the difficulty and cost associated with direct cell counts most investigations into seasonality in primary productivity use chlorophyll *a* concentrations as an index of primary producer biomass (MacIntyre *et al.*, 1996). Although microphytobenthos generally remain productive throughout the year as continuous regeneration within sediments prevents nutrient limitation, seasonal patterns linked to temperature variation have been recorded (Heip *et al.*, 1995; MacIntyre *et al.*, 1996; Underwood & Krompkamp, 1999). In contrast, phytoplankton biomass tends to be highly seasonal with temperature and nutrient limitation in the water column (Admiraal, 1984; Soetaert & Herman, 1995; Goto *et al.*, 1998). This can result in seasonal variation in the flux of chlorophyll *a* and phaeopiments between the water column and sediment media. Phaeopigments, the degradation products of chlorophyll *a*, increase with grazing and light intensity (Ingalls *et al.*, 2000), or as nutrient availability becomes limiting to photosynthesis. The chlorophyll *a* to phaeopigment ratio is, therefore, an effective indication of the rate of active photosynthesis compared with detrital inputs into a system.

Inter-annual variability in primary production is often taken to be an indication of relative ecosystem health and/or change (Agard *et al.*, 1993; Genner *et al.*, 2004), particularly in relation to environmental nutrient loads (Gee *et al.*, 1985). Due to difficulties in defining the species composition, type and flux of primary producers in the marine environment it is sometimes more feasible to monitor the population dynamics of a selected 'index' or 'indicator' species from a higher trophic level to identify relative changes in overall habitat productivity (Gray & Pearson, 1982). This process requires the assumption that some form of 'bottom-up' or 'top-down' regulation to population size and/or distribution occurs between the primary food source and index species, with changes in one directly affecting the other in some quantifiable way. In reality this relationship can be very difficult to define, particularly when food is not limiting, due to the complex interaction of physical and biological processes in the environment (Connell & Jones, 1991). Factors other than food availability may exert more stringent thresholds on population size when food is plentiful, and in this case the relationship between primary production and the abundance of the index species will be decoupled (Connell, 1961; Gosselin. & Qian, 1997). Regulatory processes are particularly difficult to detect in patchily distributed populations (Hassell, 1987).

Relatively immobile habitat-specific benthic organisms are useful indictor species, particularly in the intertidal zone due to easy accessability and as their spatial distribution and population parameters are generally more easily defined than mobile and/or pelagic forms. Primary consumers are particularly useful, as they are low in the food web and are therefore less likely to be affected by biological interactions that occur more regularly at higher trophic levels. This is especially true in estuarine and intertidal areas where lower trophic levels account for the majority of heterotrophic consumption, much of the nutrients from which are rapidly re-cycled *via* the detrital food chain (Epstein, 1997a). As many higher consumers cannot utilise primary producers as a food source directly, primary consumers represent an extremely important pathway for nutrient transfer between trophic levels (Rossi, 2002). Therefore, an understanding of primary producer-consumer dynamics within particular habitats or ecosystems may reveal much about short-, medium- and long-term changes in the environment. The aim of this chapter was to describe temporal variability in the abundance of two dominant primary consumer species in relation to

food availability, and to assess their suitability as indicator species for the sites where they were studied in response to anthropogenic changes to the Dublin Bay system such as sewage treatment, dredging or industrial inputs to the Bay.

3.2 Materials and Methods

Mudflats and sandflats are representative of the majority of the intertidal habitats occurring within Dublin Bay. A mudflat at Bull Island and a sandflat at Blackrock (see Chapter 2 for full site description) were monitored for temporal changes in the concentration of sediment surface photopigments (chlorophyll *a* and phaeopigment) and variation in the abundance of the principle primary consumer at each site. The infaunal bivalve *Tellina tenuis* was the dominant primary consumer at the Blackrock site, while the surface dwelling gastropod *Hydrobia ulvae* was most abundant at Bull Island. Both of these species were specific to the habitat types sampled, at least within Dublin Bay.

3.2.1 Field sampling

Each sampling site was divided into sampling blocks of 20 x 20m on a scale map. One block was selected randomly at the midshore level and designated as the sampling area for the entire duration of the study. Only one sampling block was chosen due to a high degree of spatial variation in the size distribution and abundance of the target species at the study site (Chapter 2). A midshore station was selected as between-site differences in the distribution of the species principally occurred between high and low shore stations (Chapter 2). A station slightly toward the mouth of the mudflat at Bull Island was sampled to account for recruitment periods, as the smallest cohort was more common at this end of the site. The station selected corresponded to station C4 (Figure 2.5) sampled in Chapter 2. As the same C4 (Figure 2.6) station at Blackrock had yielded *Tellina* abundance estimates representative of most of the rest of the site, this was the selected station at this site also. It was assumed that seasonal variation in abundance of the target species and food source at the selected station were representative of those occurring elsewhere in the whole site, regardless of tide height or distance along shore. It was not possible to sample multiple shore levels during this study due to logistical constraints.

3.2.1a Short-term variation in photopigment concentration

Daily sediment samples were analysed for chlorophyll *a* and phaeopigment concentration over 10 consecutive days during April 2000 to assess short-term variability in these variables. This information was used to define the sampling frequency for medium to long term monitoring. The sampling location at each site was determined as described in the previous section. Modified medical syringes were used to extract cores randomly from within the designated area of the flat. The base of each syringe core, which would normally act as the securing point for the needle, was removed so that it could be pushed easily into the sediment to yield a circular core with a surface area of 2.01cm^2. Although each syringe was pushed into the sediment to a depth approximately 4cm, only the top 0.5cm layer was used to determine pigment concentration. The taking of additional sediment below that necessary for analysis acted as a barrier to contamination of the surface layer, which remained uppermost in the syringe cylinder. Gentle upward pressure was applied to the plunger of the syringe as it was removed from the sediment, the suction acting to retain the sample within the tube. The open end of each labelled syringe was covered with a strip of laboratory 'Parafilm'. Sample cores were returned immediately to the laboratory and frozen within the syringes until processed in the laboratory. Ten syringe cores (2.01cm^2) were taken at each site and day, and analysed as described in section 2.2.4.

As the population turnover rate of the primary consumers at the sampling sites was assumed to be far lower than that of the primary consumers, no account was taken of species abundance during this exercise. Such short-term changes in abundance were unlikely to be relevant to seasonal and inter-annual variation in population size.

3.2.1b Bimonthly time-series sampling

As daily sampling was not practical for prolonged periods, a sampling interval of 2 weeks was selected as more feasible for determining the overall seasonal trends in chlorophyll *a* and phaeopigment concentration. This bimonthly sampling strategy was adopted at both sites when possible, particularly during the spring and summer seasons when more pronounced fluctuations appeared to occur. All samples for chlorophyll *a* and phaeopigment determination were taken during morning low tide.

A table of random numbers was used to assign sampling co-ordinates within the pre-selected area prior to arrival at the site. These were measured out as 'paces' across the area, with new co-ordinates assigned for each subsequent visit. At least 16 replicates were taken at each site every two weeks from April 2000 to September 2001.

Previous investigations (see 2.2.6a) had determined the optimum sampling unit area for *Hydrobia ulvae* to be between 40 and 80cm^2. This size of sample appeared to be suitable for the collection of significant numbers of individuals to facilitate examination of population size-frequency distribution. As the placing of quadrats and manual removal of sediment and animals within was not practical on the fluid mud of Bull Island, so a core sampler was used at this site. A section of standard household waste-pipe was fashioned as a corer with a sampling area of 70.9cm^2. Cores were taken to a depth of 8cm, with a minimum of 6 cores taken in the designated sampling block on each occasion. The positions of these cores were determined prior to arrival at the site, using the same methodology employed for sediment pigments. Each core was sieved in seawater on site using a 0.5mm sieve and the retained component returned to the laboratory in labelled plastic bags.

Tellina tenuis was sampled with a core area 70.9cm^2 to a sediment depth of 15cm at Blackrock. Although this core size was not large enough to adequately retain sufficient numbers of individuals to examine size-frequency distribution, mean shell height (SH) had been observed to vary significantly across the site previously (see Chapter 2). If a larger core had been used to collect higher numbers of individuals, the resultant size-frequency distributions were highly unlikely to represent the whole site. Robust abundance estimates from the selected sampling area were far more likely to be indicative of seasonal trends in other areas of the sand-flat, and therefore of greater application in establishment of a medium to long-term monitoring programme. A minimum of 12 cores were taken within the designated sampling station on each sampling occasion. Each core was sieved in seawater on site using a 0.5mm sieve and the retained component returned to the laboratory in labelled plastic bags.

Sediment temperature was measured at each site using an Altal Aqua Sensor. Water temperature data from the sampling period was obtained from Dublin City Council. Average air temperature, average irradiance and total sunlight hours were obtained from Met Éireann.

3.2.2 Laboratory and data analyses

3.2.2a Photopigments

Sediment samples within syringes were allowed to defrost at room temperature before removal. The lower, unwanted portion of the core was slowly ejected from the tube and discarded until only the top 0.5cm surface sample remained. This portion was placed directly into labelled centrifuge tubes. Although large, visibly conspicuous pieces of filamentous algae were removed prior to analysis, it was not always possible to be 100% sure that pieces of macroalgae were absent from all samples. Replication reduced the influence of such contamination, and outliers were removed when identified. Pigment analysis was determined following the method of Nusch (1980), Sartory and Grobbelaar (1984) and Brennan (1991). See section 2.2.4 for full methodology.

The ratio of chlorophyll *a* to phaeopigment concentration can be used as an index of productivity with high ratios implying quicker turnover, and this was calculated for each sampling date. High ratios suggest that most of the pigments are active chlorophyll *a* which generally occurs when microalgae are growing quickly. Low ratios are often observed during episodes of strong re-suspension as deeper sediment layers, which are rich in phaeopigments, are mixed with surface layers more rich in chlorophyll *a* or when deposition from the water column occurs.

3.2.2b Hydrobia and Tellina laboratory analysis

The component of each sample retained after sieving at the study sites was placed in a field tray and submerged in seawater on return to the laboratory. Live *Hydrobia ulvae* were easily identified either by actual movement or movement tracks left on the tray. The shells of dead *Hydrobia*, often white in colouration due to bleaching and lacking an operculum, were discarded. Live *Tellina tenuis* were easily identified by the

presence of flesh within the shell and isolated from the empty valves of individuals that had died prior to sampling. Significant quantities of shell material from dead individuals of both species were removed from samples. Shell height (SH) of live specimens of both species was measured from posterior to anterior shell tips using Mitutoyo Absolute Digimatic callipers to 0.1mm.

3.2.2c Data analysis

With due consideration of data normality and heterogeneity of variance, between-date comparisons were conducted using ANOVA or Kruskal-Wallis tests as appropriate, with Scheffe or Dunns post-hoc tests applied where required. Correlations between variables were tested using Pearson's product method. Where applicable, lag periods between variables were also examined by repeated analysis while offsetting the value of the relevant variable temporally. This aimed to identify changes in a response parameter caused by variation in another in the recent past. This was not possible for sediment temperature as this was recorded only on the day of sampling.

The overall site mean chlorophyll *a* concentrations from the bimonthly time-series data set were converted to microphytobenthic primary production using the extrapolation conversion equation of Colijn & de Jonge (1984):

Primary production (g C m^{-2}) = 0.6705 x Chlorophyll *a* (mg m^{-2}) + 4.01

This equation was used to to obtain estimates of primary production due to the difficulties involved in its measurement. The measurement of primary production is difficult, time consuming and methodogical problems exist. It was beyond the scope of the current study. Although other equations exist for conversion of chlorophyll to primary producton (e.g. Brotas & Catarino, 1995; Kromkamp *et al*, 1995) the extrapolation conversion equation of Colijn & de Jonge (1984) was chosen. The range of sediment chlorophyll *a* values reported in the study included the range of values at Dublin Bay and the eqution was based on different habitat types that included sites such as both Bull Island and Blackrock. Although the use of this equation was not ideal, as inherent differences could exist in the relationship between

chlorophyll *a* and primary production in the Netherlands and in Dublin Bay it was deemed necessary under the circumstances.

3.3 Results

3.3.1 Bull Island

3.3.1a Ten day consecutive sampling of photopigments

Sediment chlorophyll *a* concentrations ranged from 3.8 to 86.0 mg m^{-2} over the 10 day period at Bull Island (Figure 3.1). Significant differences were observed between dates (Kruskal Wallis, χ^2=27.8, df=9, $p \leq 0.01$). Day 1 had significantly higher mean chlorophyll *a* concentration than all other days with the exception of 1 and 7, while mean concentration on day 7 was higher than all but 1. No other significant differences between dates occurred. Phaeopigment levels on days 1 and 9 were different to all other dates, the former being significantly lower and the latter higher (Kruskal Wallis, χ^2=27.3, df=9, $p \leq 0.01$). Phaeopigment values were higher than chlorophyll *a* concentrations on all days at the Bull Island sampling station, ranging from 37.5±3.8 to 242.8±56.5mg m^{-2} (Figure 3.1). The chlorophyll/phaeopigment ratio for the 10 consecutive dates in April 2000 at Bull Island and Blackrock (shown together for comparison) can be seen in Figure 3.2. At Bull Island this ratio was highest on day 1 (Figure 3.2). The ratio on this day was significantly higher (Kruskal Wallis, χ^2=13.2, df=9, $p \leq 0.01$) than all other days, but no other statistically significant differences were determined.

3.3.1b Bimonthly time-series sampling of photopigments

Mean sediment chlorophyll *a* ranged from 7.7±4.4 to 124.6±16.5mg m^{-2} (Figure 3.3), at Bull Island during the sampling period June 2000 to September 2001. Lowest chlorophyll *a* levels occurred during April and May of each year. Chlorophyll *a* levels increased during May/June 2000 and fluctuated between 50-100mg m^{-2} throughout most of the year until decreasing during the following April. Significant differences were observed between dates (ANOVA, F=22.9, df=36, $p \leq 0.01$) and Scheffe post-hoc tests detailed these differences (Appendix 3.1). September 2001 had

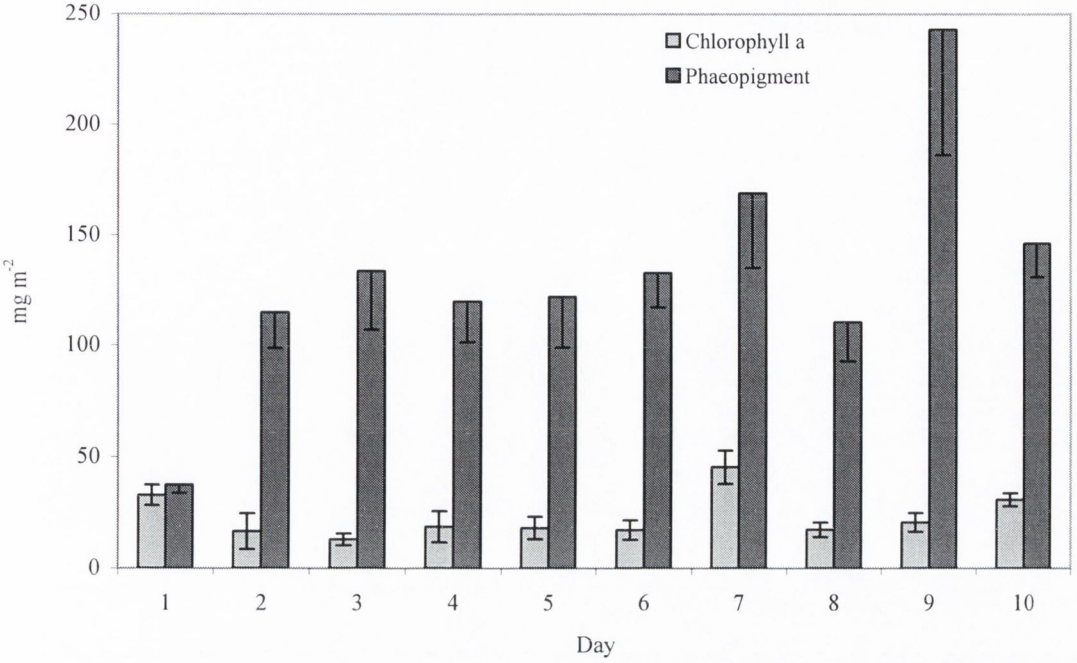


Figure 3.1. Mean sediment chlorophyll *a* and phaeopigments (mg m^{-2} ± S.E.) on 10 consecutive days at Bull Island, April 2000. N=55.

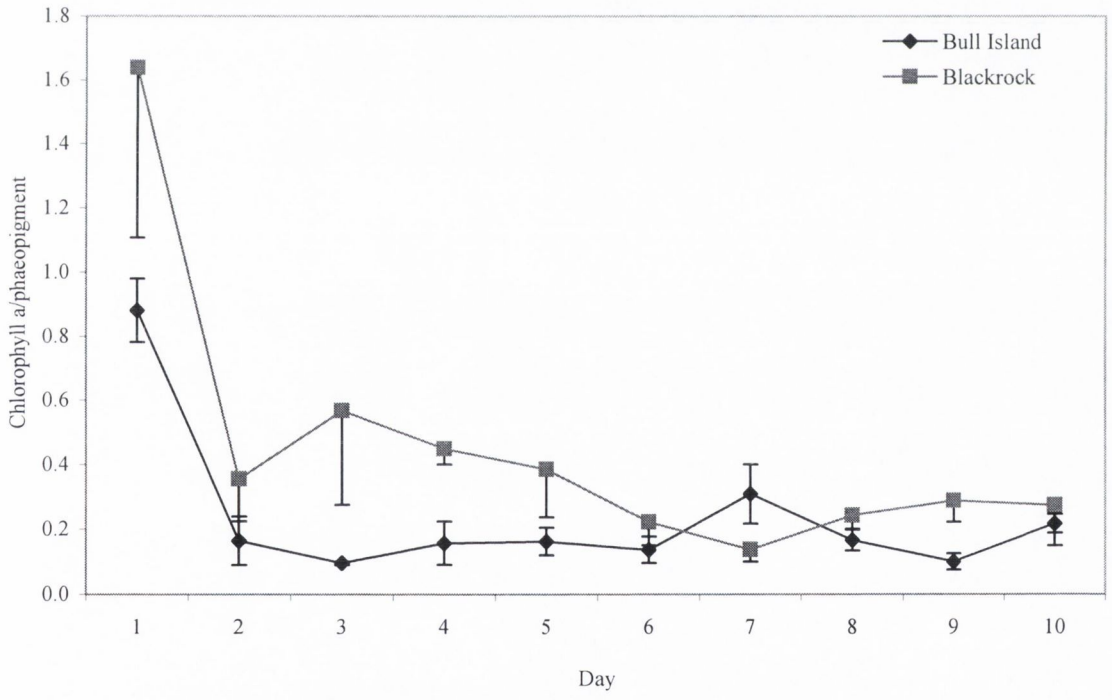


Figure 3.2. Sediment chlorophyll *a*/phaeopigment ratio at Bull Island and Blackrock on 10 consecutive days in April 2000. N=55.

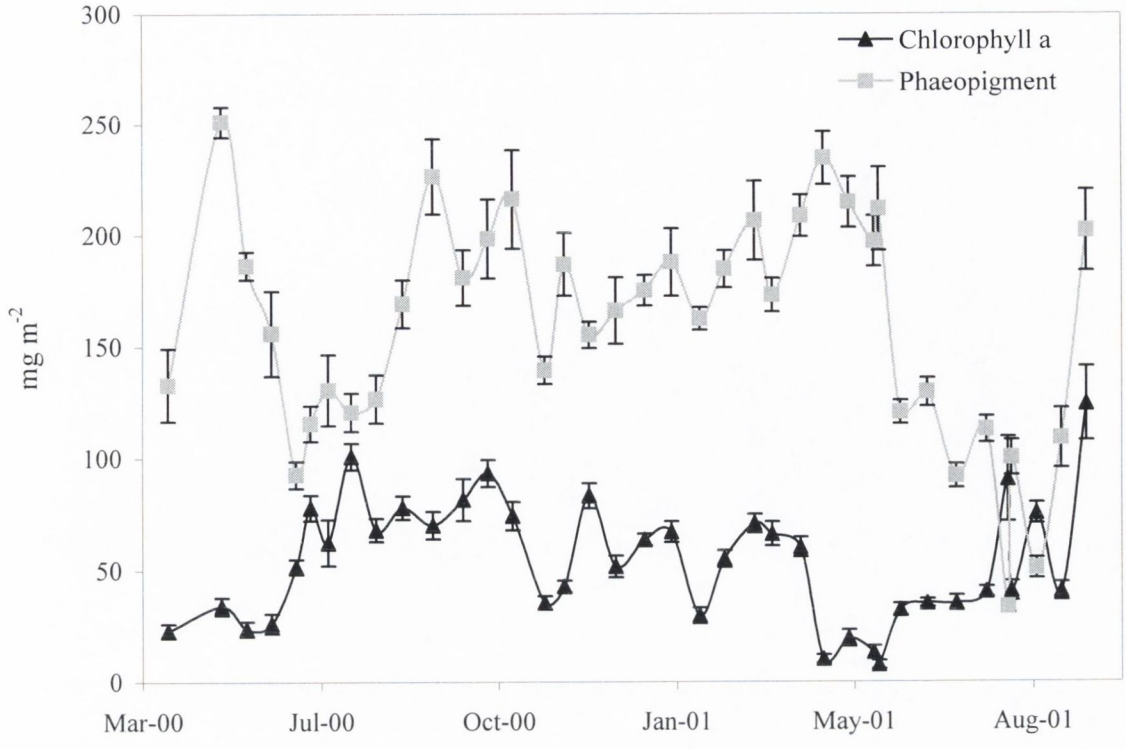


Figure 3.3. Mean sediment chlorophyll *a* (N=577) and phaeopigments (N=577) (mg m^{-2} ± S.E.) at Bull Island from May 2000 to October 2001.

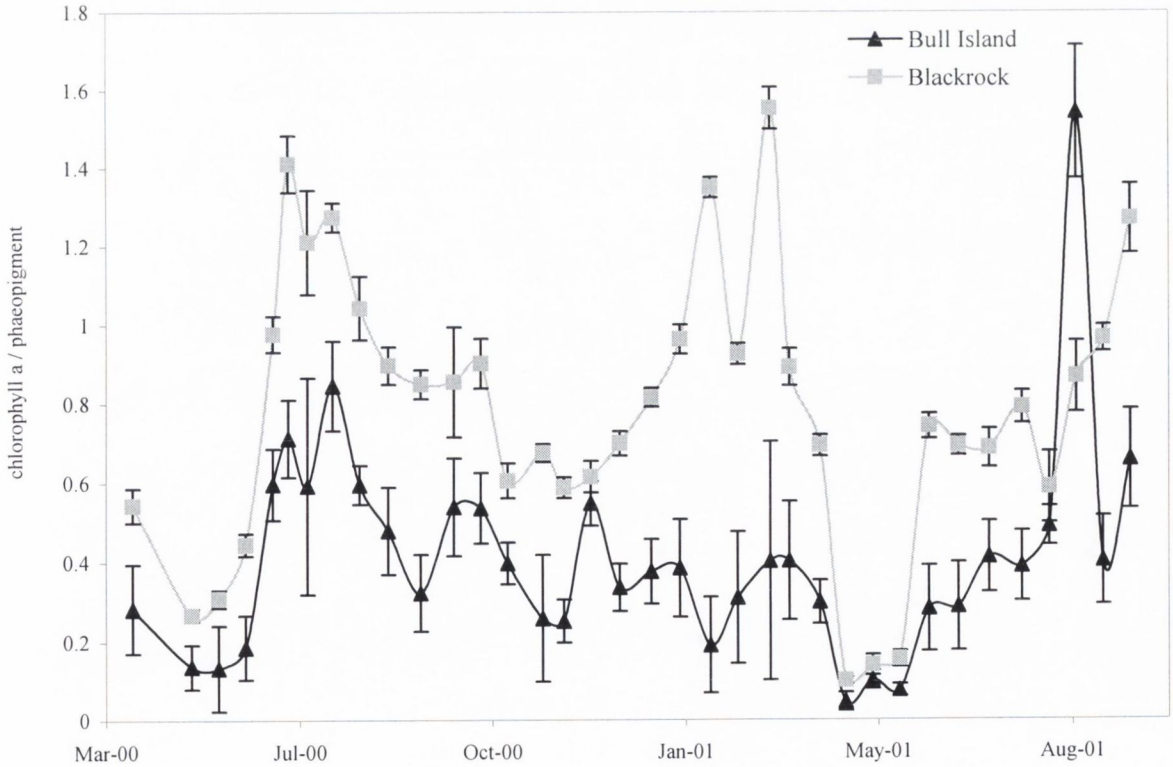


Figure 3.4. Sediment chlorophyll *a*/phaeopigment ratio at Bull Island and Blackrock from May 2000 to October 2001. N=577.

significantly higher chlorophyll *a* concentrations than most dates during the sampling period, while April 2001 was significantly lower.

Chlorophyll *a* concentration was not significantly correlated with any temperature or sunlight variable (Table 3.1). No relationships were determined during repeat analyses for lag periods of 24h, 48h, 2 or 4 weeks.

Table 3.1. Pearson's correlation between mean chlorophyll *a* concentration (mg m^{-2}), phaeopigment concentration (mg m^{-2}), chlorophyll/phaeopigment ratio and environmental variables at Bull Island, Jun-00 to Sept-01. * indicates significant at <0.05 level.

	Chlorophyll *a*	p-value	Phaeopigment	p-value	Ratio	p-value
Sediment temperature	-0.10	0.63	-0.49	≤ 0.01*	+0.34	0.09
Water temperature	+0.28	0.12	-0.47	≤ 0.01*	+0.51	≤ 0.01*
Air temperature	+0.12	0.48	-0.44	≤ 0.01*	+0.45	≤ 0.01*
Average irradiance	-0.18	0.29	-0.11	0.51	-0.02	0.91
Total sunlight hours	-0.05	0.77	+0.14	0.42	-0.12	0.48

Phaeopigment generally occurred in higher concentrations than chlorophyll *a* at the Bull Island site (Figure 3.3). Mean sediment phaeopigment levels ranged from 33.8±2.4 to 251.1±6.9mg m^{-2}. Mean concentration was lowest during July-August, fluctuating between 100 and 250mg m^{-2} for much of the rest of the year. Although significant differences were observed between sampling events (ANOVA, F=15.7, df=36, p≤0.01) these were not as frequent when compared with chlorophyll *a*. The lowest mean value, which occurred on August 11th 2001, had significantly less sediment phaeopigment than many other dates (Appendix 3.2). Phaeopigment concentrations were negatively correlated with all temperature variables recorded on the day of sampling, but not with either average irradiance or total sunlight hours (Table 3.1), or after lag periods of 24h, 48h, 2 or 4 weeks were applied to the data.

The chlorophyll *a*/phaeopigment ratio at Bull Island varied significantly (ANOVA, F=26.6, df=36, p≤0.01) over the course of the sampling period with higher ratios generally recorded during the summer months (Figure 3.4). The highest chlorophyll *a*/phaeopigment ratio was recorded on August 11th 2001, and was significantly higher than all other dates sampled. Scheffe post-hoc tests also showed that June and July 2000 also had significantly higher ratios than a number of other dates (Appendix 3.3).

The ratio was lowest at Bull Island during April and May in both 2000 and 2001. The chlorophyll *a*/phaeopigment ratio was positively correlated with water and air temperature recorded on the day of sampling (Table 3.1), although not when lag periods of 24h, 48h, 2 or 4 weeks were tested.

The average chlorophyll *a* concentrations at Bull Island in the temporal sampling period were not comparable to the average values recorded in the corresponding months in the spatial variation described in Chapter 2. Average values of 137.5±7.5, 161.4±7.3, 98.0±4.1 and 115.8±3.5mg m^{-2} were recorded in the months of January, February, March and April 2000 for investigations 2.2.5a, b, c and 2.2.6, respectively. Much lower average values of 63.9±2.7, 55.4±3.8, 71.1±4.2 and 60.6±4.4mg m^{-2} were observed for the corresponding months in 2001. Mean phaeopigment concentrations recorded during January, February, March and April 2000, were also not comparable to values observed in this study during 2001. In January 2000 average phaeopigment concentrations were 93.1±5.0 compared with 175.2±6.9mg m^{-2} for the same month in 2001. Mean phaeopigment concentrations in March and April 2000 of 100.7±6.9 and 95.5±3.7mg m^{-2} were lower than 206.5±17.7 and 208.6±9.3mg m^{-2} determined in 2001. Chlorophyll *a*/phaeopigment ratios were higher in 2000 than 2001. In the spatial variation trials described in chapter 2, ratios varied from 1.6±0.07 in January to 0.5±0.03 in February. In 2001 ratios were less variable over the corresponding months ranging from 0.3±0.03 to 0.4±0.05. It should be appreciated that the location of each of these investigations differed.

3.3.1c Bimonthly time-series of Hydrobia abundance

Mean abundance of *Hydrobia* varied significantly (ANOVA, $F=30.3$, $df=33$, $p \leq 0.01$) within the selected study area between June 2000 and September 2001 (Figure 3.5). Although a large degree of variation sometimes occurred between the densities of *Hydrobia* recorded during previous and subsequent sampling events, a general seasonal trend in abundance occurred. A minimum mean (±S.E.) density of 2,630±316 individuals m^{-2} was recorded during June 2000. Generally low densities persisted over the summer months until a rapid increase occurred in September 2000. This corresponded to the period when large numbers of juveniles were recruited into the population, resulting in the highest mean densities recorded during the study of

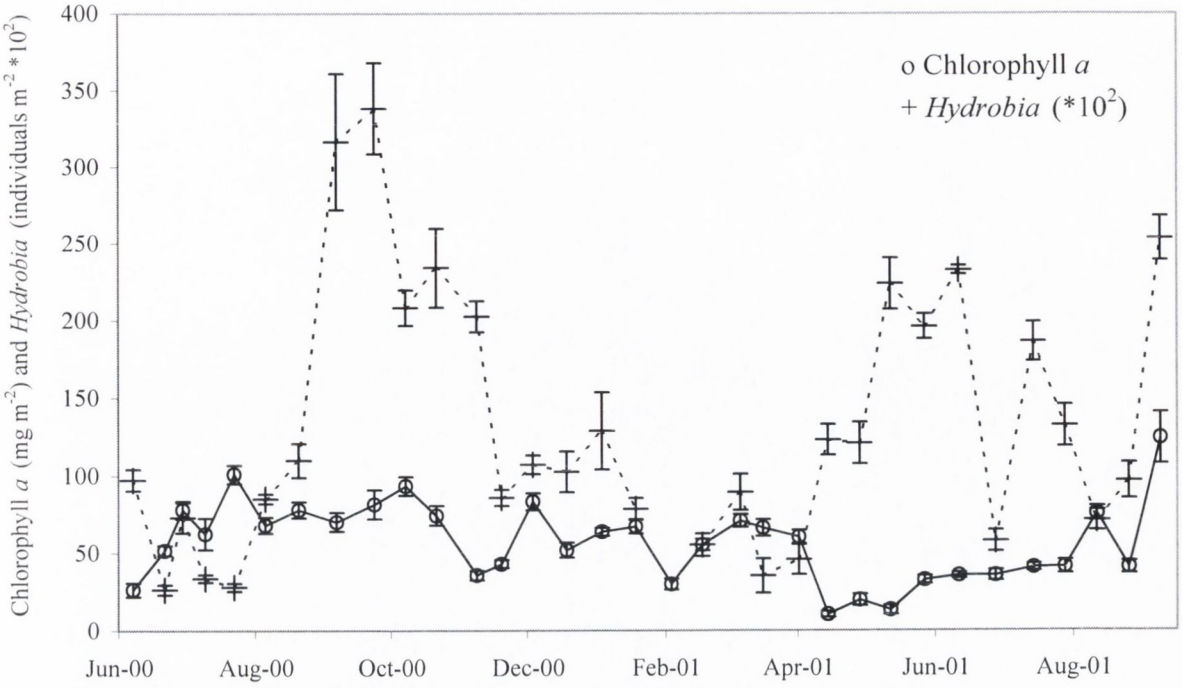


Figure 3.5. Mean sediment chlorophyll *a* concentration (mg m^{-2} ±S.E.) (N=577) and mean abundance of *Hydrobia ulvae* (m^{-2} ±S.E.) (N=30,313) at Bull Island during the period June 2000 to September 2001.

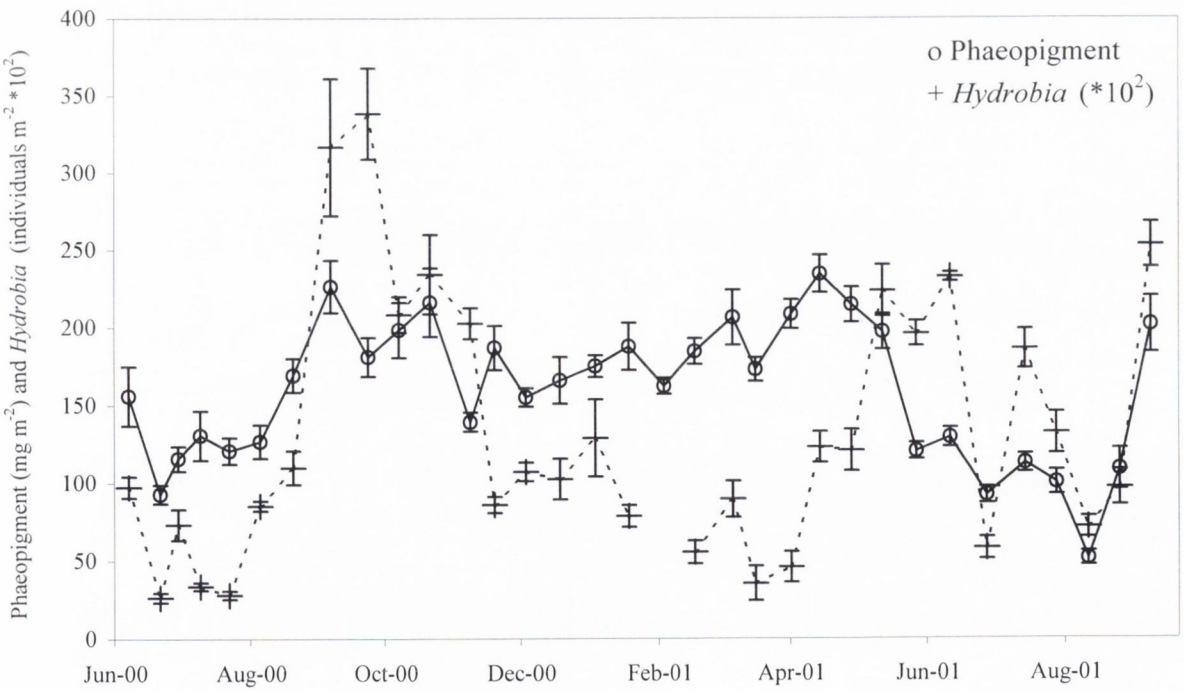


Figure 3.6. Mean sediment phaeopigment concentration (mg m^{-2} ± S.E.) (N=577) and mean abundance of *Hydrobia ulvae* (m^{-2} ±S.E.) (N=30,313) at Bull Island during the period June 2000 to September 2001.

33,831±2,965 individuals m^{-2}. A decline in numbers was then observed, falling to <10,000 individuals m^{-2} by November 2000. Densities remained low until May 2001 when early spring recruitment caused an increase in densities to >20,000 m^{-2}. Densities decreased again over the summer months to a low of 7,000m^{-2} in August 2001. In September 2001 another large increase in density occurred, again being associated with a large autumn recruitment pulse. Scheffe post-hoc tests (Appendix 3.4) showed that abundance on June 21st 2000 was significantly less than a large proportion of other dates throughout this sampling period, while those recorded in September 2000 and 2001 were significantly higher.

There was no statistically significant correlation between *Hydrobia* abundance and chlorophyll *a*, and similar trends were apparent only during parts of the sampling period (Figure 3.5). When chlorophyll *a* showed a general rise in concentration during late summer 2000, *Hydrobia* abundance also increased significantly. Both variables declined towards the end of 2000, although the former was quite variable. Opposing trends were apparent at other times of the year however, which resulted in the lack of significant correlation. In April 2001 sediment chlorophyll *a* content decreased significantly as *Hydrobia* densities increased. Over the following summer months chlorophyll *a* increased while *Hydrobia* abundance declined. Recruitment of *Hydrobia* juveniles into the population occurs during April and September so highest densities would be expected at these times regardless of chlorophyll *a* concentration. The decrease in chlorophyll *a* content in April could be due to the high number of recruits present and a similar occurance in September could be due to the recruitment pulse. Despite the application of lag phases of 24h, 48h, 2 or 4 weeks to the datasets, with each variable being interchanged to precede the other (top-down or bottom-up regulation), no significant correlations could be determined.

A positive correlation with phaeopigment concentration and *Hydrobia* abundance was recorded. Similar trends were observed during a much of the sampling period (Figure 3.6) with increasing *Hydrobia* densities generally corresponding to an increase in sediment phaeopigment concentrations although again increases in *Hydrobia* density corresponded to the recruitment pulses in April and September. The chlorophyll/phaeopigment ratio did not correlate with *Hydrobia* abundance over the temporal sampling period however. No relationship was determined between

phaeopigment content or the chlorophyll/phaeopigment ratio and *Hydrobia* abundance recorded on the day of sampling or after lag phases of 24h, 48h, 2 or 4 weeks were applied to the data.

Although a pattern appeared to exist between *Hydrobia* abundance and seawater temperature (Figure 3.7), its alternating nature resulted in no statistically significant correlation occurring. Water temperature had a peak (17°C) in late August 2000, declining over the following months to a low of < 6°C in January/February 2001 (Figure 3.7). For the 6 months after this minimum value the water temperature in Dublin Bay increased until August 2001 (15.8°C) when again it began decline. The decreasing temperature from August 2000 until the end of the year corresponded with significant reductions in *Hydrobia* abundance. Although the decline in *Hydrobia* numbers at this time most likely results from high mortality in the juvenile recruits temperature is also likely a major factor. In 2000, highest densities of *Hydrobia* were recorded after water temperature had begun to decrease, due to the autumn settlement of larger number of small individuals. As water temperature increased again in spring 2001, so did the density of *Hydrobia*, as this corresponded with the spring settlement event. When temperature began to decline in late summer 2001 the numbers of *Hydrobia* recorded began to increase, again due to a settlement event. There were also no significant correlations observed between *Hydrobia* abundance and other environmental parameters tested on the day of sampling. No relationships were determined after lag periods of 24h, 48h, 2 or 4 weeks was applied to the data.

The mean (±S.E.) abundance of 17110±1443 individuals m^{-2} *Hydrobia* recorded during the exercise conducted in April 2000 (Chapter 2), was far in excess of that recorded during April 2001 (9680±1102 individuals m^{-2}). The high abundance of the former was due primarily to the earlier establishment of a numerically strong settlement cohort, but spatial variation in sampling locations would also be a factor.

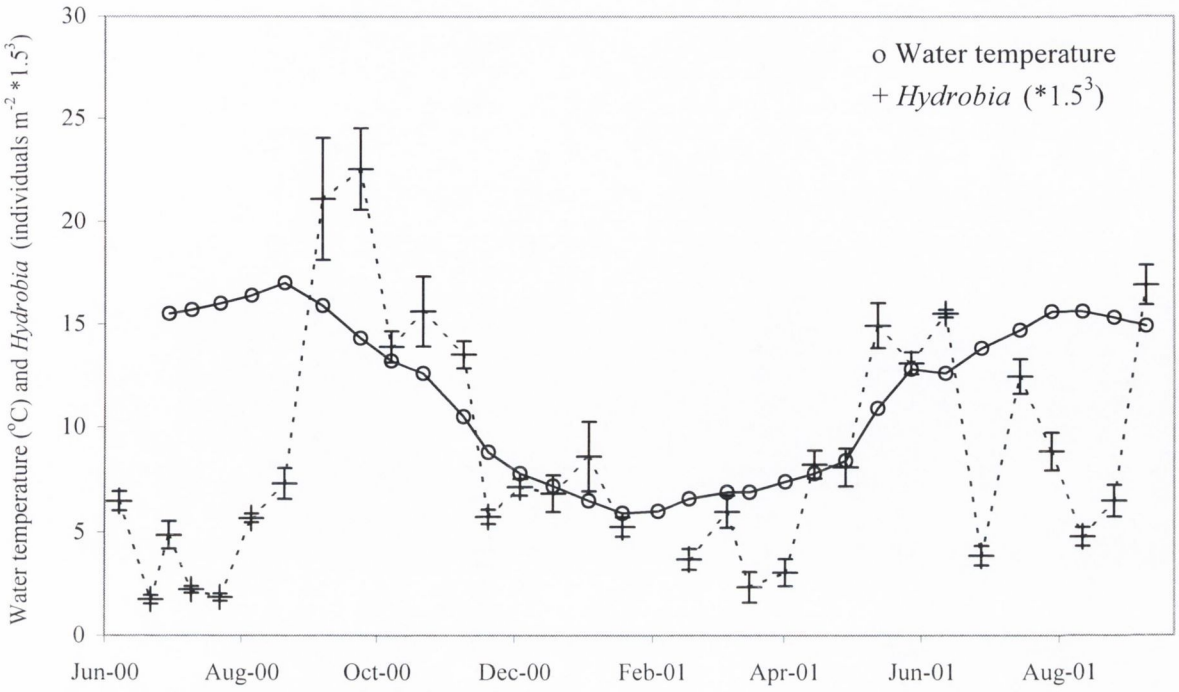


Figure 3.7. Mean (±S.E.) density of *Hydrobia ulvae* m^{-2} (N=30,313) at Bull Island and water temperature (°C) during the period June 2000 to September 2001. Temperature data courtesy of Dublin City Council.

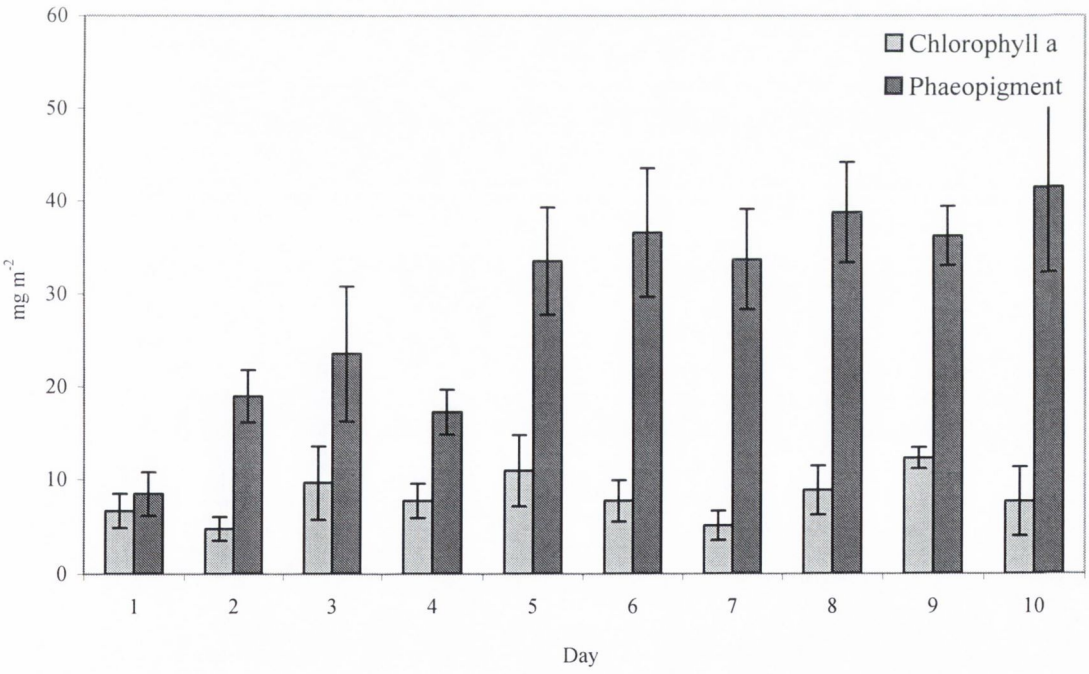


Figure 3.8. Mean chlorophyll *a* and phaeopigments (mg m^{-2} ± S.E.) on 10 consecutive days at Blackrock, April 2000. N=55.

3.3.2 Blackrock

3.3.2a Ten day consecutive sampling of photopigments

Average levels of sediment chlorophyll *a* determined at Blackrock during the 10 day period in April ranged from 4.8±1.3 to 12.3±1.1mg m^{-2} (Figure 3.8) with no significant differences occurring between-days (Kruskal Wallis, χ^2=3.4, df=9, p=0.21). Mean phaeopigment levels ranged from 8.5±2.3 to 41.5±9.2mg m^{-2} during the same period and significant between-day differences were observed (Kruskal Wallis, χ^2=29.1, df=9, p≤0.01) (Table 3.2). These pigment levels were generally less than values observed at Bull Island.

Table 3.2. Dunn post-hoc test of between-day comparison of mean phaeopigment concentration at Blackrock over a 10 day sampling period, April 2000. * indicates differences significant at ≤ 0.05 level.

Day	2	3	4	5	6	7	8	9	10
1	0.04*	0.02*	0.19	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
2		0.46	0.80	0.02*	≤0.01*	0.02*	≤0.01*	0.01*	≤0.01*
3			0.41	0.16	0.07	0.16	0.04*	0.09	0.03*
4				0.04*	0.01*	0.03	≤0.01*	0.02*	≤0.01*
5					0.67	0.98	0.46	0.72	0.33
6						0.69	0.75	0.96	0.55
7							0.48	0.73	0.34
8								0.74	0.74
9									0.54

The highest chlorophyll/phaeopigment ratio was recorded on day 1 at Blackrock (Figure 3.2). This maximum was significantly higher (Kruskal Wallis, χ^2=2.8, df=9, p≤0.01) than all other days but no other statistically significant differences between days were determined. For the 10 consecutive dates sampled during April 2000 chlorophyll/phaeopigment ratio was higher at Blackrock than Bull Island for all days except the seventh (Figure 3.2).

3.3.2b Bimonthly time-series sampling of photopigments

At Blackrock mean sediment chlorophyll *a* concentration ranged between 4.5±0.9 to 35.7±2.0mg m^{-2} over the sampling period (Figure 3.9). Lowest concentrations of chlorophyll *a* occurred during April and May of each year, with higher fluctuating values throughout the rest of the sampling period. The high values in August and

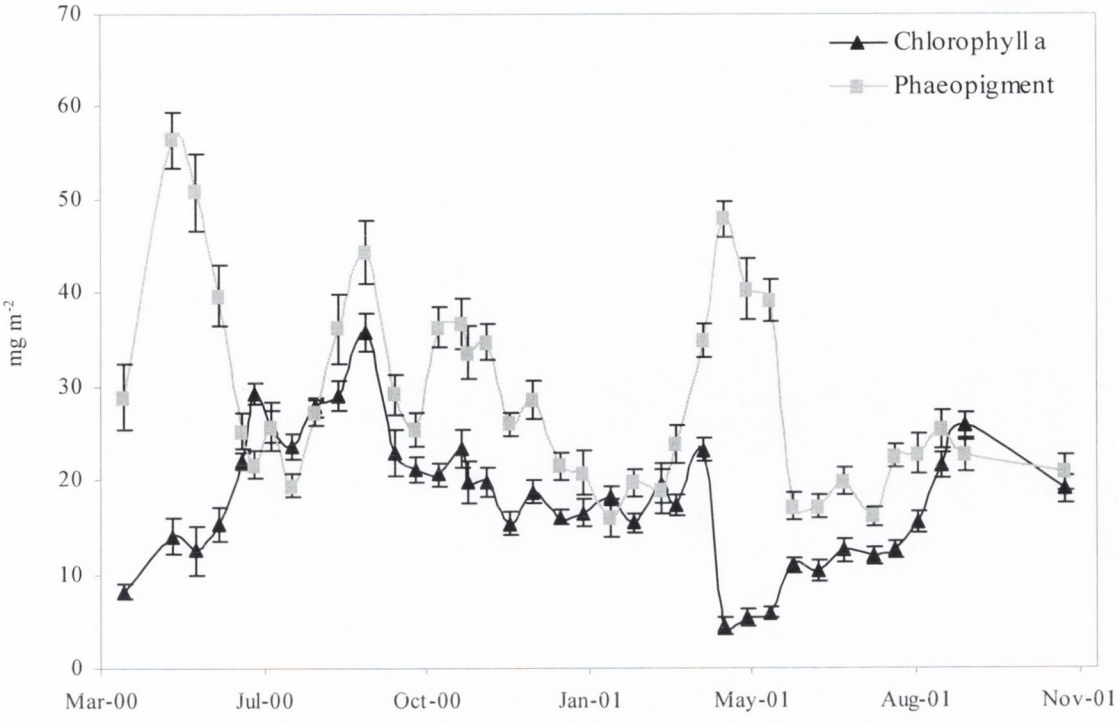


Figure 3.9. Mean sediment chlorophyll *a* and phaeopigments (mg m^{-2} ±S.E.) at Blackrock from May 2000 to October 2001. N=513.

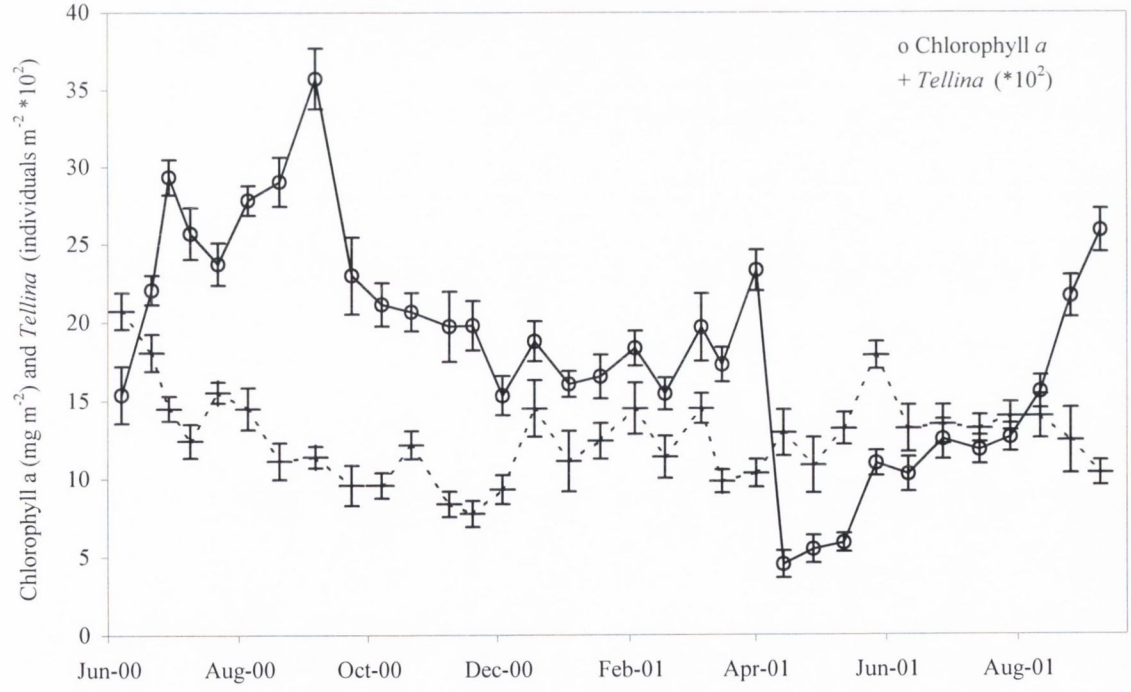


Figure 3.10. Mean sediment chlorophyll *a* concentration (mg m^{-2} ±S.E.) (N=513) and mean abundance of *Tellina tenuis* (m^{-2} ±S.E.) (N=1437) at Blackrock during the period June 2000 to September 2001.

September 2000 were significantly different to a number of other sampling dates (ANOVA, F=24.5, df=35, $p \leq 0.01$) (Appendix 3.5). Scheffe post-hoc tests also showed that the low values in April 2001 showed statistically significant differences to many other sampling events (Appendix 3.5). Chlorophyll *a* concentration was positively correlated with water temperature on the day of sampling at Blackrock but not with any other environmental parameter tested. No relationships were determined after lag periods of 24h, 48h, 2 or 4 weeks were applied to the data.

Phaeopigment concentrations were generally higher than chlorophyll *a* levels, the disparity being more pronounced at times of the year when chlorophyll *a* values were lower (Figure 3.9). Mean sediment phaeopigment content ranged from 16.0±2.1 to 56.4±2.9 mg m^{-2}. There were not as many significant differences between-dates (Appendix 3.6) as with chlorophyll *a,* although May 2000 had significantly (ANOVA, F=21.4, df=35, $p \leq 0.01$) less sediment phaeopigment concentration than most other sampling dates. No significant correlations were observed between phaeopigment concentration and any of the environmental variables examined on the day recorded or after lag periods of 24h, 48h, 2 weeks or 1 month were applied to the data.

The chlorophyll *a*/phaeopigment ratio at Blackrock varied during the sampling period (Figure 3.6). The ratio was lowest during early summer (April and May) in both 2000 and 2001, although only the concentration recorded during April 2001 was significantly different and lower than other values (ANOVA, F=8.6, df=35, $p \leq 0.01$) (Appendix 3.7). Highest ratios occurred at this site in late June and July 2000, February and March 2001 and September 2001. The chlorophyll *a*/phaeopigment ratio did not correlate significantly with any of the environmental variables recorded on the day of sampling or after the application of the lag phases.

The location of the study area for this investigation corresponded to shore level 4 in the large-scale spatial trial conducted during April 2000 (see section 2.2.6), where an average chlorophyll *a* concentration of 29.0±1.4mg m^{-2} (±S.E.) was recorded. This value was toward the upper range of mean chlorophyll *a* concentrations recorded at Blackrock during all periods in this study. Although a similar value of 23.3±1.3mg m^{-2} was recorded at the beginning of April 2001, levels had dropped to 4.5±0.9mg m^{-2}

by mid April 2001. The average values of 27.8±1.9, 58.0±0.4 and 61.2±1.1mg m^{-2} recorded in January, February and March 2000, respectively, were generally higher than those recorded in the corresponding months in 2001, when all values recorded were below 20mg m^{-2}. Average phaeopigment values of 4.5±0.7, 4.5±0.6, 3.8±0.7 and 4.5±0.2mg m^{-2} were recorded in 2000 for experiments 2.2.5a, b, c and 2.2.6, respectively. For the corresponding months of January, February, March and April in 2001 higher concentrations up to 47.9±1.9 were observed. The chlorophyll *a*/phaeopigment ratios recorded during the temporal sampling period did not correspond to values determined in Chapter 2. The average ratios observed in the spatial variation experiments of up to 9.1 were much higher than the ratios of <1.5 recorded in the current study.

3.3.2c Bimonthly time-series of Tellina abundance

Mean abundance of *Tellina* (Figure 3.10) varied significantly on different sampling occasions (ANOVA, F=5.4, df=30, $p \leq 0.01$) within the selected study area. Scheffe post-hoc tests, however, revealed that differences occurred between June 7th 2000 and November 19th 2000 only. Density ranged between 1,000–1,500 individuals m^{-2} on most sampling occasions. Highest abundance of *Tellina* occurred during June and July 2000 and at the end of May 2001, with mean density >1,500 individuals m^{-2} recorded. Densities <1,000 m^{-2} were recorded on 6 occasions, generally in the late autumn and winter of 2000. An overall trend in abundance appeared evident, despite variation between subsequent sampling dates. At the beginning of the sampling period in June 2000 densities of *Tellina* fluctuated but eventually decreased from the end of June (2072±117m^{-2}) until late November 2000 (777±84m^{-2}). From December 2000 until March 2001 abundance fluctuated between approximately 1,000 and 1,500 individuals m^{-2}. Abundance then increased until the end of May 2001, when *Tellina* density reached its second highest peak (1,787±87 m^{-2}) of the sampling period. A gradual decline was observed until the end of the sampling period.

There was no clearly identifiable trend between *Tellina* abundance and chlorophyll *a* (Figure 3.10). No obvious seasonal pattern in the abundance of *Tellina* could be attributed to cohort periodicity or juvenile recruitment. Although this species can spawn from March until September peak spawning occurs in July/August, no

evidence of settlement was recorded at any period of the year. Chlorophyll *a* increased from the start of the sampling period to a high of 35.7±11.4mg m^{-2} on September 6th 2000. During the same period *Tellina* gradually decreased in abundance. Both variables fluctuated in late 2000 and early 2001, until chlorophyll *a* concentrations decreased sharply in April 2001 as *Tellina* densities remained relatively constant. By the end of the sampling period chlorophyll *a* was increasing in concentration and *Tellina* abundance appeared to be declining. There was no relationship between *Tellina* abundance and chlorophyll *a* on the day of sampling or after a lag period of 24h, 48h, 2 weeks or 1 month. There was also no statistically significant correlation between *Tellina* abundance and phaeopigment concentration (Figure 3.11). The chlorophyll/phaeopigment ratio at Blackrock did not correlate with *Tellina* abundance. No relationship was determined between *Tellina* abundance and either phaeopigment content or the chlorophyll/phaeopigment ratio on the day of sampling or after a lag period of 1,2 or 4 weeks.

As water temperature decreased from its maximum of 17°C in late August, a general decline in *Tellina* abundance was also observed until late November (Figure 3.12). Although water temperature continued to decrease after this date, *Tellina* abundance remained stable until the end of the year. The water temperature increased from a low of 5.9°C in January 2001 and reached 12.8°C by the end of May when *Tellina* density had reached a peak of 1,787±87individuals m^{-2} (N=70). As water temperature began to decline from 15.6°C in August 2001 a corresponding decline was observed in *Tellina* densities. No statistically significant correlation between *Tellina* density and water temperature were determined, but this was unsurprising considering the oscillating nature of the parameters. *Tellina* abundance was positively correlated with air temperature and average irradiance recorded on the day of sampling but not any other environmental parameter tested. No significant correlations were determined after lag periods of 24h, 48h, 2 or 4 weeks.

The mean (±S.E.) abundance of *Tellina* recorded during the exercise conducted in April 2000 (Chapter 2), 1080±31 individuals m^{-2}, was comparable to that recorded during April 2001 (1140±138 individuals m^{-2}).

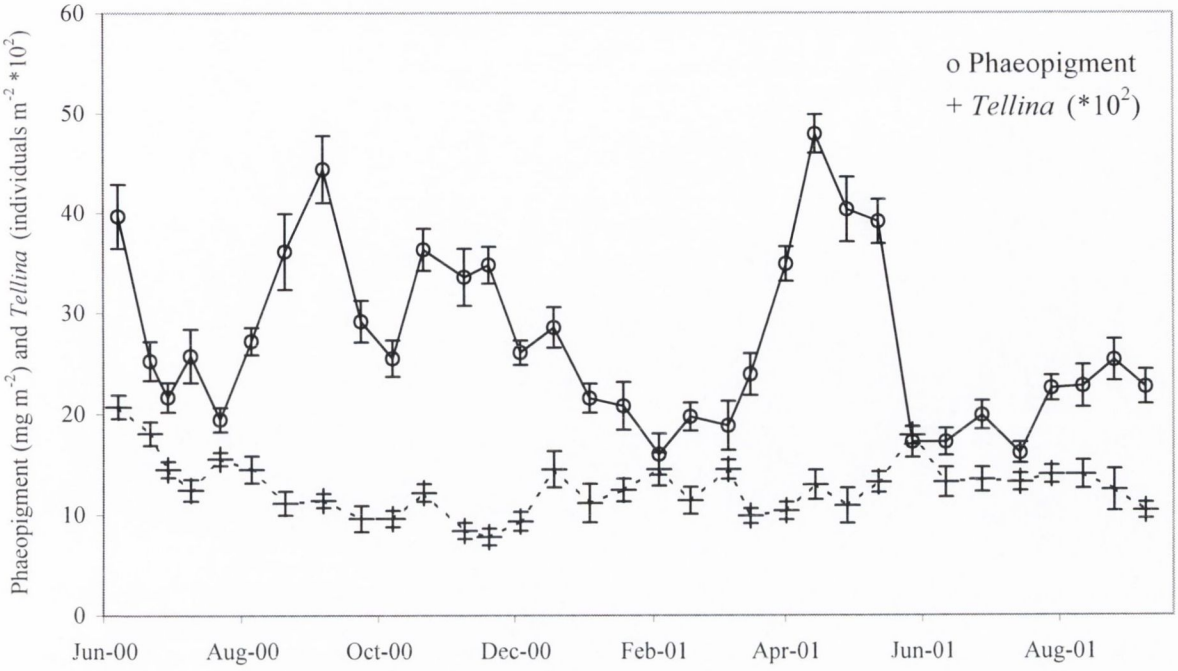


Figure 3.11. Mean sediment phaeopigment concentration (mg m^{-2} ±S.E.) (N=513) and mean abundance of *Tellina tenuis* (m^{-2} ±S.E.) (N=1437) at Blackrock during the period June 2000 to September 2001.

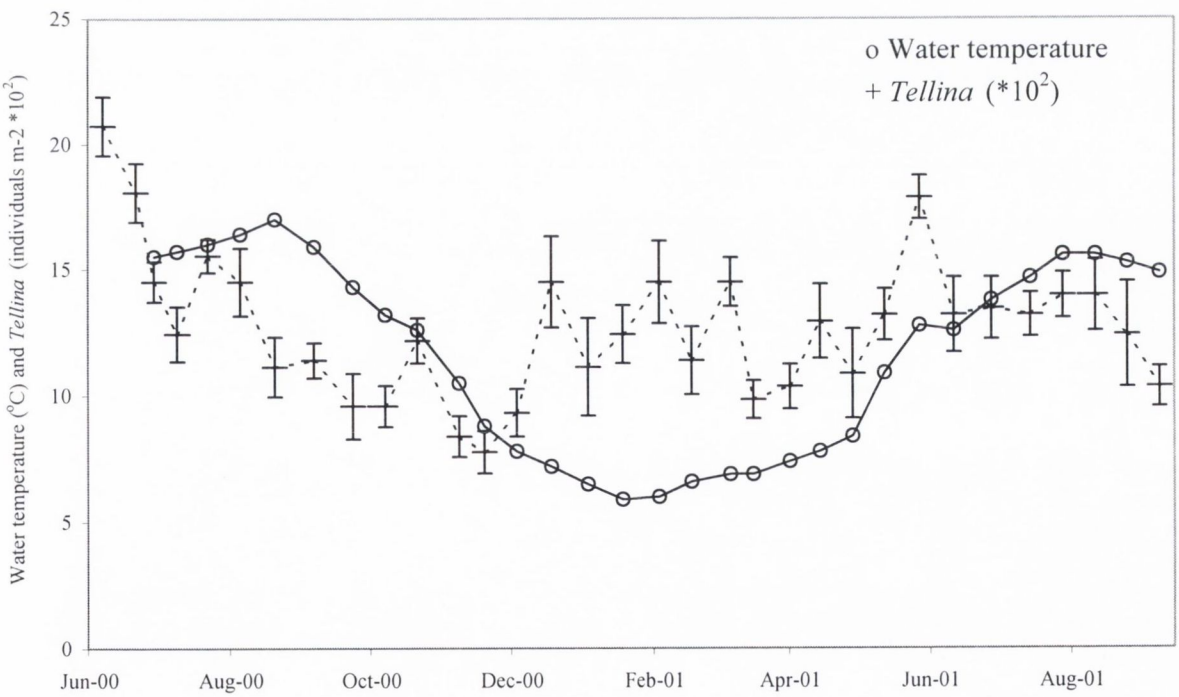


Figure 3.12. Density (±S.E.) of *Tellina tenuis* m^{-2} (N=1437) and water temperature (°C) at Blackrock during the period June 2000 to September 2001. Water temperature data courtesy of Dublin City Council.

3.3.3 Bimonthly sampling-comparison between sites

Although chlorophyll *a* concentrations were significantly higher at Bull Island than Blackrock over the bimonthly time-series sampling period (T-test, t=36.8, df=2036, $p \leq 0.01$), seasonal trends were similar and significantly correlated (Pearson-product +0.63, $p \leq 0.01$). A similar significant (Pearson-product +0.60, $p \leq 0.01$) correlation was observed between-sites for phaeopigment concentration, this again being higher at the Bull Island site (T-test, t =36.5, df=2036,$p \leq 0.01$). Although higher at the Blackrock site, the chlorophyll/phaeopigment ratio was also significantly correlated with that recorded at the Bull Island site.

Mean (±95% C.I.) primary productivity of the microphytobenthos over the temporal sampling period, calculated using the equation of Colijn & de Jonge (1984), was 40.4±6.0 and 16.2±1.5g C m^{-2} for Bull Island and Blackrock, respectively. Although the values at Bull Island were higher than at Blackrock they were also more variable with a range of 9.2 to 87.6 compared with 7.0 to 28.0g C m^{-2} at Blackrock. Highest productivity values corresponded with high chlorophyll *a* concentrations, which generally occurred during the summer months at both sites.

3.4 Discussion

Wide seasonal fluctuations in both photopigment concentration and primary consumer abundance were recorded at the study site. Similar trends in chlorophyll *a* and consumer abundance appeared to occur during the winter months, while the opposite were generally apparent in the spring and summer. Although this may suggest that alternating periods of bottom-up and top-down regulation of primary producers and consumers may occur at the sites, no firm conclusions can be made. Although underlying trends appeared to occur, the changes were often masked by high variance (Hassell, 1987). Rossi (2002) recorded similar short-term fluctuations in sediment chlorophyll *a*. The use of random samples in the presence of high heterogeneity can lead to large sampling variances that can make seasonal and annual differences difficult to detect (Bowman & Lewis, 1977). Any oscillating primary producer-consumer relationship would probably be obscured by the influence of numerous physical and biological parameters, the individual and combined action of which are yet to be adequately described at the study sites. For example, the re-suspension of

microphytobenthos and input of 'new' phytoplankton biomass on each tidal cycle has been shown to result in considerable variability in chlorophyll *a* (Miller *et al.*, 1996). Although the factors regulating photopigment concentration and primary consumer abundance are poorly understood, this study suggests that further medium to long-term monitoring may reveal the community dynamics at these sites.

Some previous studies have detected no short-term daily fluctuations in chlorophyll *a* between tidal events (Hargrave *et al.*, 1983). The variability in photopigment concentration observed in the current study over a 10 day period was probably a result of the deposition and re-suspension of phytoplankton rather than significant environmentally driven changes in the microphytobenthos. A high level of spatial and temporal variability in microphytobenthic biomass has been recorded previously (MacIntyre *et al.*, 1996; Guarini *et al.*, 2002), and in some cases medium-term cyclical patterns have been identified (Cole & Cloern, 1987). Similar to the present study, most microphytobenthic populations reach peak biomass in spring or summer (Cadee & Hegeman, 1977; Colijn & de Jonge, 1984; Sullivan & Moncreiff, 1988; Sundback & Jonsson; Gould & Gallagher, 1990; De Jonge & Beusekom, 1992; Pinckney & Zingmark, 1993a,b; Underwood & Paterson, 1993; Cariou-Le Gall & Blanchard, 1995; de Jong & de Jonge, 1995; Santos *et al.*, 1996). Some authors have also documented a second bloom of shorter duration in autumn (Cadee & Hegeman, 1977; Herndl *et al.*, 1989; Cammen, 1991; de Jong & de Jonge, 1995). Although the fluctuations recorded in the current study hampered clear isolation of seasonal patterns, a rise in sediment chlorophyll *a* was also observed in autumn at both sites. Similarly, low chlorophyll *a* concentrations observed as those in April/May have been reported previously (Gould & Gallagher, 1990; Cammen, 1991). Although spatial and temporal variation in sediment photopigment concentration is a common occurrence in many intertidal flats, some studies report no significant differences over periods of up to a year (Cadee & Hegeman, 1974; Varela & Penas, 1985; de Sousa *et al.*, 1998 Conde *et al.*, 1999).

Phaeopigment concentrations have been recorded as being both higher (Santos *et al.*, 1996) and lower (Cadee & Hegeman, 1974) than sediment chlorophyll *a* concentrations, and no clear or universal trend has been identified between these variables. Sediment phaeopigment concentrations have also been observed to follow

the same pattern as chlorophyll *a* and be of similar magnitude (Cadee & Hegeman, 1977; Sundback & Jonsson, 1988; Cammen, 1991; Pinckney & Zingmark, 1993; Galois *et al.*, 2000), although this was not the case in the current study. Phaeopigment concentrations were generally higher than chlorophyll *a* concentrations at both sites although the difference was more pronounced at the Bull Island mudflat. The presence of primary consumers has been shown to increase sediment phaeopigment content under laboratory conditions (Ingalls *et al.*, 2000; Cartaxana *et al.*, 2003). It is generally assumed that the grazing activity and subsequent egestion by the species concerned is the cause of this increase, although disturbance through significant locomotory activity of mobile species has also been postulated to contribute to the degradation of chlorophyll *a* by perturbation of the sediment surface. Although the abundance of *Hydrobia* correlated with the concentration of phaeopigment in the current study, a direct causative relationship was not established in the absence of supporting experimental data, particularly as no clear related correlation with chlorophyll *a* concentration was observed. Due to the large number of variables that can affect abundance and grazing rates it is unlikely that a simple correlation between food available and abundance would occur. The lower relative abundance and sedentary nature of *Tellina* may have accounted for the lack of correlation with phaeopigment, although the degradation of phaeopigment into by-products by detritivores and bacteria or the grazing action of other consumer species may decouple any relationship with the abundance of primary consumers.

The large disparities in photopigment concentration and the abundance of *Hydrobia* between the spatial sampling conducted in early 2000 and that recorded in the corresponding months of the following year were an indication of the high degree of inter-annual variability that can occur (Connell, 1961; Bowman & Lewis, 1977; Fogarty *et al.*, 1991). The winter of 2000 was much milder than that of 2001, with generally higher environmental temperature and irradiance. Elevated chlorophyll *a* and lower phaeopigment concentration during the early months of 2000 when compared with 2001, suggested that a higher degree of active photosynthesis had occurred earlier in 2000. Reduced duration of planktonic development in the warmer waters of 2000 probably accounted for the disparity between *Hydrobia* abundance estimates between years. Although settlement of young of the year individuals was well established during April 2000, it was only just beginning in the same period

during 2001. The earlier recruitment of 2000 may also have been a result of earlier reproduction and larval release by adults in response to elevated temperatures and/or food availability. Many marine invertebrate species are known to synchronise larval release to times when food is plentiful, particularly when larvae are planktotrophic (Rumrill, 1990; Robinson & Tully, 2000a). Alternatively, the lower number of settlers recorded during 2001 may have been due to inter-annual variability in recruitment success. This is an important regulator of population size for many marine invertebrate species, particularly those that are habitat-specific and/or inhabit the intertidal zone (Roughgarden *et al.*, 1988). Variability in planktonic phase mortality is known to be linked to environmental factors such as temperature, food limitation and predator abundance (Pechenik, 1987; Rumrill, 1990). Physical transport mechanisms can remove individuals to unfavourable locations (Gaines *et al.*, 1985; Caley *et al.*, 1996). Although most larvae have mechanisms to maximise the probability of encountering a favourable habitat, the most severe being direct development to the benthic form to avoid planktonic dispersal completely, many are forced to settle onto sub-optimal habitats where they cannot persist (Rumrill, 1990; Robinson & Tully, 2000b). Although the similar abundances recorded for *Tellina* between years may suggest that population numbers are more stable for this larger, longer lived species, regular recruitment failure have been observed in other populations (Stephen 1928; McIntyre, 1970; Barnett & Watson, 1986; Wilson, 1996; Dekker & Beukema, 1999).

The correlation analyses applied in the current study were suitable only for identifying linear trends between data sets and were particularly sensitive to inherent variability, common to biological datasets. Seasonal trends and relationships between variables often conform to asynchronous oscillating functions (Robinson & Tully, 2000c), but often require long datasets to be formally identified and described (Genner *et al.*, 2004). The time-series dataset presented in the current study was relatively short in duration, and was not of sufficient length to warrant the application of formal time-series analysis techniques. These are useful tools for identifying repeating trends of varying irregularity. Detection of autocorrelation, the relationship between previous and subsequent observations within a time-series dataset over varying lag periods, serves firstly to identify non-randomness in data and then to define the appropriate model to apply if patterns are detected. The data presented in the current study

suggested that there were probably repeating patterns in the abundance of both photopigments and primary consumers, although these were only described subjectively. If the primary aim of the study had been to identify and quantify inter-annual variation in abundance then a longer time-series of data would have been collected, smoothed and subjected to formal analysis. Data were not smoothed in the figures detailed in this study, as the actual between-sampling data variability in the parameters studied was considered important for planning a future monitoring programme. Smoothing the data did show possible seasonal patterns, especially in the abundances of both *Hydrobia* and *Tellina,* a longer time series would be necessary to confirm it. In the chlorophyll concentrations at both sites the only pattern was the low values in April followed by an increase and further data would be required to determine patterns for the rest of the year.

Bimonthly sampling of primary producers and consumers was time consuming when the fieldwork and laboratory processing elements required were combined. It would not be feasible to maintain this level of monitoring continually without substantial support from a larger, well-equipped team. The time-series data presented suggested that a bimonthly sampling frequency would probably not be required for medium- to long-term monitoring at these sites. Monthly or quarterly sampling during winter, the spring settlement, midsummer and at the autumn settlement event would likely reveal sufficient information to determine seasonal trends in the abundance of both the primary consumers and photopigments when a sufficiently long time-series of information was gathered. Due to the wide inter-annual variations in the magnitude and timing of settlement and recruitment of marine invertebrates, it may be difficult to accurately quantify these processes by quarterly sampling. Robinson & Tully (2000) suggested that the very wide intra-annual fluctuations in growth and survival of benthic marine invertebrates during the summer months, especially of recently settled individuals, might indicate that the more stable over-wintering population would give a better indication of inter-annual variation in population size when long-term monitoring with low sampling frequency was required. Gosselin & Qian (1997) listed numerous studies where the population abundance, distribution and community structure where controlled or significantly influenced by juvenile mortality during the summer and autumn. The type, frequency and spatial extent of sampling will be dictated by the purpose of the monitoring programme itself.

Although the overall mean chlorophyll *a* concentrations determined at Bull Island and Blackrock, 54.3±4.4 and 18.1±1.1 mg m^{-2} (±S.E.) respectively, were comparable to previously published values for intertidal marine and estuarine habitats, they were towards the lower end of the range (Colijn & de Jonge, 1984; Heip *et al.*, 1995; MacIntyre *et al.*, 1996, Underwood & Krompkamp, 1999). MacIntyre *et al.*, (1996) collated data from 25 studies worldwide and reported ranges from <1 to 560 mg m^{-2}. These data related principally to mudflats, but some sandflats were also included. Some of the variation between sites was related to the depth of sediment sampled, with depths of 2mm, 5mm and 10mm the most common. Although active chlorophyll *a* has been found in sediments up to 12cm deep (Joint, 1978; Barranguet *et al.,* 1997) active photosynthesis by microphytobenthos only occurs in illuminated sediment, which is restricted to the top 2-3mm in most sediments types (MacIntyre *et al.*, 1996; Paterson *et al.*, 1998). Although benthic algae migrate vertically to the surface when sediment is exposed at low tide and descend before it's flooded (Palmer & Round, 1965; Cadee & Hegeman, 1974), most recent studies have sampled to a depth of 5mm as migration in and out of the photic zone is limited to this depth (Baillie & Welsh, 1980; Pinckney & Zingmark, 1993b). The seasonal timing and frequency of sampling, which could also have impacted on the mean concentrations observed, also varied between studies.

Exposure to waves and strong currents generally increases the mean size of sediment grains at a habitat and decreases benthic algal production. As a result, the clay/silt content of sediments has been shown to correlate positively with microalgae production (Heip *et al.*, 1995; Santos *et al.*, 1996), with mudflats generally supporting higher sediment chlorophyll *a* concentrations than sandflats (Cadee & Hegeman, 1977; Hargrave *et al.*, 1983; Delgado, 1989). Exceptions, to this general rule are often associated with nutrient enrichment (Cammen, 1991; Pinckney & Zingmark, 1993). The Bull Island mudflat contained higher levels of chlorophyll *a* than the Blackrock sandflat. Phaeopigments were also higher at Bull Island than Blackrock. Higher phaeopigment concentrations have been recorded previously in mudflats compared with nearby sandflats (Pinckney & Zingmark, 1993). The resultant increase chlorophyll *a*/phaeopigment ratios at the Blackrock site indicated higher percentages of average active chlorophyll *a* (38%) to total sediment pigments when

compared with Bull Island (25%). These values were higher than recorded on intertidal flats in Spain (10 to 20%, Varela & Penas, 1985), but lower than on the east coast of America (34-80%, Cammen, 1991). The differences between-sites in the current study may have suggested that either chlorophyll *a* degraded more rapidly at the Bull Island site but passed through the detrital food chain more slowly, that other sources of detritus where deposited into the habitat, or that physical or biological action removed detritus more efficiently from the Blackrock site (Riedle, 1971). The chlorophyll *a*/phaeopigment ratio can be influenced strongly by the input of algal detritus (Cadee & Hegeman, 1977), with low ratios generally being associated with high deposition. As a result chlorophyll *a* to phaeopigment ratios are usually higher in more dynamic sand environments compared with depositional mudflats (Delgado, 1989; Koster & Meyer-Reil, 2001). Although differences in photopigment concentration were observed between-sites, seasonal trends in chlorophyll *a* and phaeopigment were similar. This would suggest that some larger-scaled environmental processes impose similar influences on all habitats within the Bay, but that the responses remain habitat-specific.

The microphytobenthos are a preferred food source for deposit feeding macrofauna (Miller *et al.*, 1996), and a number of studies have recorded a direct impact by particular species on chlorophyll *a* concentrations (Morrisey, 1988b; Miller *et al.*, 1996; Ingalls *et al.*, 2000). Gould & Gallagher (1990) suggested that a spring increase in the population of grazing organisms significantly affected biomass due to the initially slow growth of diatoms after the over-wintering period. However, Miller *et al.*, (1996) stated that although deposit feeders can reduce microphytobenthos abundance, in many cases growth and transport are sufficient to prevent biomass declines to values less than ~50% of ungrazed controls. In the current study, the highest abundance of *Hydrobia* recorded during autumn 2000 did not coincide with a decline in benthic chlorophyll *a* concentration, while the rapid increase in *Hydrobia* that occurred in the spring did. Chlorophyll *a* also declined dramatically at the Blackrock site during the same period, although a substantial increase in *Tellina* densities was not recorded. Although direct evidence for top-down regulation of microphytobenthos was not determined in the present study, the data would suggest agreement with the findings of Gould & Gallagher (1990) and further investigation is warranted.

Fluctuations in the populations of the two primary consumer species studied were generally less pronounced during the winter periods when temperatures were lower. It is generally accepted that as the metabolism of marine invertebrates decreases, within the boundaries of their thermal tolerance, growth and mortality also decrease. It was not possible to determine a direct relationship between temperature and the population size of the primary consumers studied. Although temperature will affect population size indirectly through changes in development and activity rates of the species, and their competitors, they are well adapted to tolerate seasonal as well as daily changes in the environmental parameters such as temperature (McLusky, 1989). It is likely that wide ranges of interacting environmental parameters elicit various influences on the population size of the primary consumers studied, and these combined, may mask the influence of each in isolation.

The abundance of *Hydrobia* observed in the current study at Bull Island was similar to that described for other populations previously. Although some degree of variation occurred it was not clear whether this was due to geographic, temporal or methodological criteria. Sola (1996) recorded densities between 1,500-19,000 individuals m^{-2} in a Spanish estuary, while Haubois *et al.*, (2004) observed maximum densities of 16,000m^{-2}. Barnes (1998) recorded similar maximum densities of 15,000m^{-2} in England. Although densities up to 288,000m^{-2} (Lillebo *et al.*, 1999) and 294,667m^{-2} (Cardosa *et al.*, 2002) were recorded at two sites in Portugal the authors did not distinguish between live animals and the empty shell casings of previous mortalities. It is highly probable that these results were strongly erroneous considering the long persistence of *Hydrobia* shells in the environment after death. The abundances recorded in the current study would have been many times higher under similar circumstances. Drake & Arias (1995) recorded very low densities of between 185-353individuals m^{-2} on mudflat in Spain. Such low figures in comparison with other studies may indicate either previous or current sub-optimal habitat conditions at this site.

The abundances of *Tellina* recorded in the current study were high compared with most published values across the geographical distribution of this species, although temporal changes cannot be discounted considering the age of some survey data. The

highest published densities were recorded in Scotland at almost 8,000 individuals m^{-2} (Stephen, 1928), although values of this magnitude have not been recorded in recent years. In a study of several populations in different sites around Scotland the maximum densities observed were <800m^{-2} (McIntyre, 1970). Dekker & Beukema (1999) recorded densities <100indivdiauls m^{-2} in the Netherlands over a 30 year period. Previously surveys of the *Tellina* population examined in Dublin Bay by Wilson (1996) observed densities of approximately 500 individuals m^{-2} over a sampling period of 20 years, although sampling was only conducted once annually and the exact period not stated. A previous study of the same population based on monthly samples taken over a 1 year period recorded densities <400 individuals m^{-2} (Wilson, 1995). In the current study the lowest density recorded (770m^{-2}) was considerably higher than reported at any time previously by Wilson (1995;1996). Unless major changes in the population size occurred between the period between 1996 and 2000 differences in sampling methodology must have accounted for these differences. For example, in the current study a sieve with mesh size 0.5mm was used compared with 1.0mm by Wilson (1995; 1996). Dekker & Beukema (1999) showed that the latter failed to adequately sample the smaller size classes. Small sized individuals were not, however, a major component of the population at the Blackrock site in the present study however. As Wilson (1995, 1996) gave no indication of the shore level sampled, spatial variation in abundance may have accounted in part for the disparity.

The productivity estimates for the two sites in the current study of 40.4±6.0g C m^{-2} y^{-1} (±C.I.) and 16.2±1.5g C m^{-2} y^{-1} for Bull Island and Blackrock, respectively, were toward the lower end of the range of values reported in the literature (Colijn & de Jonge, 1984, MacIntyre *et al.*, 1996, Underwood & Kromkamp, 1999), and significantly less than the overall mean worldwide estimate of 100g C m^{-2} y^{-1} cited for microphytobenthic production (Charpy-Roubaud & Sournia, 1990). The primary controlling factors of autotrophic production are the biomass of the algal populations (Cadee & Hegeman, 1974; Colijn & de Jonge, 1984; Cole & Cloern, 1987) and light availability (Cole & Cloern, 1987; Guarini *et al.*, 2002). Factors such as nutrient availability and salinity gradients are probably of secondary importance to the microphytobenthos as nutrient concentrations are high in estuarine sediments (Jeffrey *et al.*, 1995; Goto *et al.*, 1998), and salinity tolerances of benthic microalgae species

have been recorded in the range 0 to 40‰ (Admiraal, 1977). Seasonality in sediment chlorophyll *a* has been positively related to temperature in a number of studies (Colijn & de Jonge, 1984), but no clear relationship has been reported in others (Goto *et al.*, 1998). Although the photosynthetic rate of microphytobenthos can double with a 10°C increase in temperature (MacIntyre *et al.*, 1996; Underwood & Krompkamp, 1999) it has been shown to remain constant despite temperature decreases (Goto *et al.*, 1998). This illustrates how the relationship between environmental production and environmental parameters can be complex. The geographic latitude at which the current and previous studies were conducted could also account for variations in productivity estimates. The majority of microphytobenthic primary production studies in temperate environments have been conducted at lower latitudes than the current study. Studies at higher latitudes than Dublin Bay, for example in Scotland, produced lower productivity estimates of 31g C m^{-2} y^{-1} for mudflats (Leach, 1970) and 4-9g C m^{-2} y^{-1} for a sandflat (Steele & Baird, 1968). Studies conducted at higher latitudes with greater production have generally been influenced by heavy anthropogenic enrichment. Relatively low microphytobenthic biomass (measured as chlorophyll *a*) was recorded at both sites in the present study and, therefore, low productivity levels were expected. Although most studies have found a relationship between primary productivity and chlorophyll *a* (microphytobenthic biomass), this is not always the case. For example high sediment chlorophyll *a* levels of 400 to 600mg m^{-2} corresponded to low annual production values of 31g C m^{-2} y^{-1} in a Scottish mudflat (Leach, 1970). Higher productivity rates have also been recorded with tidal elevation as longer durations of light availability allow the benthic algae to photosynthesise for longer periods (Heip *et al.*, 1995; Guarini *et al.*, 2002). It is possible that the conversion equation of Colijn & de Jonge (1984) used to convert chlorophyll *a* concentrations recorded at Bull Island and Blackrock to primary productivity was not appropriate for application to either site. Although it was not feasible to measure primary productivity directly in the current study, the environment in the Netherlands where the equation was derived would be considered similar to Dublin Bay. Despite the low productivity and microphytobenthic biomass in the intertidal system recorded, the primary producers support large numbers of consumer species. Further site-specific evaluation of the productivity of the study sites is required to gauge their relative importance in the energy and nutrient budgets of the bay.

Novel data pertaining to the seasonal abundance of two important primary consumers and photopigment concentration has been obtained during the current study. Although it is not yet possible to determine the factors regulating fluctuations in these parameters, either intra- or inter-annually, initial indications suggest that more medium- to long-term sampling could reveal much about the dynamics of the study sites. Although the relationship between primary producer and consumer abundance was not described, it appears that there is some potential for the use of consumers as indictor species. More prolonged data collection of biological and physical parameters at the site may permit the identification of causes of and responses to variations in the environment through time-series analysis. If achieved, such models can be used to predict future changes in the environment due to natural and anthropogenically induced perturbations.

Appendix 3.1. Scheffe post-hoc tests of differences in chlorophyll *a* concentration between sampling dates at Bull Island * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	1.0	1.0	0.9	1.0	0.1	1.0	1.0	1.0	0.8	0.1	1.0	1.0	1.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	*
24-May-00	2		1.0	1.0	0.5	1.0	*	0.9	0.6	1.0	0.3	*	0.6	1.0	1.0	0.1	1.0	1.0	0.9	1.0	1.0	0.7	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	*
07-Jun-00	3			1.0	0.2	1.0	*	0.8	0.3	0.9	0.1	*	0.2	1.0	1.0	*	1.0	0.9	0.7	1.0	1.0	0.4	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	*
21-Jun-00	4				1.0	1.0	0.4	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.9	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
29-Jun-00	5					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	*	*	*	0.5	0.7	0.7	0.9	0.9	1.0	0.9	0.4
09-Jul-00	6						1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.9	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
22-Jul-00	7							1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.1	1.0	0.5	1.0	1.0	*	0.7	1.0	1.0	0.9	*	*	*	*	*	*	0.1	0.1	1.0	0.1	1.0
05-Aug-00	8								1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	*	0.3	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
20-Aug-00	9									1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0	*	*	*	0.7	0.8	0.8	1.0	1.0	1.0	1.0	0.6
06-Sep-00	10										1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.6	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3
23-Sep-00	11											1.0	1.0	0.5	0.9	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	*	*	*	0.3	0.4	0.4	0.8	0.8	1.0	0.8	0.7
07-Oct-00	12												1.0	*	0.1	1.0	0.5	1.0	1.0	*	0.8	1.0	1.0	1.0	*	*	*	*	*	*	*	*	1.0	*	1.0
21-Oct-00	13													0.7	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	*	*	*	0.5	0.7	0.7	0.9	1.0	1.0	1.0	0.1
08-Nov-00	14														1.0	0.1	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.6	1.0	*
19-Nov-00	15															0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
03-Dec-00	16																1.0	1.0	1.0	*	1.0	1.0	1.0	1.0	*	*	*	0.1	0.1	0.1	0.4	0.5	1.0	0.5	0.6
18-Dec-00	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.6	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
03-Jan-01	18																		1.0	0.9	1.0	1.0	1.0	1.0	*	0.3	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
18-Jan-01	19																			0.8	1.0	1.0	1.0	1.0	*	0.2	*	0.9	1.0	1.0	1.0	1.0	1.0	1.0	*
03-Feb-01	20																				1.0	0.6	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	*
17-Feb-01	21																					1.0	1.0	1.0	0.3	0.9	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
06-Mar-01	22																						1.0	1.0	*	*	*	0.8	0.9	0.9	1.0	1.0	1.0	1.0	*
16-Mar-01	23																							1.0	*	0.2	*	0.9	1.0	1.0	1.0	1.0	1.0	1.0	*
01-Apr-01	24																								0.1	0.6	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
14-Apr-01	25																									1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	*
28-Apr-01	26																										1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	*
12-May-01	27																											1.0	1.0	1.0	1.0	1.0	*	1.0	*
27-May-01	28																												1.0	1.0	1.0	1.0	0.4	1.0	*
11-Jun-01	29																													1.0	1.0	1.0	0.6	1.0	*
27-Jun-01	30																														1.0	1.0	0.6	1.0	*
14-Jul-01	31																															1.0	0.9	1.0	*
28-Jul-01	32																																0.9	1.0	*
11-Aug-01	33																																	0.9	0.1
25-Aug-01	34																																		*

Appendix 3.2. Scheffe post-hoc tests of differences in phaeopigment concentration between sampling dates at Bull Island * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	0.9	*	0.1	0.6	0.4	0.4	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.3	*	*	*	*	*	1.0
24-May-00	2		1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	0.1	1.0	1.0
07-Jun-00	3			1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0
21-Jun-00	4				1.0	1.0	1.0	1.0	1.0	*	0.7	*	*	1.0	0.2	1.0	0.9	0.6	0.2	0.9	0.3	*	0.7	*	*	*	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*
29-Jun-00	5					1.0	1.0	1.0	1.0	0.7	1.0	0.9	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.7	0.1	0.5	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
09-Jul-00	6						1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
22-Jul-00	7							1.0	1.0	0.9	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.4	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
05-Aug-00	8								1.0	0.9	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.9	0.3	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
20-Aug-00	9									1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0
06-Sep-00	10										1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.9	0.1	0.5	0.2	*	0.4	1.0
23-Sep-00	11											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.9	*	1.0	1.0
07-Oct-00	12												1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.1	0.7	0.3	*	0.6	1.0
21-Oct-00	13													0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.7	*	0.2	0.0	*	0.1	1.0
08-Nov-00	14														1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.9	1.0	1.0	1.0	1.0	1.0	1.0	0.6	1.0	1.0
19-Nov-00	15															1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.9	0.7	*	0.9	1.0
03-Dec-00	16																1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0
18-Dec-00	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	*	1.0	1.0
03-Jan-01	18																		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.9	*	1.0	1.0
18-Jan-01	19																			1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.9	0.7	*	0.9	1.0
03-Feb-01	20																				1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	1.0
17-Feb-01	21																					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.7	*	0.9	1.0
06-Mar-01	22																						1.0	1.0	1.0	1.0	1.0	0.7	0.9	*	0.4	0.1	*	0.3	1.0
16-Mar-01	23																							1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	*	1.0	1.0
01-Apr-01	24																								1.0	1.0	1.0	0.6	0.9	*	0.4	0.1	*	0.3	1.0
14-Apr-01	25																									1.0	1.0	0.0	0.2	*	*	*	*	*	1.0
28-Apr-01	26																										1.0	0.4	0.7	*	0.2	*	*	0.1	1.0
12-May-01	27																											0.9	1.0	0.1	0.7	0.3	*	0.6	1.0
27-May-01	28																												1.0	1.0	1.0	1.0	1.0	1.0	0.8
11-Jun-01	29																													1.0	1.0	1.0	0.9	1.0	1.0
27-Jun-01	30																														1.0	1.0	1.0	1.0	0.1
14-Jul-01	31																															1.0	1.0	1.0	0.6
28-Jul-01	32																																1.0	1.0	0.2
11-Aug-01	33																																	1.0	*
25-Aug-01	34																																		0.5

Appendix 3.3. Scheffe post-hoc tests of differences in chlorophyll/phaeopigment ratio between sampling dates at Bull Island * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	1.0	0.6	0.2	0.9	*	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.4
24-May-00	2		1.0	0.6	0.2	0.9	*	0.8	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.3
07-Jun-00	3			0.4	0.1	0.9	*	0.8	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.2
21-Jun-00	4				1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	0.8	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	*	*	*	0.9	0.9	1.0	1.0	1.0	*	1.0	1.0
29-Jun-00	5					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.4	1.0	0.9	1.0	1.0	0.1	0.8	1.0	1.0	0.7	*	*	*	0.6	0.6	1.0	1.0	1.0	*	1.0	1.0
09-Jul-00	6						1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.2	0.4	0.3	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
22-Jul-00	7							1.0	1.0	0.7	1.0	1.0	0.7	0.1	*	1.0	0.3	0.6	0.6	*	0.2	0.7	0.8	0.2	*	*	*	0.1	0.1	0.8	0.7	1.0	*	0.8	1.0
05-Aug-00	8								1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	0.0	0.2	0.1	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
20-Aug-00	9									1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.9	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
06-Sep-00	10										1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
23-Sep-00	11											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	0.5	0.4	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
07-Oct-00	12												1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.1	0.3	0.2	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
21-Oct-00	13													1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
08-Nov-00	14														1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.6
19-Nov-00	15															1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.5
03-Dec-00	16																1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	0.1	0.3	0.1	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
18-Dec-00	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
03-Jan-01	18																		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
18-Jan-01	19																			1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
03-Feb-01	20																				1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.1
17-Feb-01	21																					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.9
06-Mar-01	22																						1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
16-Mar-01	23																							1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0
01-Apr-01	24																								1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	0.8
14-Apr-01	25																									1.0	1.0	1.0	1.0	0.8	0.9	0.3	*	0.9	*
28-Apr-01	26																										1.0	1.0	1.0	1.0	1.0	0.7	*	1.0	*
12-May-01	27																											1.0	1.0	0.9	1.0	0.5	*	1.0	*
27-May-01	28																												1.0	1.0	1.0	1.0	*	1.0	0.8
11-Jun-01	29																													1.0	1.0	1.0	*	1.0	0.8
27-Jun-01	30																														1.0	1.0	*	1.0	1.0
14-Jul-01	31																															1.0	*	1.0	1.0
28-Jul-01	32																																*	1.0	1.0
11-Aug-01	33																																	*	*
25-Aug-01	34																																		1.0

Appendix 3.4. Scheffe post-hoc tests of differences in *Hydrobia* abundance between sampling dates at Bull Island * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
07-Jun-00	1	1.0	1.0	1.0	1.0	1.0	1.0	*	*	0.7	0.2	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.8	0.2	1.0	1.0	1.0	1.0	1.0	*
21-Jun-00	2		1.0	1.0	1.0	1.0	1.0	*	*	*	*	*	1.0	1.0	1.0	0.8	1.0	0.2	1.0	1.0	1.0	1.0	0.6	0.9	*	*	*	1.0	*	0.5	1.0	1.0	*
29-Jun-00	3			1.0	1.0	1.0	1.0	*	*	0.2	*	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	0.2	*	1.0	0.7	1.0	1.0	1.0	*
09-Jul-00	4				1.0	1.0	1.0	*	*	*	*	*	1.0	1.0	1.0	0.9	1.0	0.5	1.0	1.0	1.0	1.0	0.9	1.0	*	*	*	1.0	*	0.8	1.0	1.0	*
22-Jul-00	5					1.0	1.0	*	*	*	*	*	1.0	1.0	1.0	0.9	1.0	0.4	1.0	1.0	1.0	1.0	0.8	1.0	*	*	*	1.0	*	0.7	1.0	1.0	*
05-Aug-00	6						1.0	*	*	0.4	0.1	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.5	0.1	1.0	0.9	1.0	1.0	1.0	*
20-Aug-00	7							*	0.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.2
06-Sep-00	8								1.0	0.8	1.0	0.7	*	*	*	*	*	*	*	*	*	*	*	*	1.0	0.3	1.0	*	0.3	*	*	*	1.0
23-Sep-00	9									0.6	1.0	0.5	*	*	*	*	*	*	*	*	*	*	*	*	0.9	0.2	1.0	*	0.2	*	*	*	1.0
07-Oct-00	10										1.0	1.0	0.8	1.0	1.0	1.0	0.6	1.0	0.2	0.8	*	*	1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0	0.5	0.8	1.0
21-Oct-00	11											1.0	0.2	0.7	0.6	1.0	0.1	1.0	*	0.3	*	*	0.8	0.9	1.0	1.0	1.0	*	1.0	0.9	0.1	0.3	1.0
08-Nov-00	12												0.9	1.0	1.0	1.0	0.7	1.0	0.3	0.9	*	*	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	0.6	0.9	1.0
19-Nov-00	13													1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.8	0.3	1.0	1.0	1.0	1.0	1.0	*
03-Dec-00	14														1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.7	1.0	1.0	1.0	1.0	1.0	0.1
18-Dec-00	15															1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.6	1.0	1.0	1.0	1.0	1.0	0.1
03-Jan-01	16																1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5
18-Jan-01	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	0.7	0.2	1.0	0.9	1.0	1.0	1.0	*
03-Feb-01	18																		1.0	1.0	0.8	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
17-Feb-01	19																			1.0	1.0	1.0	1.0	1.0	*	0.2	*	1.0	0.6	1.0	1.0	1.0	*
06-Mar-01	20																				1.0	1.0	1.0	1.0	0.5	0.9	0.3	1.0	1.0	1.0	1.0	1.0	*
16-Mar-01	21																					1.0	1.0	1.0	*	*	*	1.0	0.2	1.0	1.0	1.0	*
01-Apr-01	22																						1.0	1.0	*	*	*	1.0	0.2	1.0	1.0	1.0	*
14-Apr-01	23																							1.0	0.9	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.2
28-Apr-01	24																								1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	0.4
12-May-01	25																									1.0	1.0	0.1	1.0	1.0	0.2	0.5	1.0
27-May-01	26																										1.0	0.2	1.0	1.0	0.5	0.9	1.0
11-Jun-01	27																											*	1.0	0.9	0.1	0.3	1.0
27-Jun-01	28																												0.6	1.0	1.0	1.0	*
14-Jul-01	29																													1.0	0.9	1.0	1.0
28-Jul-01	30																														1.0	1.0	0.4
11-Aug-01	31																															1.0	*
25-Aug-01	32																																*

Appendix 3.5. Scheffe post-hoc tests of differences in chlorophyll *a* concentration between sampling dates at Blackrock * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	1.0	1.0	0.2	0.9	1.0	0.4	0.3	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7
24-May-00	2		1.0	1.0	*	0.7	1.0	0.2	0.1	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4
07-Jun-00	3			1.0	0.1	0.9	1.0	0.3	0.2	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.8	0.8	1.0	1.0	1.0	1.0	1.0	1.0	0.6
21-Jun-00	4				1.0	1.0	1.0	1.0	1.0	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.2	0.1	0.6	0.4	0.7	1.0	1.0	1.0
29-Jun-00	5					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.1	0.8	0.1	0.2	0.7	0.1	0.9	0.4	1.0	*	*	*	*	*	*	*	*	0.1	1.0	1.0
09-Jul-00	6						1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.9	1.0	1.0	0.9	1.0	1.0	1.0	*	*	*	*	*	0.2	0.1	0.2	0.9	1.0	1.0
22-Jul-00	7							1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.3	0.2	0.7	0.5	0.7	1.0	1.0	1.0
05-Aug-00	8								1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	1.0	0.4	0.5	0.9	0.2	1.0	0.7	1.0	*	*	*	*	*	*	*	*	0.2	1.0	1.0
20-Aug-00	9									1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.9	0.2	0.4	0.8	0.1	1.0	0.6	1.0	*	*	*	*	*	*	*	*	0.2	1.0	1.0
06-Sep-00	10										0.7	0.1	0.1	0.3	*	*	*	*	*	*	*	*	*	0.6	*	*	*	*	*	*	*	*	*	0.2	1.0
23-Sep-00	11											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.3	0.2	0.7	0.5	0.8	1.0	1.0	1.0
07-Oct-00	12												1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.6	0.4	0.9	0.8	0.9	1.0	1.0	1.0
21-Oct-00	13													1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.7	0.5	1.0	0.9	1.0	1.0	1.0	1.0
08-Nov-00	14														1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.2	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
19-Nov-00	15															1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	0.9	0.8	1.0	1.0	1.0	1.0	1.0	1.0
03-Dec-00	16																1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.7	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5
18-Dec-00	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0
03-Jan-01	18																		1.0	1.0	1.0	1.0	1.0	1.0	0.2	0.5	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7
18-Jan-01	19																			1.0	1.0	1.0	1.0	1.0	0.2	0.4	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8
03-Feb-01	20																				1.0	1.0	1.0	1.0	*	*	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
17-Feb-01	21																					1.0	1.0	1.0	0.4	0.6	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5
06-Mar-01	22																						1.0	1.0	*	*	*	0.9	0.8	1.0	1.0	1.0	1.0	1.0	1.0
16-Mar-01	23																							1.0	0.1	0.2	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
01-Apr-01	24																								*	*	*	0.1	*	0.4	0.2	0.4	1.0	1.0	1.0
14-Apr-01	25																									1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	*	*
28-Apr-01	26																										1.0	1.0	1.0	1.0	1.0	1.0	0.6	*	*
12-May-01	27																											1.0	1.0	1.0	1.0	1.0	0.7	*	*
27-May-01	28																												1.0	1.0	1.0	1.0	1.0	0.4	*
11-Jun-01	29																													1.0	1.0	1.0	1.0	0.3	*
27-Jun-01	30																														1.0	1.0	1.0	0.9	*
14-Jul-01	31																															1.0	1.0	0.7	*
28-Jul-01	32																																1.0	0.9	*
11-Aug-01	33																																	1.0	0.5
25-Aug-01	34																																		1.0

Appendix 3.6. Scheffe post-hoc tests of differences in phaeopigment concentration between sampling dates at Blackrock * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	0.9	*	*	*	*	*	0.7	1.0	*	*	0.5	0.5	0.3	*	*	*	*	*	*	*	*	0.3	1.0	0.9	0.9	*	*	*	*	*	*	*	*
24-May-00	2		1.0	*	*	0.1	*	0.2	1.0	1.0	0.4	*	1.0	1.0	0.9	*	0.2	*	*	*	*	*	*	1.0	1.0	1.0	1.0	*	*	*	*	*	*	*	*
07-Jun-00	3			0.8	0.6	1.0	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.9	1.0	0.3	0.2	*	0.1	*	0.7	1.0	1.0	1.0	1.0	*	*	0.1	*	0.4	0.5	0.9	0.5
21-Jun-00	4				1.0	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	0.6	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
29-Jun-00	5					1.0	1.0	1.0	1.0	0.3	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	0.4	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
09-Jul-00	6						1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
22-Jul-00	7							1.0	0.9	0.1	1.0	1.0	0.8	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	*	0.2	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
05-Aug-00	8								1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
20-Aug-00	9									1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.3	0.8	0.7	1.0	1.0	1.0	1.0	1.0	0.4	0.4	0.8	0.3	1.0	1.0	1.0	1.0
06-Sep-00	10										1.0	0.7	1.0	1.0	1.0	0.7	1.0	0.1	0.1	*	*	*	0.5	1.0	1.0	1.0	1.0	*	*	*	*	0.2	0.3	0.6	0.2
23-Sep-00	11											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
07-Oct-00	12												1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	0.8	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
21-Oct-00	13													1.0	1.0	1.0	1.0	0.8	0.7	*	0.4	0.3	1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.5	*	0.9	0.9	1.0	0.9
08-Nov-00	14														1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0	0.8	1.0	1.0	1.0	1.0
19-Nov-00	15															1.0	1.0	0.9	0.9	0.1	0.7	0.6	1.0	1.0	0.9	1.0	1.0	0.3	0.3	0.8	0.1	1.0	1.0	1.0	1.0
03-Dec-00	16																1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18-Dec-00	17																	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
03-Jan-01	18																		1.0	1.0	1.0	1.0	1.0	0.9	*	0.1	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18-Jan-01	19																			1.0	1.0	1.0	1.0	0.9	*	0.1	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
03-Feb-01	20																				1.0	1.0	1.0	0.1	*	*	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
17-Feb-01	21																					1.0	1.0	0.7	*	*	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
06-Mar-01	22																						1.0	0.5	*	*	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
16-Mar-01	23																							1.0	*	0.5	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
01-Apr-01	24																								1.0	1.0	1.0	0.3	0.3	0.7	0.1	1.0	1.0	1.0	1.0
14-Apr-01	25																									1.0	1.0	*	*	*	*	*	*	*	*
28-Apr-01	26																										1.0	*	*	*	*	0.2	0.3	0.7	0.3
12-May-01	27																											*	*	0.1	*	0.4	0.5	0.9	0.5
27-May-01	28																												1.0	1.0	1.0	1.0	1.0	1.0	1.0
11-Jun-01	29																													1.0	1.0	1.0	1.0	1.0	1.0
27-Jun-01	30																														1.0	1.0	1.0	1.0	1.0
14-Jul-01	31																															1.0	1.0	1.0	1.0
28-Jul-01	32																																1.0	1.0	1.0
11-Aug-01	33																																	1.0	1.0
25-Aug-01	34																																		1.0

Appendix 3.7. Scheffe post-hoc tests of differences in chlorophyll/phaeopigment ratio between sampling dates at Blackrock * indicates significant <0.05.

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
10-May-00	1	1.0	1.0	1.0	0.7	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8
24-May-00	2		1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
07-Jun-00	3			1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
21-Jun-00	4				1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.7	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
29-Jun-00	5					1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
09-Jul-00	6						1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
22-Jul-00	7							1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	0.3	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
05-Aug-00	8								1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.8	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
20-Aug-00	9									1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
06-Sep-00	10										1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
23-Sep-00	11											1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
07-Oct-00	12												1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
21-Oct-00	13													1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
08-Nov-00	14														1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
19-Nov-00	15															1.0	1.0	1.0	1.0	0.9	1.0	0.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
03-Dec-00	16																1.0	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18-Dec-00	17																	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
03-Jan-01	18																		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18-Jan-01	19																			1.0	1.0	1.0	1.0	1.0	0.8	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
03-Feb-01	20																				1.0	1.0	1.0	1.0	*	*	*	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0
17-Feb-01	21																					1.0	1.0	1.0	0.8	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
06-Mar-01	22																						1.0	0.7	*	*	*	0.9	0.7	0.7	0.9	0.4	1.0	1.0	1.0
16-Mar-01	23																							1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
01-Apr-01	24																								1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
14-Apr-01	25																									1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.7	*
28-Apr-01	26																										1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	0.1
12-May-01	27																											1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.1
27-May-01	28																												1.0	1.0	1.0	1.0	1.0	1.0	1.0
11-Jun-01	29																													1.0	1.0	1.0	1.0	1.0	1.0
27-Jun-01	30																														1.0	1.0	1.0	1.0	1.0
14-Jul-01	31																															1.0	1.0	1.0	1.0
28-Jul-01	32																																1.0	1.0	1.0
11-Aug-01	33																																	1.0	1.0
25-Aug-01	34																																		1.0

Chapter 4 Determining growth and mortality for two intertidal mollusc species.

4.1 Introduction

Knowledge of population parameters is essential for understanding both individual species and ecosystem dynamics. Growth and mortality rates are indications of population 'turnover' and regulation, and together with density dependent and independent factors determine population numerical size, distribution and biomass. The influence of these parameters fluctuates both spatially and temporally in response to predictable and stochastic events. Due to their importance in shaping populations growth and mortality estimates are common, inputs to population modelling, particularly in the absence of robust time-series size-frequency and abundance data.

The growth rate of the vast majority of species is proportionally greater in the early stages of the lifecycle when individuals are relatively small (Cobb *et al.*, 1974,1983; Perkins-Visser *et al.*, 1996; Gosselin & Quain, 1997). Slower growth rates at larger sizes are generally associated with decreased metabolic rate and the redirection of a varying proportion of the overall energy budget from somatic to reproductive growth as sexual development occurs. Growth curves serve to describe probable size-at-age, although due to the wide biological variability in individual growth rates, particularly in larger\older individuals, a large degree of error is generally associated with the mean estimates. Most species of invertebrate display continuous growth apart from members of Phylum Arthropoda. Due to biological and environmental factors the growth period may be constrained to particular times of the year, resulting in a seasonal pattern of growth and maturation (Robinson & Tully, 2000a). Temperature and food availability are generally considered to be the most influential factors in determining seasonal growth rate (Barnett, 1985, Dekker & Beukema, 1999). Although smaller individuals generally tend to be proportionally more active than larger conspecifics, the later will generally posses proportionally greater tissue mass and cause a greater impact in the habitat to which they belong (Robinson & Tully, 2000b & c).

Physical and biological factors determine both the growth rate and maximum obtainable size of a species. Variation in temperature during and after development is

known to significantly affect both variables strongly for most cold-blooded invertebrates through changes in metabolic rate (Wear, 1974; Robisnon & Tully, 2000a; Genner *et al*, 2004). Salinity, oxygen concentration, aerial exposure and physical disturbance are also known to elicit strong effects in certain circumstances (Pechenik, 1987; Roughgarden *et al.*, 1988; Rumrill, 1990). Inherent genetic variation in growth rate and maximum size can occur between isolated populations of the same species within the same geographical and environmental range, and between individuals within the same population (Gosling, 2003). Parental body size, particularly on the maternal side, is known to affect gamete and subsequent offspring size for many invertebrates (Wear, 1974; Rumrill, 1990). Although the physiology, form and function of a species will generally determine maximum longevity and size, temperature and resource availability dictate whether these potentials are reached in the absence of premature death. Temperature affects the metabolic rate of all invertebrates to some extent; higher temperature generally results in faster growth, maturation, lifecycle and lower maximum size (Pechenik, 1987; Robisnon & Tully, 2000a; Gosling, 2003). Although insufficient food is normally the cause of growth restriction, other factors, particularly space and competitive interactions in the intertidal zone, are equally if not more important for some invertebrate species (Cobb & Tamm, 1974; Perkins-Visser *et al.*, 1996; Steele 1999).

The majority of growth curves are constructed by measuring individuals of a known age, determined by counting some quantifiable validated growth mark or by knowing the exact time of birth. Although almost all species lay down some form of growth annuli, it may not be possible to detect or discern these in a meaningful way when the species is small, short-lived or the annuli obscured in some other way. For some species, particularly the crustaceans, growth bands are not laid down at all. Length-based assessment of age is an alternative method of obtaining growth rate\curve information in cases where age cannot be determined by growth marks and time of birth is not known (Robinson & Tully, 2000a). This method requires that groupings of individuals of the same age, or cohort, can be identified and isolated from the composite population size-frequency distribution. Whether age or length-based methods are used to construct a growth curve, direct methods of age validation such as growth rate data is desired to remove subjectivity and validate results of indirect methods.

The highly dynamic nature of the marine environment results in the vast majority of individuals within any invertebrate population failing to attain their maximum theoretical body size (Gosselin & Qian, 1997). Exposure of an individual to physical extremes outside of its physiological or morphological tolerances generally results in rapid deterioration in condition and death (Robinson & Tully, 2000b). Negative changes in physical and biological resource availability, will also result in mortality if novel sources cannot be found to mitigate intra- and\or inter-specific competition. Predation, disease, and genetic defects are also known to cause varying degrees of mortality within populations (Rumrill, 1990). Although fluctuations in the combined affect of the all of above variables results in the determination of population size at any one time, mortality in the early juvenile stages is believed to be the major determinant of future population size for many marine invertebrate species (Keough & Downes, 1982; Gosselin & Qian, 1997). Due to overlaps in resource utilisation, predation and inter-specific competition, fluctuations in the population size of one species may significantly impact on another (Barnes & Hughes, 1988).

Herbivorous primary consumers play an important role in marine environments by providing a pathway for nutrient transfer to higher trophic levels, which generally contain species that cannot utilise primary production directly. The population turnover of primary consumers therefore determines changes in the potential nutrients available to higher trophic levels. Even when an individual passes through its lifecycle without being consumed by a higher trophic feeder, its decomposition after death will provide a source of nutrients for detritivores within the habitat to which it belonged. The aim of this chapter was to determine the growth and mortality of two herbivorous mollusc species that represent the dominant primary consumers in the distinct habitats where they occur.

4.2 Materials and Methods

Both study sites, Bull Island mudflat and Blackrock sandflat, are located in Dublin Bay (see 2.2.1 for full site description). Although both contain several species of primary consumer, the former is dominated by the surface deposit feeder, *Hydrobia ulvae*, the later by the infaunal bivalve *Tellina tenuis*. It is not completely clear what feeding strategy *Tellina tenuis* employs, although there is some suggestion that it

combines filter and deposit feeding behaviours (Trevallion, 1971). The highly mobile species *Hydrobia* attains a relatively small maximum body size (approx. 8mm shell height (SH)) compared with *Tellina tenuis* (approx. 20mm SH).

4.2.1 Size-at-age Hydrobia ulvae

Several hundred *Hydrobia* were collected from various randomly selected locations at the Bull Island site and returned to the laboratory. Despite macro- and microscopic examination under various light sources it was not possible to identify or distinguish any growth marks on the shell or opercular plate. Although a number of techniques, including etching, peels, cross-sectioning, staining and polishing were subsequently used in an attempt to identify growth annuli, no significant results were achieved. For this reason a length-based assessment of size-at-age was conducted using time-series size-frequency distribution data (see Chapter 3 for sampling methodology). For comparative purposes, inter-annual estimates of growth and mortality were derived using the pooled population data presented in Chapter 2.

Size-frequency histograms of *Hydrobia* shell height (SH) were constructed for data collected as described section 2.2.6 (spatial variability) and for individuals collected on each sampling date in section 3.2.1c (temporal variability). Bar widths were set at 0.1mm (SH) as this interval was observed to give the best resolution of growth increments without data fragmentation. Data was smoothed by 3-point running average to account for the natural biological variability at size within the population. Smoothed histogram bar counts were used as input data to the computer package MIX 2.3 (MacDonald & Green, 1988) in order to isolate normal distributions thought to represent cohorts/year classes from each composite size-frequency distribution. Separation is accomplished by maximum likelihood estimation. For the temporal dataset (3.2.1), mean size in each cohort over time was used as input parameters to the computer package 'FISAT' (Food and Agriculture Organisation for the United Nations) for linkage of means. Outputs from this method are refined mean size-at-age estimates after subjective linking is complete. This constituted the growth estimate produced by cohort analysis.

The mean size-at-age estimates from the previous step were used as input parameters to the 'Appeldoorn's' method (Appeldoorn, 1987) routine in the FISAT package to generate growth parameter estimates for modelling purposes. This iterative routine attempts to minimise error to converge at output parameters for the production of a seasonalised von Bertalanffy (1938) function, which is commonly used to describe growth curves for the entire lifecycle of a species. It is a useful method for standardising growth parameters and methodologies, allowing more direct comparison with other populations and species. Estimated growth parameters include the asymptotic length ($L\infty$), calculated from the observed reduction in growth rate with increasing age, which is essentially the maximum size of infinitely old *Hydrobia*. The rate at which $L\infty$ is approached is estimated from the overall curvature (K). The period of minimal growth is defined as the winter point (WP). The amplitude of seasonal oscillations in growth (C) takes values between 0 and 1, with higher values indicating more pronounced seasonality. The full equation takes the form:

$$L(t)=L\infty*[1-\exp\{-K*(t-t_0)-(CK/2\pi)*\sin(2\pi*(t-ts))\}]$$

The term 'ts' is the summer point, the fraction of the year that has elapsed when growth rate is maximal (the reciprocal of the output parameter 'Winter Point' (WP)).

4.2.2 Size-at-age Tellina tenuis

Histograms of time-series size-frequency distribution collected during the sampling described in Chapter 3 (see 3.2.1b for methodology) were constructed for *Tellina tenuis* only to offer a source of comparison for future studies. Fragmentation of data and high spatial variability in size-frequency prevented the identification of individual cohorts for this species rendering the use of cohort analysis inappropriate due to high subjectivity. This had been expected from the results of the Chapter 2, which revealed significant differences in size-frequency distribution at relatively small sampling distances. Results from the large-scale examination of spatial variability presented in Chapter 2 were used in an attempt to estimate inter-annual growth and mortality.

Visible markings believed to represent annuli were apparent on the external surface of *Tellina* shell valves. Differential growth rates between summer and winter periods often cause the formation of visually identifiable ridges (annuli) in shelled mollusc species, particularly for bivalves (Gosling, 2003). Using these to age specimens assumes that the approximate time and size at settlement is known, and that annuli are only laid down in response to the annual change in seasons and not other factors such as food availability or disturbance. Although annuli are easily distinguished in some cases, the process is generally more difficult in smaller, thin-shelled species (Gosling, 2003). This can result in a high degree of subjectivity when assigning age.

Two 'readers' examined a wide of range of shells, discussing the criteria for assigning a positive age 'count' or annuli for *Tellina.* During a winter phase, 100 fresh individuals from the upper, mid and low shore level were collected from the Blackrock site. These were examined and assigned age using the reading criteria. 'Winter phase' individuals were used in the hope that the recent end of the fast growth phase could be identified, validating that the marks were attributable to changes in season alone. A total of 20 individuals were aged in each of the 5 arbitrary size classes <4mm, 4–8mm, 8–12mm, 12–16mm and >16mm SH (see Chapter 2). Each shell valve was examined visually under standard bench lighting and without magnification. Attempts to improve readability using various magnification and directional light sources did not improve clarity of banding. Each individual was aged on eight separate occasions (at least 2 weeks apart) by the 'Primary' reader, and the results compared to determine whether 'convergence' of age estimates occurred. Distances between the 'winter marks' were measured with digital callipers to estimate annual growth. During each reading the second reader randomly re-arranged the order of the shell valves, which were coded in pencil on the underside. Although efforts were made to read shell valves 'blind' it should be appreciated that the shell size itself negated the application of a truly 'blind' trial. Attempts to enhance the clarity of annuli were made. Although a number of techniques, including etching, peels, cross-sectioning, staining and polishing were used, no increase in band clarity was achieved. Many of the processes were too abrasive or harsh for the delicate thin shell valves of *Tellina*, which became increasing fragile once dry and crumbled easily.

4.2.3 Growth in the laboratory

Due to the subjectivity of assigning size-at-age to any wild population where the exact time of birth of individuals is not known, validation methods are required. Laboratory trials were conducted during the present study to provide validation in the form of size-specific growth rate data. These trials also served to determine the influence key environmental variables had in the regulation of growth so that rates more applicable to field conditions could be obtained. For the purposes of analyses, growth increment was expressed as a percentage increase in original SH, growth rate obtained by the division of the former by experimental duration. After testing for normality and heterogeneity of variance, data were arcsine transformed to satisfy the requirement of continuous datasets for ANOVA.

4.2.3a Measurement accuracy of repeated shell measurements-determining growth

Prior to conducting growth experiments, repeated size measurements were conducted on a sub-sample of each species studied to determine the actual error associated with determining the widest shell dimension. The Mitutoyo Absolute Digimatic digital callipers used were accurate to 0.01mm, but visually re-locating the widest point on small, non-uniform shell surfaces was likely to result in a reduction in this accuracy. Knowledge of this accuracy was essential considering the slow growth that was likely to occur in larger individuals. This may have resulted in the assigning of low growth to individuals that had not grown at all. The same reader measured fifty individuals representing the full size range of both species 'blindly' on three separate occasions. No previous indication of size readings were permitted at subsequent readings, with a second 'non-reader' re-arranging the order that the shells were to be measured in and maintaining data sheets accordingly. Due to the fragile nature of the shells, they were examined for possible damage between readings and replaced accordingly.

4.2.3b Laboratory growth-Hydrobia

Large numbers of *Hydrobia ulvae* and sediment samples were collected from the mudflat at Bull Island. Sediment was sieved (0.5mm) on site and the retained component placed in seawater and returned to the laboratory. Surface sediment, to a depth of 1cm, was collected separately on site. This surface sediment was sieved on

return to the laboratory using a 0.5mm sieve and any algae or fauna removed (including *Hydrobia*).

A square panel was cut from the lids of 19.6cm^2 Petri-dishes and covered with 0.5mm mesh so that water and food could enter the dish but snails could not escape. An even layer of sieved sediment approximately 3mm deep was placed at the base of each dish. Weighted trays were used to submerge Petri-dish enclosures to a depth of 15cm in a closed-circulation seawater system. A 16h light:8h dark cycle was operated. Salinity was held constant at 32$^{o}/_{oo,}$ and the tank aerated. 'Phytoplex™' aquaria brand marine micro-plankton complex was added in excess every two days to tank water to supplement natural algal growth/food supply and ensure that food availability remained high. This food source was used for ease and convenience and did not appear to have any adverse effects on snails in pre-experiment trials. As the experimental design dictated that food to be in excess it was thought necessary to supplement the natural algal growth with this artificial food source, however it was not determined if or in what quantities this food source was utilised by the animals. Arbitrary size classes of 1mm were used for growth experiments as the cohort means did not represent all individuals in the population due to biological variability in size-at-age.

4.2.3c Physiological limitation of sustained high growth rates-Hydrobia

A total of 25 individuals in each arbitrary size class 1±0.3mm, 2±0.3mm, 3±0.3mm, 4±0.3mm, 5±0.3mm and 6±0.3mm (SH) were selected randomly from stock animals during August 2003 and placed in experimental containers to acclimate to an experimental temperature of 16^{o}C for 1 week. A single individual was placed in each dish, representing a relatively low density of 500 individuals m^{-2}. After the acclimation period, shell height (SH) was measured from posterior to anterior shell tips using Mitutoyo Absolute Digimatic callipers to 0.1mm and the individuals returned rapidly to their assigned dish. Individuals were fed in excess and survivors measured after 1, 2 and 3 months.

4.2.3d Size-specific growth at temperature-Hydrobia

A total of 60 individuals in each arbitrary size class 1±0.3mm, 2±0.3mm, 3±0.3mm, 4±0.3mm, 5±0.3mm and 6±0.3mm (SH) were selected randomly from stock animals and placed in experimental containers to acclimate to experimental temperature for 1week. A single individual was placed in each dish, representing a low density of 500 individuals m^{-2}. After the acclimation period shell height (SH) was measured from posterior to anterior shell tips using Mitutoyo Absolute Digimatic callipers to 0.1mm and the individuals returned rapidly to their assigned dish. Fifteen individuals from each size class were then maintained at constant temperatures of 20, 16, 12 or 6°C for a period of one month during late August 2003, and fed in excess. These temperatures were selected as representative of the range of water temperatures recorded in Dublin Bay (see Chapter 3 for data). The low temperature tank was representative of the winter period from December 2000 to April 2001. Although highest mean temperature in Dublin Bay were recorded as 17°C, readings were taken in deeper (10m) waters than those that occur at the Bull Island site. It is probable that temperatures exceed 17°C in the lagoon, particularly on summer days at low tide however. A high temperature treatment of 20°C was, therefore, used. The 12 and 16°C treatments were used to represent the rest of the yearly range in temperature. After a period of one month, all surviving individuals were removed and re-measured.

In some cases, growth of species is constrained by the size of the vessel in which they are held captive. To ensure that growth was not restricted by the size of the Petri-dish enclosures, a concurrent experiment was conducted with a smaller number of larger (56.7cm^2) Petri-dishes. Three *Hydrobia* of from each size class above were placed in an individual large dish and added to each of the 4 temperature treatments, replicating the density of 176 individuals m^{-2} for 1 individual in the smaller dishes. These larger dishes were interspersed amongst the smaller dishes in each treatment tank.

4.2.3e The effect of density-Hydrobia

The growth rate of *Hydrobia* was examined at 3 different densities under a constant temperature of 12°C during July 2003. Densities of 2,500, 7,500 and 15,000 individuals m^{-2} were selected. Densities in the field population during seasonal monitoring (see Chapter 3) ranged from 2,630–33,830 individuals m^{-2}. The lowest density treatment was comparable to that experienced in the field during low

abundance (2,500), the medium density (7,500) to the relatively stable over-wintering period. Although the highest density treatment was just less than half that recorded during peak abundance in the field, this was often due to the presence of high numbers of newly settled (<1.6mm SH) individuals. It was not practical to recreate densities exceeding 15,000 individuals m^{-2} in the laboratory, particularly those consisting of large numbers of small individuals. Additionally, as the timing of this experiment preceded the autumn (August) settlement, individuals of 1mm SH were not available in the field. The high density treatment was considered a good representation of peak abundances of the combined spring Young of the Year (YOY) and previously settled cohorts however.

Hydrobia were divided into 5 size classes 2±0.3mm, 3±0.3mm, 4±0.3mm, 5±0.3mm and 6±0.3mm SH. Each 19.6cm^2 (small) Petri-dish contained the same number of *Hydrobia* from each size class. At the lowest density (2,500m^{-2}) one snail of each size was placed in each Petri-dish, totalling 5 *Hydrobia* per dish. For the medium density (7,500 m^{-2}) three snails from each size class were added. Individuals of the same size class were marked using 'Humbrol Super Enamel' modelling paint so that they could be distinguished from one another. It was assumed that the application of a very small amount of this inert non-toxic paint to the tip of the shell spiral was unlikely to affect subsequent survival, growth or behaviour in experimental conditions. A number of individuals marked in this way and observed prior to the experiment appeared to be unaffected. To ensure no bias between treatments, the single individuals representing each size class in the lowest (2,500) density treatment were also marked in the same way. At the highest density (15,000m^{-2}), 6 individuals from each size group were marked individually and placed in each dish. Ten replicates of each density treatment were established. Temperature was kept constant at 12°C, with all other conditions matching those described in 4.2.3a.

4.2.3f The effect of food availability and density on growth-Hydrobia

Surface sediment and *Hydrobia* samples were removed from the Bull Island site during November 2003. This time was chosen as previous results suggested that no growth would be occurring in natural circumstances during this period. Any subsequent growth in the laboratory would therefore suggest that the factors examined

were limiting growth in the field. Sediment (0.5mm) was sieved in the laboratory and all filamentous algae and *Hydrobia* removed. An untreated layer of mud 1cm deep was placed in 6 aquaria with base dimension 250cm^2, and 3 filled to a depth of 15mm with freshly collected seawater from the Bull Island site. Filtered seawater was added to the remaining other 3 tanks containing normal, unaltered mud. A further 3 aquaria of identical dimension were filled with mud that had been subjected to an autoclaving process to remove all chlorophyll *a* and other potential food sources, and then filled with filtered seawater. 'Phytoplex' marine aquaria phytoplankton was added to the tanks according to Table 4.1 to enforce high, low and 'no food' availability regimes. *Hydrobia* of various sizes, representative of the size-frequency observed when isolating individuals from the sediment in the process described above, were added to each tank in sufficient numbers to represent the high, medium and low densities shown in Table 4.1. Prior to the addition of *Hydrobia*, 10 individuals were selected at random from each 1mm size class between 1-6mm and marked with 'Humbrol Super Enamel' modelling paint to be uniquely identifiable. The shell height of each of these individuals was recorded to 0.1mm. All treatment tanks were kept at a constant temperature of 16°C for a period of 1month, after which time surviving *Hydrobia* were removed and marked individuals re-measured.

Table 4.1. Treatments applied to 9 tanks examining growth of *Hydrobia* under varying food availability and density regimes.

Tank No.	Treatment Code	Sediment property	Seawater property	Feeding regime 5ml 'Phytoplex'	*Hydrobia* density (individuals m^{-2})
1	1-L	Normal mud	Normal seawater	Daily	2,500
2	1-M	Normal mud	Normal seawater	Daily	7,500
3	1-H	Normal mud	Normal seawater	Daily	15,000
4	2-L	Normal mud	Filtered seawater	Weekly	2,500
5	2-M	Normal mud	Filtered seawater	Weekly	7,500
6	2-H	Normal mud	Filtered seawater	Weekly	15,000
7	3-L	Autoclaved mud	Filtered seawater	No	2,500
8	3-M	Autoclaved mud	Filtered seawater	No	7,500
9	3-H	Autoclaved mud	Filtered seawater	No	15,000

4.2.3g Laboratory growth-Tellina

Sediment was sieved (1.0mm) at the Blackrock site and the retained component placed in water and returned to the laboratory. Surface sediment to a depth of 5cm was collected separately. This sediment was sieved (0.5mm) in the laboratory and any algae or fauna collected discarded. Sediment was added to 3 large (0.5x1.0m) aquaria to a depth of 10cm, and covered with seawater to a depth of 15cm. A 16h light:8h dark cycle was enforced. Salinity was held constant at $32^{o}/_{oo,}$ and the tank aerated. 'Phytoplex™' aquaria brand marine micro-plankton complex was added each day to tank water to supplement natural algal growth/food supply and ensure that food was not limiting to growth.

4.2.3h The effect of temperature on growth-Tellina

Tellina of varying sizes were measured using digital callipers to the nearest 0.1mm, and divided arbitrarily into five 4mm (SH) size classes, <4mm, 4-8mm, 8-12mm, 12-16mm and >16mm (see Chapter 2). Approximately 106 individuals were placed in each of three separate seawater aquaria (described above), resulting in a density of <500 individuals m^{-2}. This density was lower than observed at Blackrock at any time of year (see Chapter 3), ensuring that density-dependant restriction of growth did not occur. 'Humbrol Super Enamel' modelling paint was used to identify individuals of the same size class within treatments. The 3 tanks were held at constant temperatures of $6^{o}C$, $12^{o}C$, and $20^{o}C$ respectively. These temperatures were selected as representative of the range of seasonal water temperatures recorded in Dublin Bay. The tanks were examined each day and dead individuals removed from the surface. After a period of 3 months all remaining *Tellina* were removed and re-measured.

Although an identical series of experiments were planned for *Tellina* as those conducted for *Hydrobia* (c-f above), these were not possible due to difficulties in maintaining *Tellina* in artificial conditions.

4.3 Results

4.3.1 Hydrobia size-at-age

When examined visually prior to data smoothing, most composite *Hydrobia* size-frequency distributions appeared to comprise a variable number of distinct, normally distributed size groupings (Figure 4.1). Distinct recruitment events, indicated by the periodic addition of large numbers of small sized (0.5-2.0mm SH) individuals, were clearly detected during August-September of both 2000 and 2001, and April-May of 2001. It was generally less easy to discern individual cohorts by visual examination of unsmoothed data during the winter months, when overall densities were low. Growth of *Hydrobia* in each cohort can be observed over the summer months but is less clear during other times of the year. After data smoothing, distinct cohort/year groups were identified from all composite size-frequency distributions using MIX. In a few cases it was not possible to fit normal distribution to individual cohorts with low density. These, generally, represented the oldest and largest group within the population, which affected the significance of the overall test result and the successful resolution of other cohorts (Table 4.2). Difficulty in fitting normal distributions to this oldest group also occurred when densities in the cohort were high, due to the distribution becoming skewed and failing to conform to normality. This was due to the rapidly increasing rarity of very large individuals (>5mm SH) in this group in comparison with the population as a whole.

On July 14th 2001 the start of the autumn *Hydrobia* recruitment was detected by the appearance of a few individuals much smaller than observed in previous samples, but the main peak of settlement seemed yet to occurr. A normal distribution could not be fitted to this fragmented component (Figure 4.2) due to low abundance. This increased significantly in subsequent samples as further settlement occurred and the cohort became fully established. When the lower or higher ends of the size-frequency corresponded to the above conditions and fitting normal distribution was not possible, data were truncated prior to analysis so that other cohorts could be identified. For the remaining data Chi-square values for goodness-of-fit were significant to the 95% level. These values represented maximum likelihood solutions. The proportion of individuals from each sample assigned to the various cohorts presented by MIX, as a function of the overall sample size, was used to compartmentalise the original density

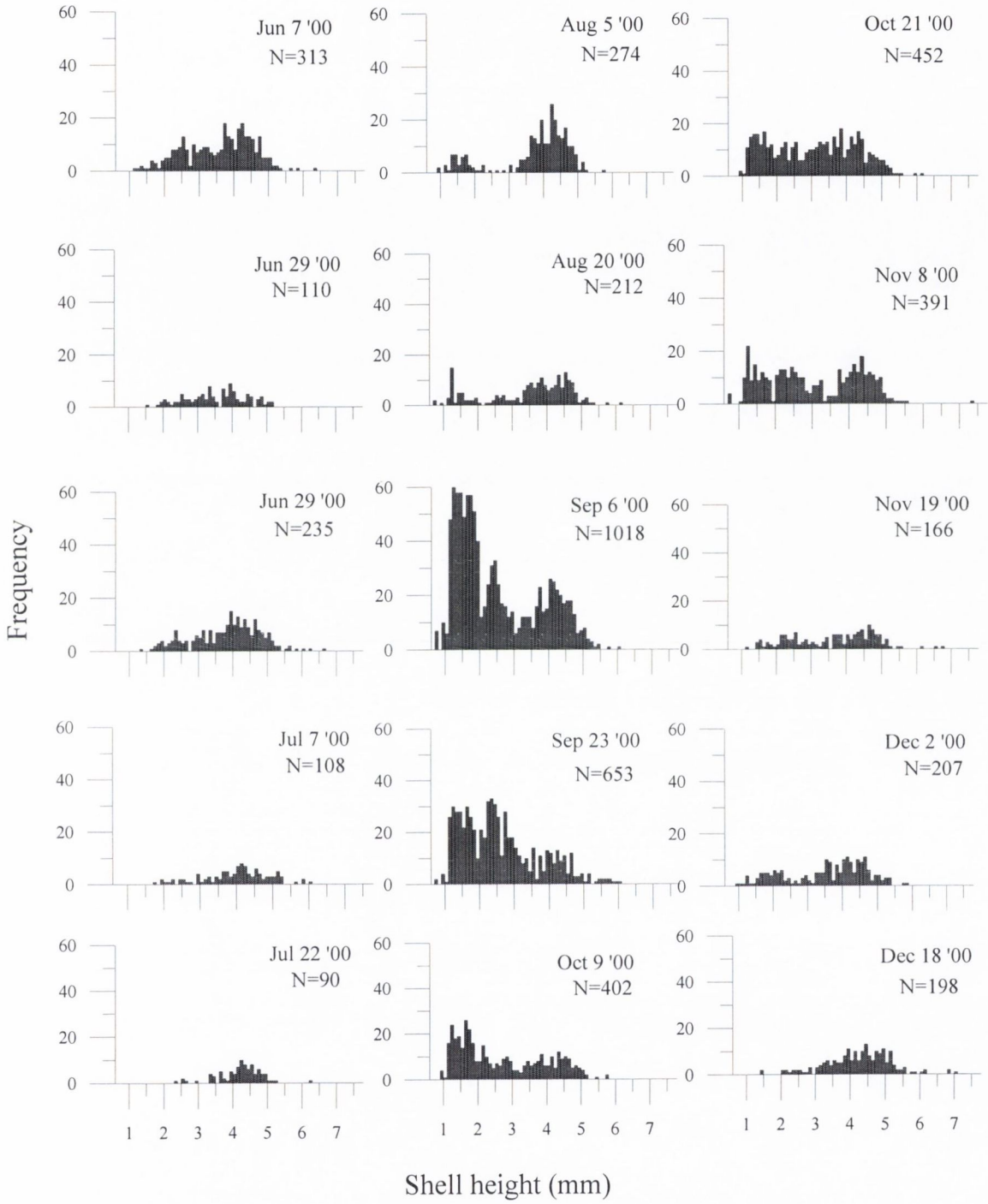


Figure 4.1. Size-frequency histograms of *Hydrobia* from Bull Island, Dublin; June 2000 – September 2001. (continued overleaf).

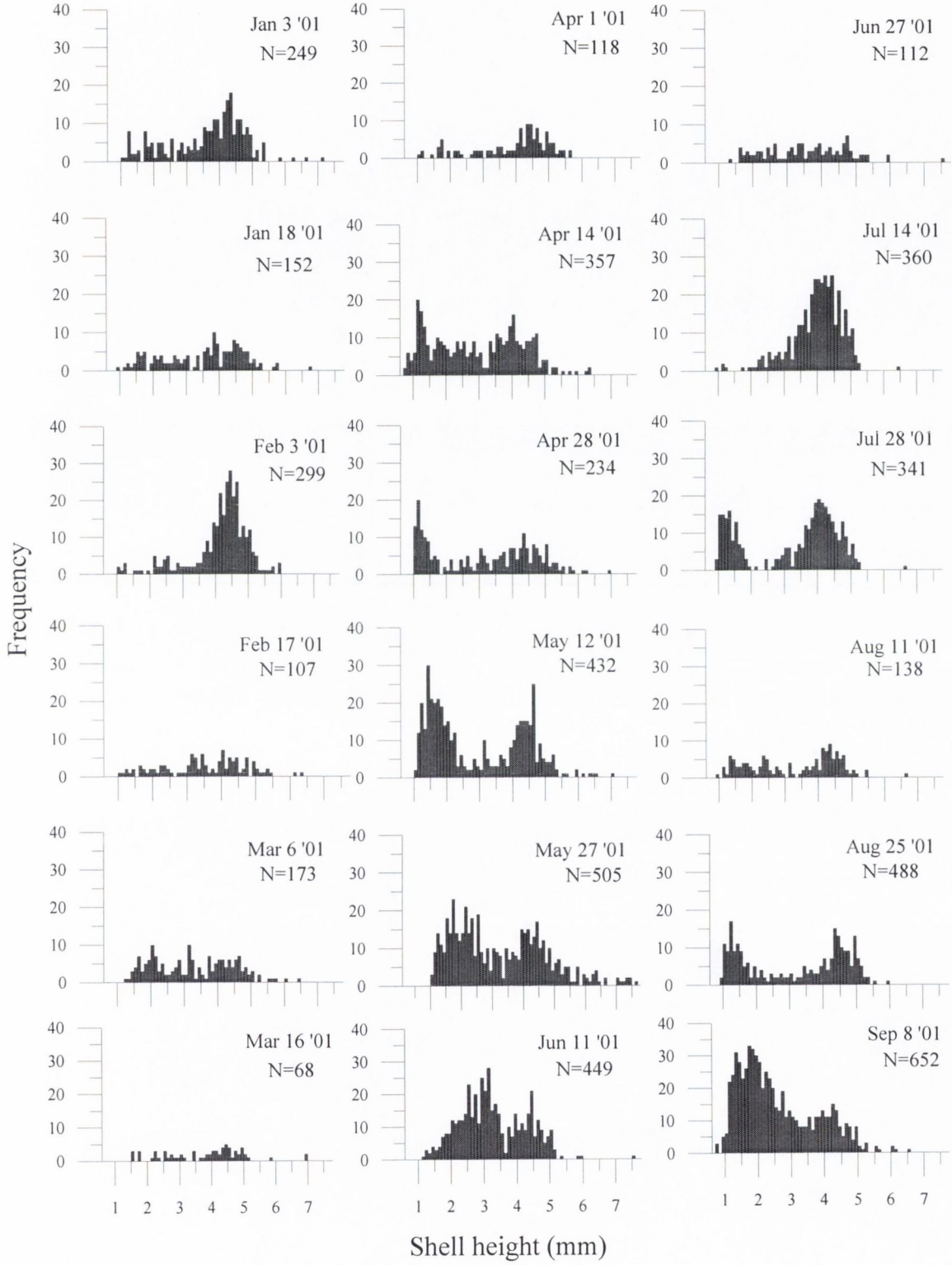


Figure 4.1.Size-frequency histograms of *Hydrobia* from Bull Island, Dublin; June 2000–September 2001. (continued).

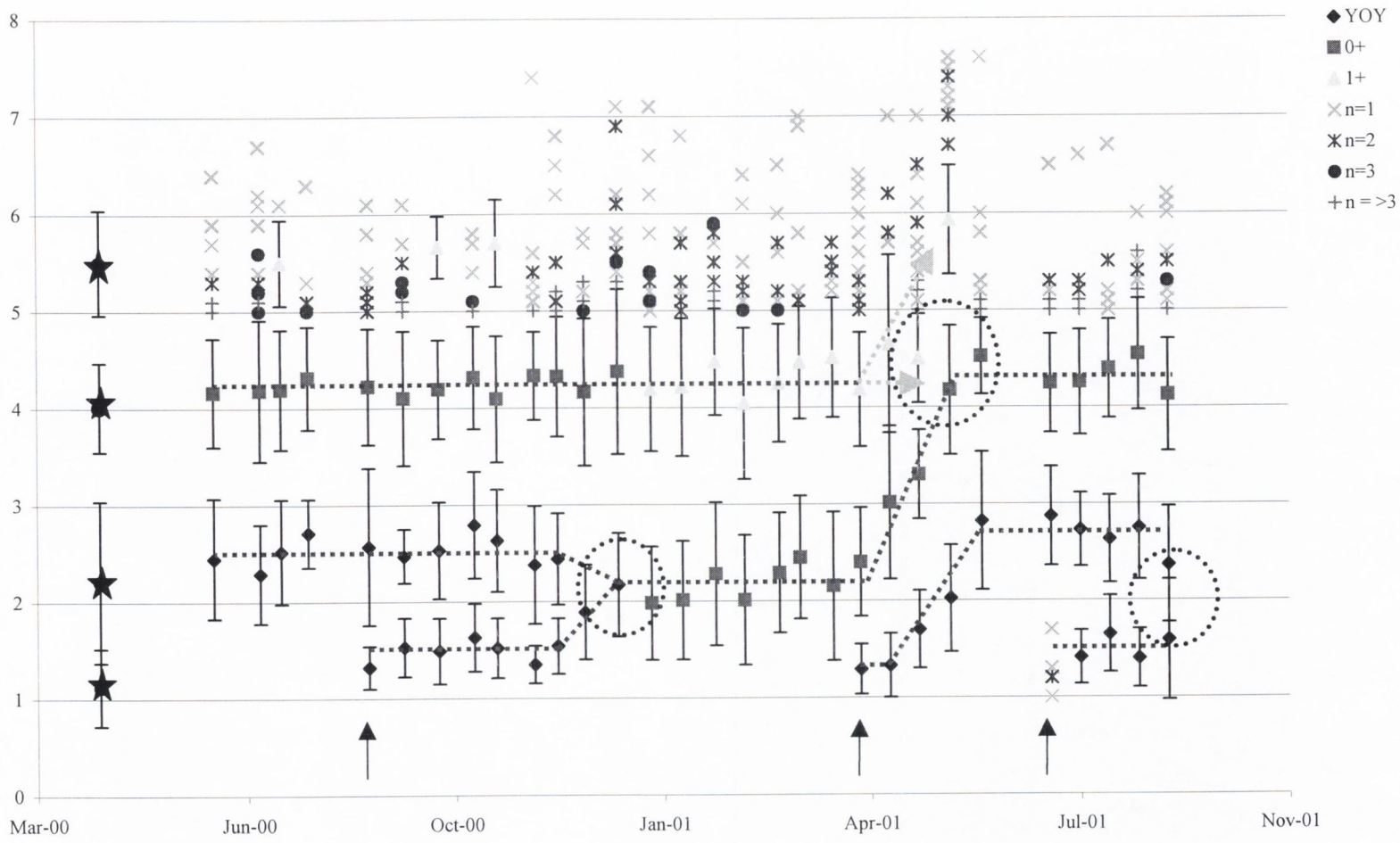


Figure 4.2. Mean cohorts size (±S.D.) identified from size-frequency data. Stars represent mean (±S.D) S.H. within each of the cohorts described in chapter 2. Points represent individual *Hydrobia* plotted when range too extensive or insufficient N to create a significantly distinct cohort. Dotted lines represent likely growth curves of cohorts. Circles represent merging of cohorts. Arrows indicate settlement events.

estimate obtained in the field. The estimated cohort densities corresponding to individuals truncated prior to analysis were also included in Table 4.2 for completeness.

Table 4.2. Density of *Hydrobia* m^{-2} within each cohort identified from size-frequency data. Proportions in brackets represent groups without sufficient N to create a distinct cohort or skewed distribution.

Date	Sample N	YOY Autumn	YOY Spring	Age 0+	Age 1+	Age 2+
07-Jun-00	313		3678	5614	[438]	
29-Jun-00	235		1534	5406	[365]	
07-Jul-00	108		769	2222	366	
22-Jul-00	90		322	2260	[215]	
20-Aug-00	218	1483	2493	6700	[308]	
06-Sep-00	1018	18374	6352	13081		
23-Sep-00	653	10285	15359	7172	1015	
09-Oct-00	402	8477	5123	6894	[333]	
21-Oct-00	452	6323	5925	10608	562	
08-Nov-00	391	2937	8447	7758	[1114]	
19-Nov-00	166	636	2563	4885	[516]	
02-Dec-00	207		3003	7722		
18-Dec-00	198		1046	8842	[369]	
03-Jan-01	249			3341	8798	[761]
18-Jan-01	152			2622	4764	[488]
17-Feb-01	107			2012	3182	[349]
06-Mar-01	173			4293	3899	[771]
16-Mar-01	68			1230	1814	[479]
01-Apr-01	118			1669	2916	
14-Apr-01	357		3046	3736	5166	[379]
28-Apr-01	234		3504	2776	5843	
12-May-01	432		1074	2619	8326	[10362]
27-May-01	505		9399	7829	1531	[863]
11-Jun-01	450		16353	5722	[837]	[349]
14-Jul-01	360	[783]	2835	14473	[560]	
28-Jul-01	341	3816	1060	7976	[397]	
11-Aug-01	138	1837	1408	3382	[522]	
25-Aug-01	250	3614	1467	4634		
08-Sep-01	652	8208	9956	6435	[735]	

A maximum of 4 *Hydrobia* cohorts were separated from composite size-frequency distributions during any given period. These varied significantly in density (Table 4.2) and mean size (Figure 4.2), during the study. When sampling began in June 2000 there were 2 distinct cohorts (Figure 4.2). These appear to have consisted of the YOY spring cohort (2.5±0.6mm SH) recruited during April 2000, and the 0+ cohort recruited into the population during 1999 (4.2±0.6mm SH). There were also low

numbers of larger individuals (5-8mm SH), probably from recruitment in 1998. This 1+ group (Table 4.2), were not present in sufficient numbers to form a significantly distinguishable normally distributed cohort using MIX. The same groups were present, without showing signs of growth, until settlement of the YOY autumn cohort was detected on the 20 August. Density in this cohort reached a maximum of 18,374 individuals m^{-2} on the September 6th 2000. Although numbers in this newly detected cohort declined rapidly after this date, a corresponding increase in abundance in the YOY spring cohort was observed. It is likely that the YOY cohorts merged over this time due to differential growth rates and high mortality rates in juveniles. Without evidence for immigration of large numbers of <2mm SH individuals from outside the study area to supplement the spring YOY cohort, this would suggest that mortality did not account for all losses to the YOY autumn cohort. Many individuals appear to have merged with and be indistinguishable from the YOY spring cohort, even though outside the obvious growth phase (Figure 4.2). This merger was therefore likely a result of differential mortality and high variability in shell height within the cohort. The autumn YOY cohort merged fully with the YOY spring cohort during December (2.2±0.5mm SH), and persisted into the following year to become the 0+ cohort (Table 4.2).

Table 4.3. Average growth rate of *Hydrobia* (mm day^{-1}) derived from field data.

Data Source	Cohort	Original SH (mm)	Mean growth rate (mm $day^{-1}x10^{3}$)	Growth phase (days)	Density (ind. m^{-2})
Cohort analysis	YOY	1.3±0.3	21.0±5.0	60	10,000-24,000
	0+	2.2±0.8	30.0±13.3	60	
	1+	4.5±0.6	20.0±10.0	60	
	2+	5.7±0.6	-	60	
Model-Von Bertalanffy	YOY & 0+	1.6±0.7	12.0±12.0	210	-
	1+	4.1±1.5	9.0+25.0	210	
	2+	6.0±2.2	-	210	
Inter-annual estimate	YOY	1.1±0.4	18.0+6.7	60	-
	0+	2.2±0.8	30.0+13.3	60	
	1+	4.0±0.6	25.0+10.0	60	
	2+	5.5±0.5	-	60	

Overall, linkage of mean SH (±S.D.) suggested that there were prolonged periods of the year when little or no growth occurred within the population (Figure 4.2). Growth phases were detected only between April and June 2001 (60days) for all cohorts, although apparent merging of the 1+ and 2+ cohorts confused interpretation for larger

individuals. Between April and June 2001 the mean size of the 1+ cohort appeared to increase from 4.5±0.6mm SH to 5.9±0.6 mm, or 0.02mm day^{-1} (31% of initial SH) (Table 4.3), but after the beginning of June it was not possible to isolate this cohort to statistical significance. The average SH of the 0+ cohort increased from 2.2±0.8mm to 4.5±0.4mm during the same period, corresponding to a growth rate of 0.03mm day^{-1} or 110% of original mean SH (Table 4.3). The YOY spring settlement of 2001 that established at the start of the growth period, and peaked in abundance on the 11 June with 16,353 individuals m^{-2}, also appeared to grow rapidly over the ensuing 2 months. Mean SH increased from 1.3±0.3mm to 2.8±0.7mm during growth phase, a rate of 0.021mm^{-1} day or 118% of original SH for the season on average (Table 4.3). No growth was detected after June, with the only significant addition to biomass occurring with the YOY autumn recruitment from late July 2001, although no significant growth in this cohort was detected before September. The spring and autumn YOY 2001 cohorts showed earlier signs of merging in September, but this coincided with the termination of the sampling programme.

The seasonalised von Bertalanffy growth function yielded a growth curve with high associated error when applied to the YOY autumn cohort (Figure 4.3). This was mainly due to the very truncated growth phase observed within the actual population and the merging of cohorts, which were poorly fitted in the growth model. In fact, the model predicted longer periods of growth (7 months) compared with those with no growth (5 months), which is a common error due to the oscillating nature of the model. Although the extent of the growth phase did not match field data derived from cohort analyses alone (2 months growth, 10 months no growth), the predicted mean sizes-at-age during the winter (no growth) periods for the 3 yr modelled, 1.6±0.7 mm, 4.1±1.5 mm and 6.0±2.2 mm (Table 4.3), were comparable to field data of 1.3±0.3 mm (prior to the cohort mixing with the 2nd cohort), 4.5±0.6 mm, and 5.7±0.6 mm respectively. The error associated with these size estimates was, however, very high and increased at larger sizes. Low growth rates of 0.012 and 0.009mm day^{-1} were generated for the 2yrs modelled due to the prolonged duration (210 days) of the growth phase estimate (Table 4.3).

Mortality estimates based on seasonal changes in cohort strength were invalid during periods when possible merging of cohorts occurred. It was not possible to distinguish

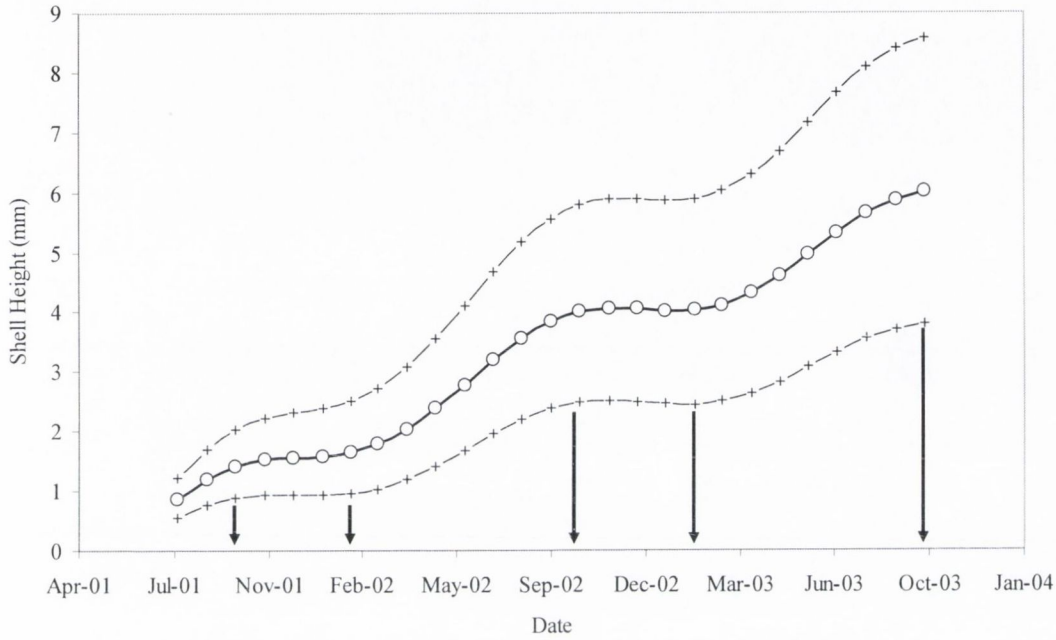
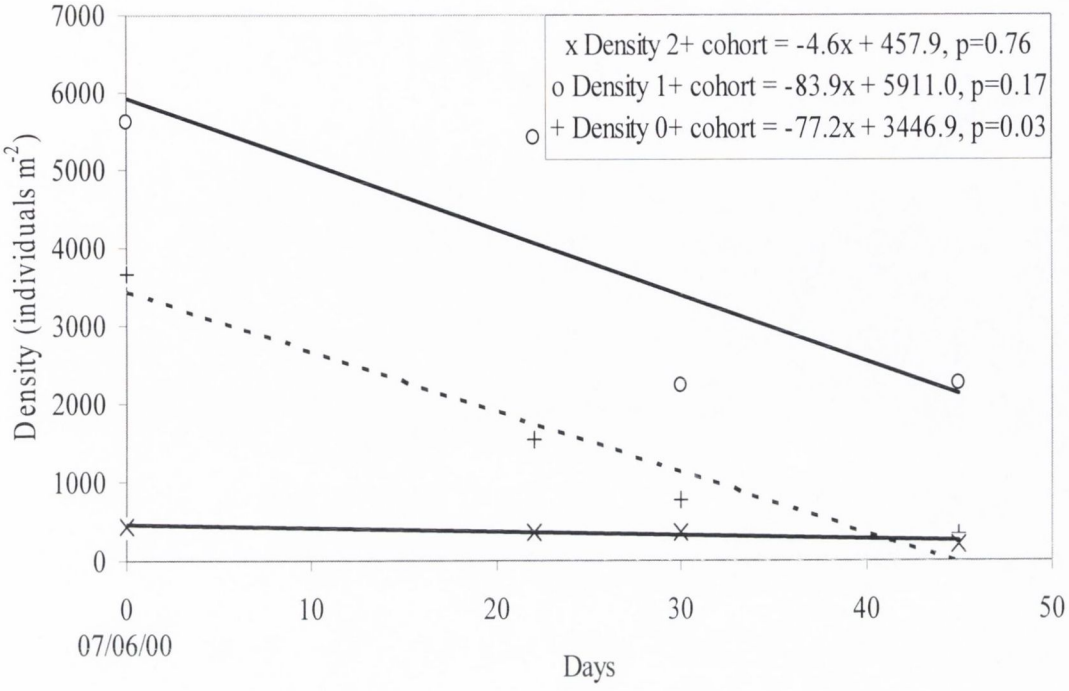


Figure 4.3. Seasonalised von Bertalanffy growth function for the autumn cohort of *Hydrobia ulvae*, Bull Island. Circles equal mean estimate, crosses ±S.E. Downward arrows indicate the start and end of successive growth seasons.

the relative influences causing reduction in cohort strength at these times. Even during periods when merging was not evident, it is possible that short-term localised changes in cohort-specific distribution patterns or migration may have influenced cohort strength recorded at the site. The strength of each cohort during the 2 periods of the study when merging was not evident is shown in Figure 4.4 a, b. When a linear decrease in numbers (mortality) and no net loss/gain due to other factors was assumed, only the 0+ cohort from 2000 and the 1+ cohort from 2001 showed significant relationships. The former corresponded to a mortality rate of 77 individuals day^{-1}, the later 60. Due to obvious limitations, these figures were treated with appropriate caution.

The shell heights (SH) of over 25,000 *Hydrobia* (15,921, 4,470, 4,391 and 333 for cohorts 0+, 1+, 2+ and 3+, respectively) were recorded during the course of the investigations described in 2.2.6, which was conducted during April 2000. Mean density decreased from 10,851 individuals m^{-2} ± 1,236, in the 0+ cohort to 3,047 m^{-2} ± 331, 2,991 m^{-2} ± 266 and 227 m^{-2} ± 52 in the 1+, 2+ and 3+ cohorts, respectively. No clear relationship existed between the densities in each cohort, however, invalidating calculation of inter-annual mortality (Figure 4.5). Mean SH±S.E. at this time (April 2000) increased from 1.1mm±0.4 in cohort 1, to 2.2mm±0.8, 4.0mm±0.6 and 5.5mm±0.5 in cohorts 2, 3 and 4, respectively, corresponding closely to SH recorded during routine monthly sampling conducted in April 2001. A statistically significant inter-annual linear correlation was observed between the mean shell heights in each cohort (Figure 4.6), which approximated growth when variability in annual cohort specific growth rate was assumed non-significant. When a growth period of 60 days was assumed, growth rates of 0.018, 0.046 and 0.091mm day^{-1} for the 0+, 1+ and 2+ cohorts, respectively, were derived (Table 4.3). These corresponded to a 100% increase in shell height during the first year's growth for the 0+ cohort, 82% for the 1+, and 38% for the 2+. These growth rates were comparable with those estimated from cohort analysis of time-series size-frequency data (Table 4.3).

a)



b)

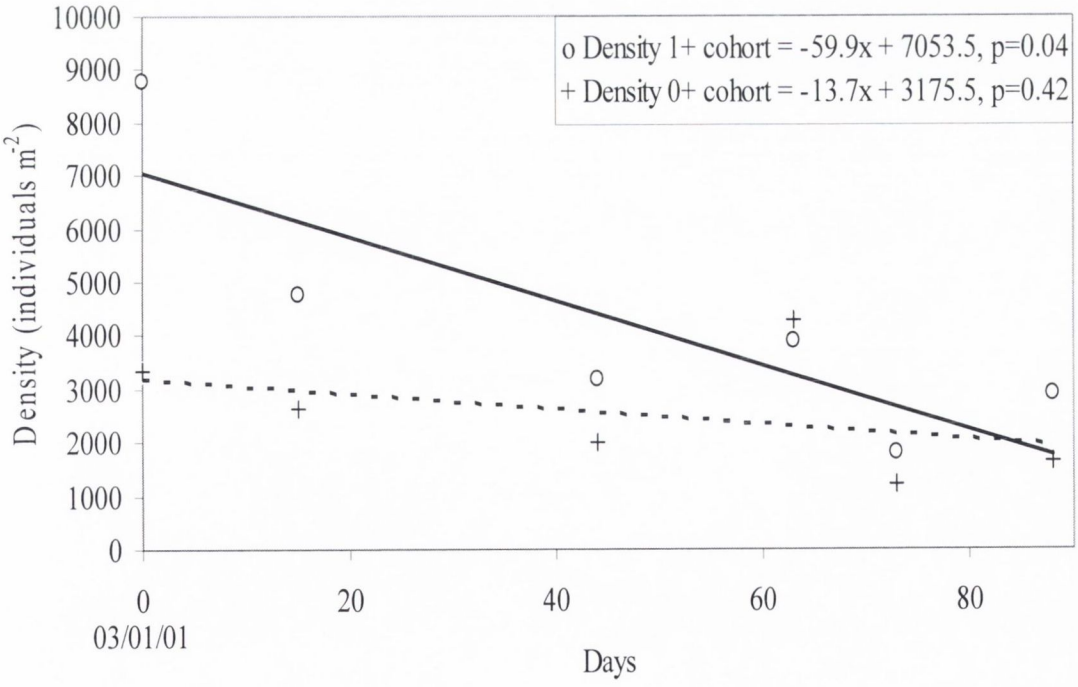


Figure 4.4 a-b. Linear regression lines fitted to density in *Hydrobia* cohorts at Bull Island during 2 phases when significant merging of cohorts was not evident. a) N=746, b) N=867.

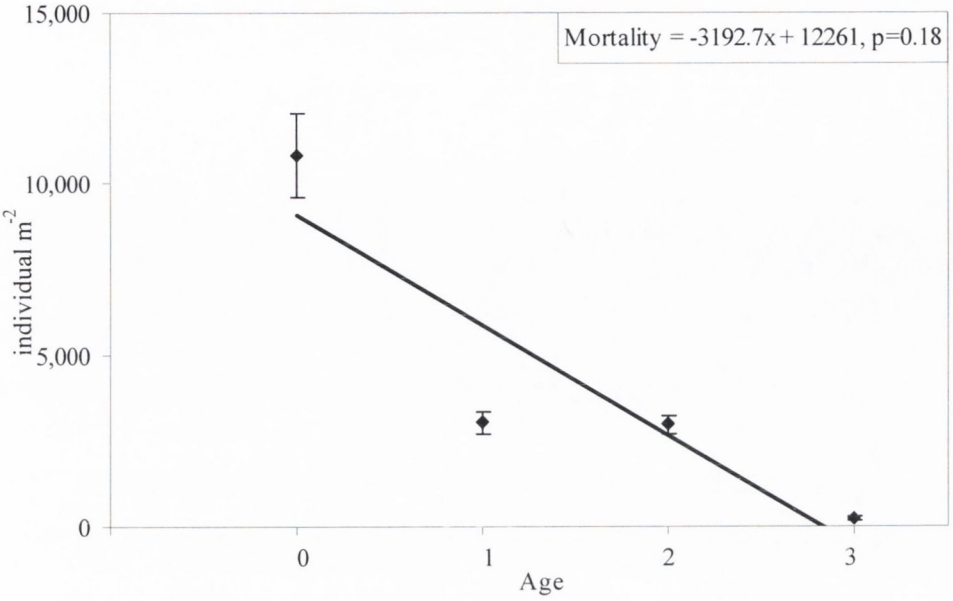


Figure 4.5. Mean density (individuals m^{-2}±C.I) of *Hydrobia* per cohort. Data collected as described in section 2.2.6. N=25,105.

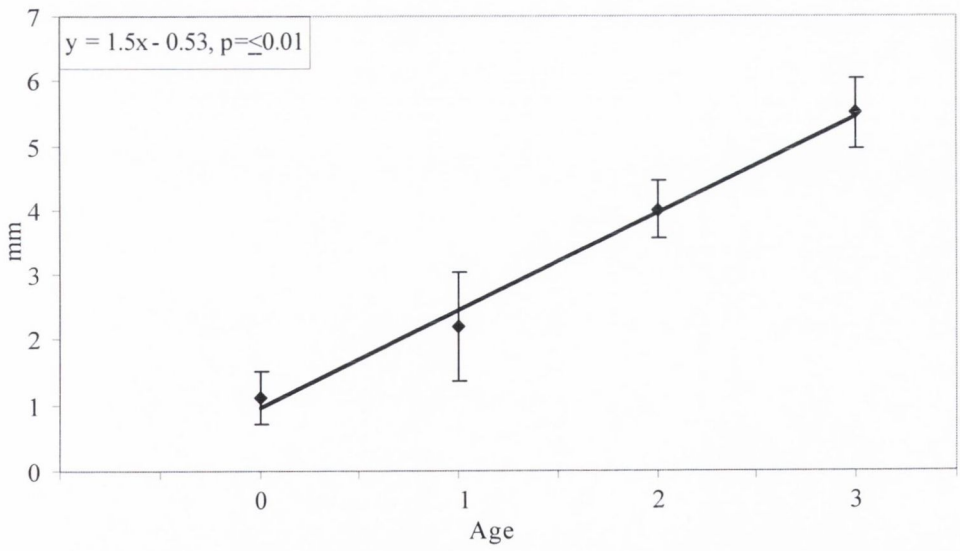


Figure 4.6. Estimate of inter-annual growth for *Hydrobia*. Calculated from mean SH per cohort ± C.I, data collected as described in section 2.2.6. N=25,105.

4.3.2 Tellina size-at-age

4.3.2a Size-frequency distribution

Size-frequency histograms of *Tellina* distributions sampled between June 2000 and September 2001 were often fragmented and difficult to interpret (Figure 4.7), as was expected from previous studies at the Blackrock site (see Chapter 2). High variation in size-frequency and abundance occurred over relative short periods of time. Recruitment pulses were difficult to define, and even when low numbers of very small individuals occurred, they often rapidly disappeared prior to the next sampling occasion (see June 2000, Figure 4.7). As expected, it was not possible to separate normal distributions from the composite size-frequency distribution using MIX for any of the sampling efforts. When divided into the arbitrary cohorts assigned in Chapter 2 (<4mm, 4–8mm, 8-12mm, 12–16mm and >16mm SH) abundance remained highly variable, although some weak trends appeared to occur. The <4mm SH cohort, assumed to represent settlers, had low densities of <250 m^{-2} throughout the study, which was considered surprising in comparison with the much higher abundance in larger, previously settled size classes. Strong and weak size classes in particular years are common in this species with total recruitment in some years frequent (Stephens, 1928; Barnett & Watson, 1986). The 4-8mm and 8–12mm SH cohorts had highest densities in late May-June, and general decreased through the rest of the year. The 12–16mm SH cohort showed lowest densities over the winter months, increasing over the summer months to a high in August. Abundance within the >16mm cohort fluctuated at a low level (<200 m^{-2}) over the entire sampling period.

4.3.2b Age readings

Although multiple age band readings were conducted it was not possible to increase the precision of estimates from successive age readings for the majority of individuals examined. The first eight readings produced significantly different age-length curves, without decrease in error between successive readings occurring (Kruskal-Wallis, χ^2=33.3, df=7, $p \leq 0.01$). Difficulty in distinguishing annuli from disturbance rings resulted in a high degree of subjectivity when assigning age and it was not possible for the reader to become more proficient in reading annuli despite practice. Although 'winter phase' individuals were used, the end of the fast growth phase could not

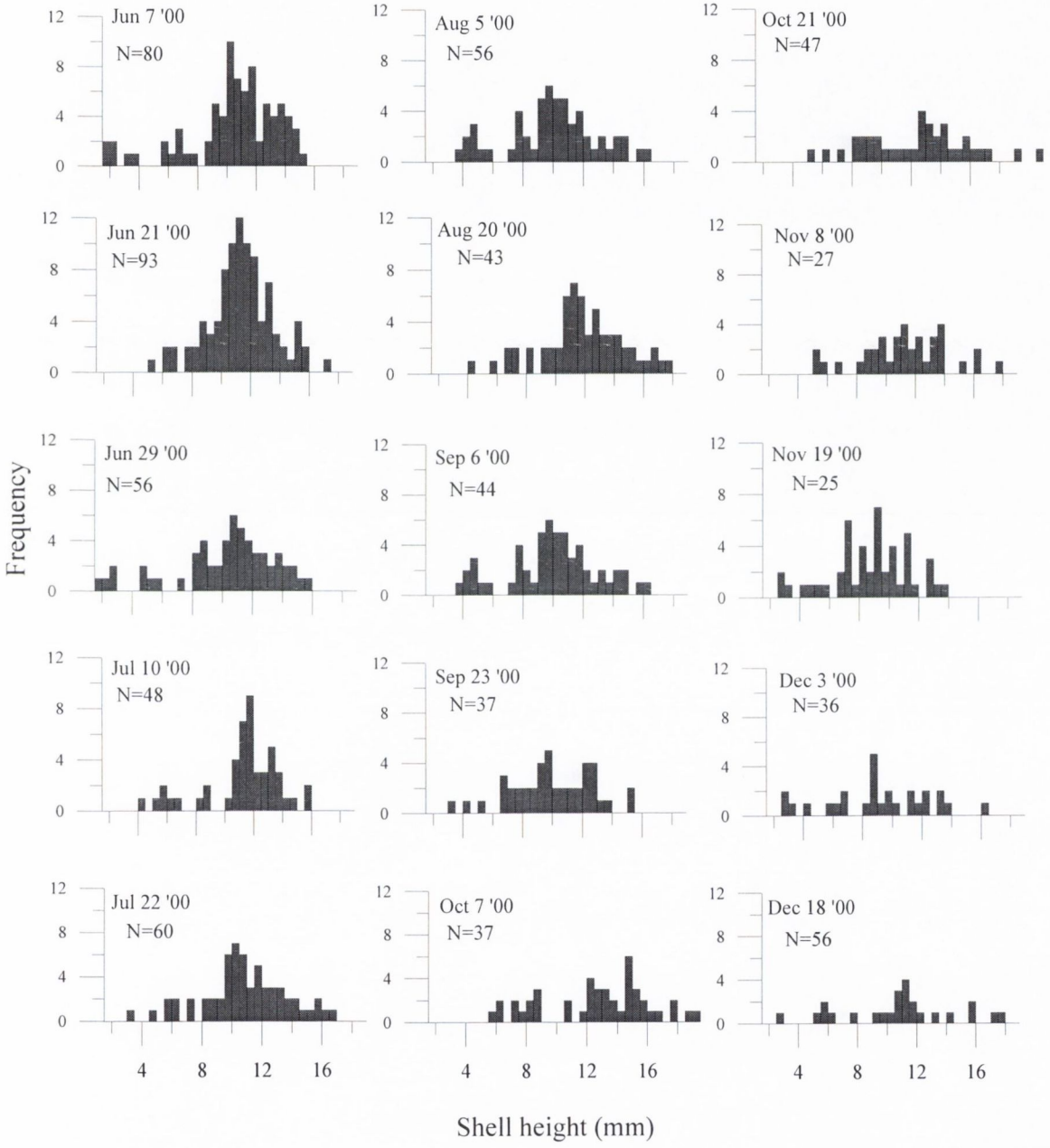


Figure 4.7. Size-frequency (shell height mm) histograms of *Tellina* from Blackrock, Dublin; June 2000 – September 2001. (continued overleaf).

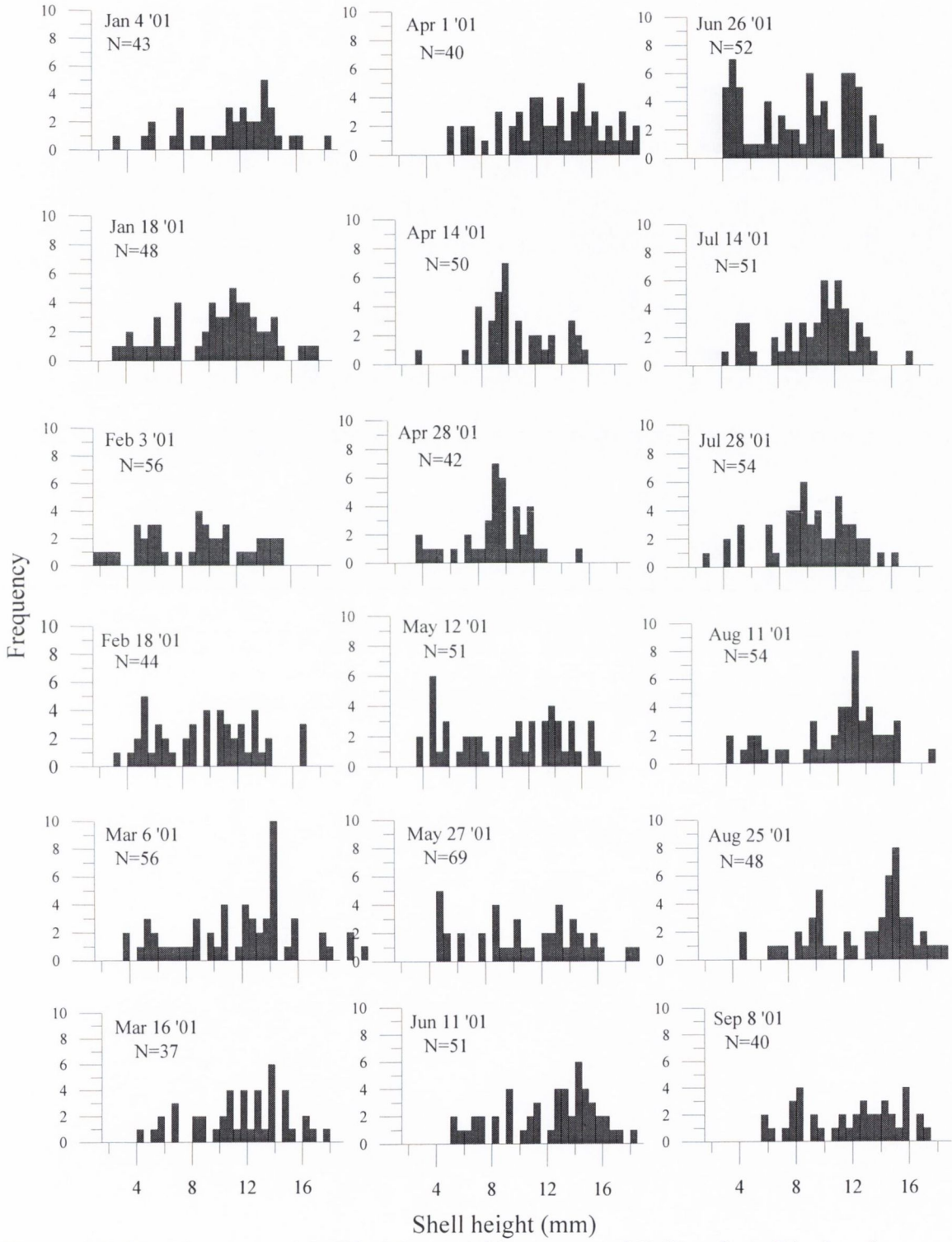


Figure 4.7. Size-frequency (shell height mm) histograms of *Tellina* from Blackrock, Dublin; June 2000 – September 2001. (continued).

always be identified, especially in larger (>10mm SH) individuals. The mean 'size-at-age' measured showed an increasing divergence of estimates as age increased (Figure 4.8). Kolmogorov-Smirnov tests showed significant differences in size-at-age between readings at all age classes. Several readings conducted by the second reader also resulted in unacceptable variation in age estimates.

4.3.3 Measurement accuracy of repeated shell measurements-determining growth

The range of differences between repeated size readings for *Tellina* and *Hydrobia* are shown in Table 4.4. Measurement error was greater than the accuracy of the digital callipers used for both species, but less than 0.1mm for all readings and for all individuals measured. This integer was therefore established as the measurement accuracy for all data relating to growth of these species. Individuals failing to increase in size by this amount during any laboratory growth experiment were considered not to have grown.

Table 4.4. Range of differences in shell height (mm) recorded during repeated measurements of the same sub-sample (N= 60) of *Hydrobia* and *Tellina*.

Species	Reading 1-2	Reading 1-3	Reading 2-3
Hydrobia	0–0.08	0–0.05	0–0.09
Tellina	0–0.06	0-0.09	0–0.07

4.3.4 Hydrobia growth and mortality estimates-laboratory data

4.3.4a Size-specific growth-phase duration

Growth rate was not significantly different between months 1 and 2 for any size class when individuals were held at 16°C, low density and with food saturation (Figure 4.9). Mean growth rate actually increased slightly in the 1 and 5mm size classes, from 0.0272±0.0026 to 0.0333±0.0029 mm day^{-1} and 0.0061±0.0009 to 0.0069±0.0018 mm day^{-1}, respectively (Table 4.5). Although the 1mm SH size group displayed the highest growth rate between month 1 and 2, 0.0333±0.0029 mm day^{-1}, the growth rate of the 2mm size class had been highest during the first month, 0.0309±0.0018 mm day^{-1}. A significant decrease in growth rate was observed for all size classes between months 2 and 3. The magnitudes of these decreases were not

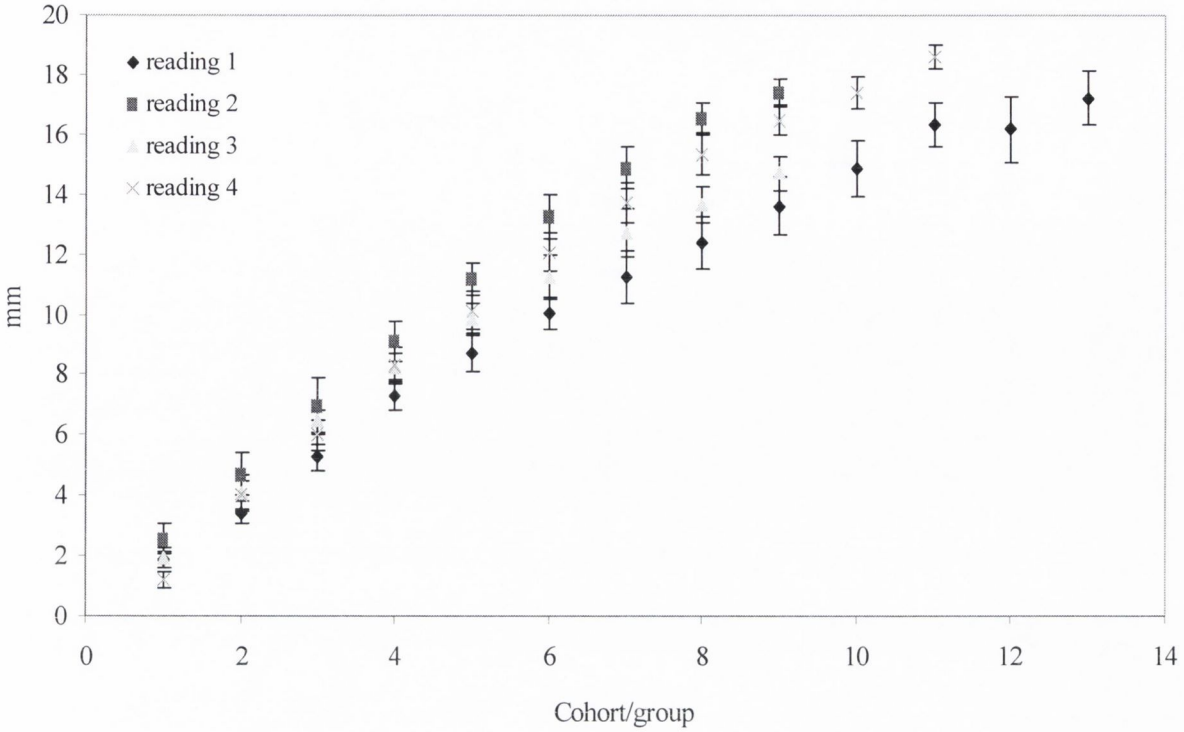


Figure 4.8. Size-at-age for *Tellina* estimated by reading shell growth rings on 100 individuals. Only the 4 most significant age-at-size curves were plotted for clarity.

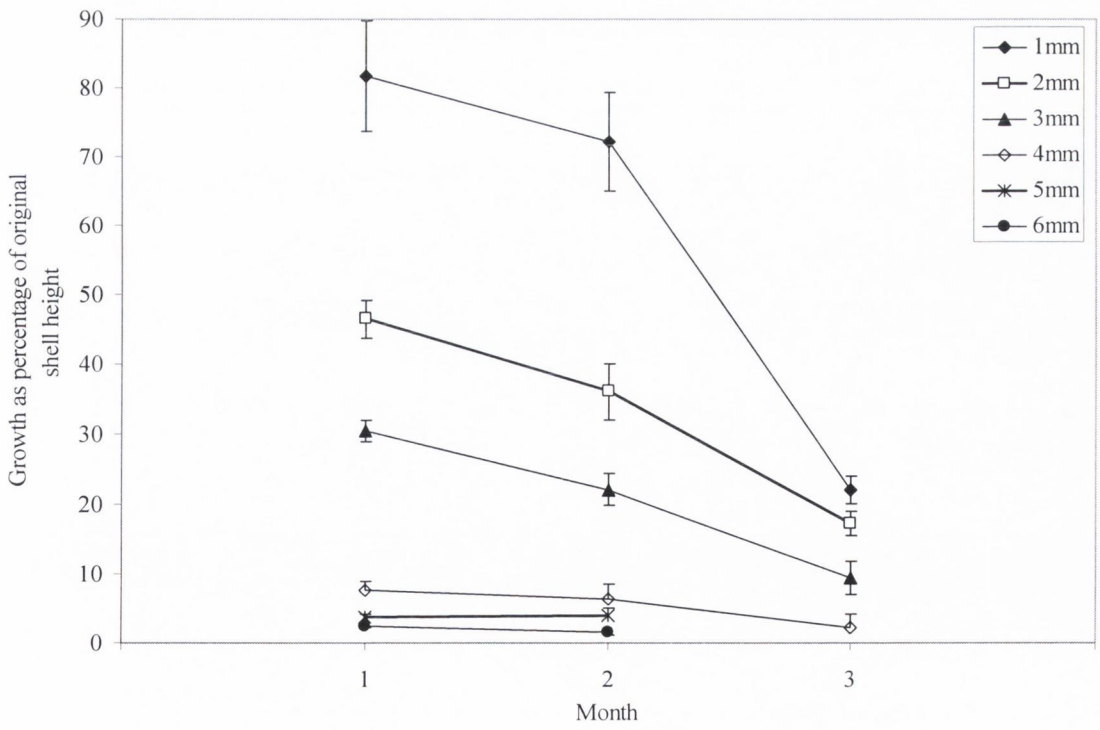


Figure 4.9. Growth of *Hydrobia ulvae* in the laboratory over a 3 month period at 16°C with food saturation. Density 500 individuals m^{-2}. N=150.

proportional to increasing body size (Figure 4.9). For example, individuals in the 1 mm size class had increased in size to approximately 1.8 mm SH after the first month growth. The second month's growth was very similar to that of 2 mm SH individuals during the first month, leaving individuals from the original 1mm SH size class with a mean SH of approximately 2.7mm. The growth rate achieved by these individuals in the third month, 0.0075+0.0018 mm day^{-1} (Table 4.5), was lower than that achieved by individuals with an original SH of 3mm in either the first or second month. Growth in the original 1, 2, 3 and 4mm SH size classes was generally between 50-75% lower than could have been expected between months 2-3, the 5 and 6mm SH classes failing to display significant growth to eliminate the possibility of measurement error. Significant mortality did not occur during the course of this experiment, with >90% of individuals persisting until the end of the third month. Although extension of the experimental duration was planned, a cooling unit failure lead to significant temperature fluctuations during the fourth month and the experiment was abandoned.

Table 4.5. Mean growth (±S.E.) rate of *Hydrobia* (mm $day^{-1}x10^{3}$) in arbitrary size classes (mm SH) at 16°C for 3 months under saturated food conditions. N=150.

Month	1mm	2mm	3mm	4mm	5mm	6mm
1	27.2±2.6	30.9±1.8	15.1±1.6	10.3±1.6	6.1±0.9	4.7±0.4
2	33.3±2.9	28.3±2.7	12.6±2.3	9.0±2.8	6.9±1.8	3.1±0.8
3	7.5±1.8	7.6±1.2	3.8±2.4	2.9±2.7	-	-

4.3.4b Size-specific growth-temperature

Size-specific mean increase in SH was significantly different between temperature treatments and size classes (2-way ANOVA, F=32.3, df=3, $p<0.01$) although the interaction between size and temperature was not significant (Figure 4.10). There was a significant difference in growth in the 1mm SH size class between the 6 and 16°C, and the 16 and 20°C temperature treatments, and between the 16 and 6°C treatments for the 2 mm SH size class. Although mean increase in SH was greatest in the 16°C treatment for all other size classes, differences were not statistically significant (ANOVA, F=1.27, df=2, p=0.30). Lowest mean growth occurred in either the 6 or 20°C treatments depending on original SH (Figure 4.10). The proportional increase in size in the 1 mm SH size class was significantly greater than all other sizes in the 6, 12

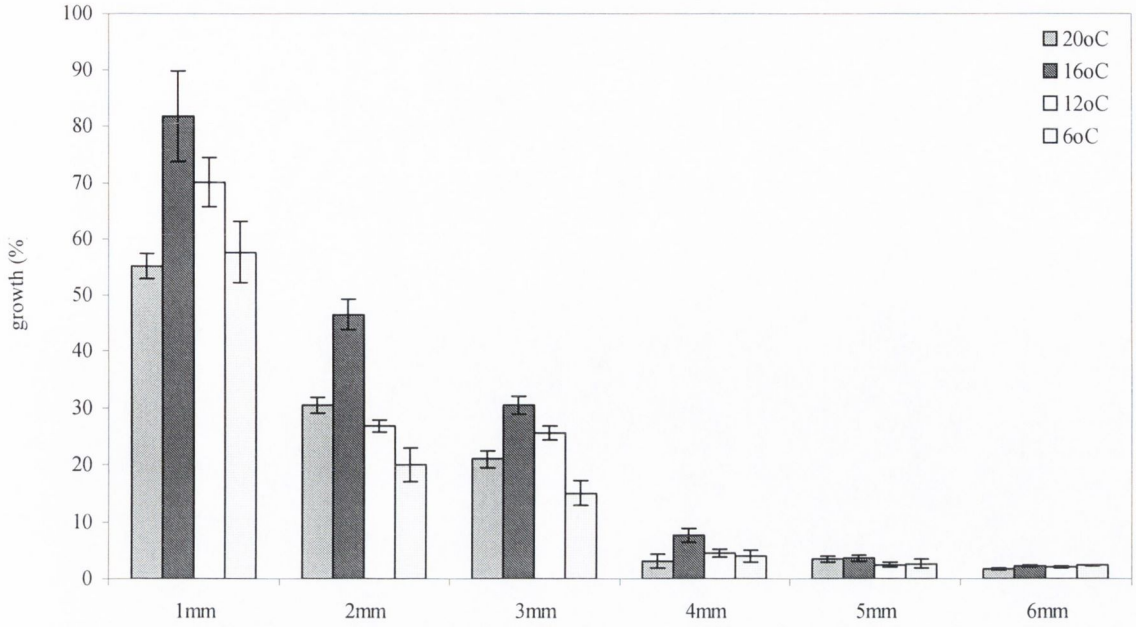


Figure 4.10. Percentage increase in growth of *Hydrobia,* as a proportion of original SH, in different arbitrary size classes over a one month period with varying temperature in the laboratory. N=360.

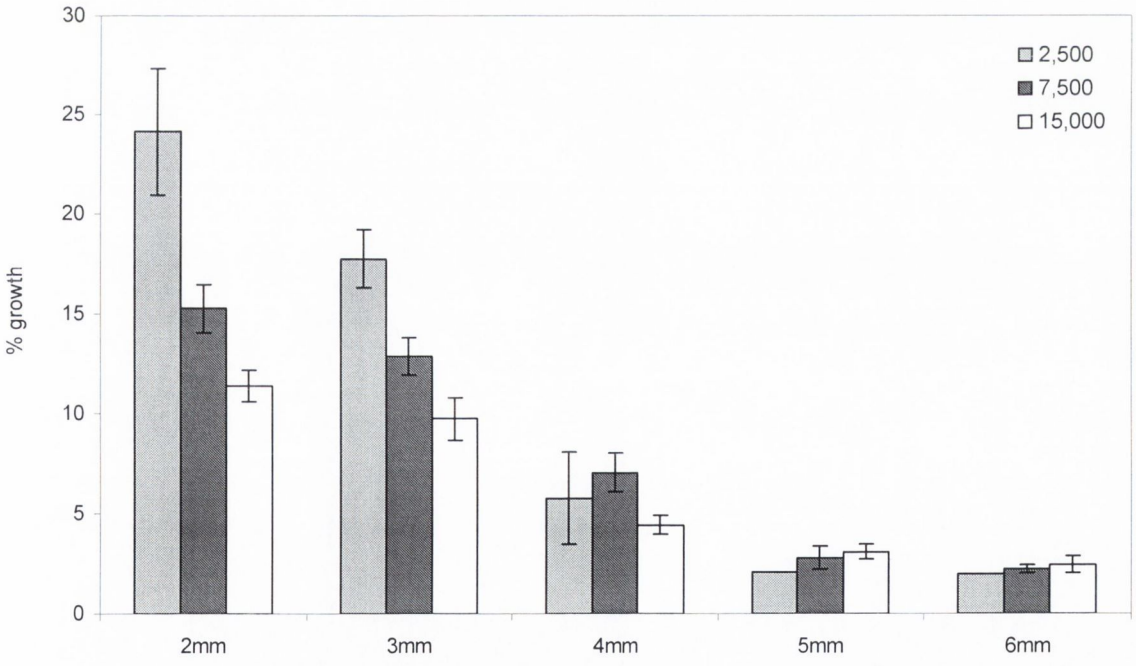


Figure 4.11. Percentage increase in growth of *Hydrobia,* as a proportion of original SH, in different arbitrary size classes, held at 3 varying densities (individuals m^{-2}) during a one month period in the laboratory. N=500.

and 16°C temperature treatments, and from all but the 2 mm size class at 20°C. Growth in the 2mm size class was significantly higher than the 4, 5 and 6 mm classes in the 12, 16 and 20°C treatments, with no differences occurring in the coldest treatment. The 3 mm size class increased proportionally more than all larger size classes in the 16°C treatment only.

Table 4.6. Mean growth (±S.E.) rate of *Hydrobia* (mm $day^{-1}x10^{3}$) in arbitrary size classes (mm SH) from 4 constant temperature treatments for one month under saturated food conditions. N=85.

Temperature	1mm	2mm	3mm	4mm	5mm	6mm
20 °C	18.1±2.1	10.0±1.1	6.9±0.8	1.1±0.4	1.2±0.1	0.6±0.1
16 °C	27.0±2.7	15.5±0.9	10.2±0.5	2.6±0.4	1.2±0.2	0.8±0.1
12 °C	23.1±2.3	8.8±0.8	8.4±0.9	1.5±0.2	0.8±0.1	0.7±0.1
6 °C	18.9±1.5	6.6±0.9	4.9±0.7	1.3±0.3	0.9±0.1	0.8±0.1

The 1mm SH size class displayed the largest increases in size, with a mean increase of 82±8% in the 16°C temperature treatment. This corresponded to a growth rate of 0.027±0.003mm day^{-1} (Table 4.6), which was very similar to growth rates derived from field data for the 0+ cohort (Table 4.3). The average growth rate of 1mm sized snails over all temperatures corresponded to a growth rate of 0.022 mm day^{-1} which was also comparable with the growth rates of the smallest cohort identified from field data (Table 4.3). Mean growth rate decreased with increasing SH for size classes 2 to 6mm, respectively, in all temperatures (Table 4.6). Highest growth rates in the 2 and 3mm (SH) size classes also occurred in the 16°C treatment, 0.015±0.0009 mm day^{-1} and 0.010±0.0004 mm day^{-1} respectively. These growth rates were lower than inter-annual estimates for the 0+ cohort of 0.046 mm day^{-1}. Growth rates in the 4mm size class were all <0.003 mm day^{-1} (Table 4.6). Corresponding rates for similar sized individuals, the 1+ cohort (~4mm SH), were higher in both field based approximations, although were similar to the von Bertalanffy model estimates (Table 4.3). Lowest growth occurred in the 6mm size class with mean increases of <3% in all temperatures (Figure 4.10). Growth rates recorded for each size class in the large Petri-dishes were not significantly different to those for smaller dishes presented in Table 4.6. Hence surface area was not a factor limiting growth in this experiment.

4.3.4c Size-specific growth-density

Size-specific mean increase in SH was significantly different between density treatments and size classes (2-way ANOVA, F=234.4, df=5, $p \leq 0.01$) although the interaction between size and temperature was not significant (Figure 4.11). For the 2 mm SH size class, significant differences occurred between all treatments with growth decreasing from 24.1±3.2% to15.3±1.2% to 11.4±0.8% from the low to high density treatments respectively. This corresponded to growth rates of 0.008 mm day^{-1}, 0.005 mm day^{-1} and 0.004 mm day^{-1} respectively (Table 4.7). Individuals with an original SH of 3 mm grew significantly more in the low density (17.8±1.5%, 0.006 mm day^{-1}) treatment when compared to the highest (9.7±1.0%, 0.003 mm day^{-1}) (Table 4.7), although no differences occurred between the mid-density treatment and either high or low density. No significant differences in growth between density treatments were observed for the 4, 5 or 6 mm SH size classes. Growth in these size classes was significantly less than for the 2 and 3 mm classes in the medium density treatment. The proportional increase in shell height was significantly higher in the 2 mm SH size class compared with those of an original SH ≥4 mm in the low and high density treatments. Considering the temperature and food availability, growth rates recorded during this experiment were significantly lower than those recorded over similar durations in the experiments described in 4.3.3a & b above. Density (500 individuals m^{-2}) used in these previous experiments was much lower than utilised here.

Table 4.7. Mean growth (±S.E.) rate of *Hydrobia* (mm $day^{-1}x10^{3}$) in each size class at densities low (2,500m^{-2}), medium (7,500m^{-2}) and high (15,000m^{-2}). N=500.

Density	2mm	3mm	4mm	5mm	6mm
2,500 m^{-2}	8.1±1.1	5.9±0.5	1.9±0.8	0.7±0.3	0.6±0.2
7,500 m^{-2}	5.1±0.4	4.3±0.3	2.3±0.3	0.9±0.2	0.7±0.1
15,000 m^{-2}	3.8±0.3	3.2±0.4	1.5±0.2	1.0±0.1	0.8±0.1

4.3.4d The effect of food availability and density on growth

Although increase in size above that required to distinguish growth from measurement error occurred in the treatments with low and high food availability, this was not the case for the 'no food' treatment regardless of size class and/or density treatment (Figure 4.12). The latter treatment was omitted from subsequent multi-factorial ANOVA analysis for this reason. Both variation in density and food

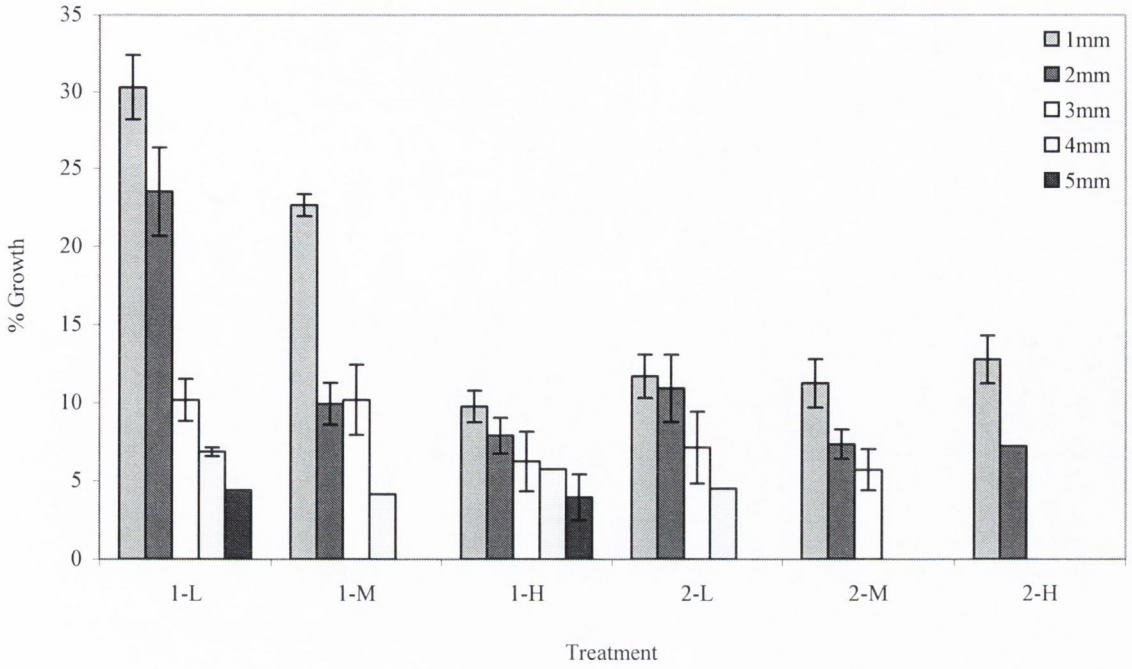


Figure 4.12. Percentage increase in growth of *Hydrobia,* as a proportion of original SH, in different arbitrary size classes, held at 3 varying densities (L=2,500m^{-2}, M=7,500m^{-2}, H=15,000m^{-2}) and 3 enrichment treatments (1=enriched, 2=normal, 3=depleted) during a one month period. Treatment 3 is not shown as no growth occurred in any size class in this treatment. N=540.

availability resulted in significant differences in size-specific growth rate for the remaining treatments when these parameters were considered in isolation (both $p \leq 0.001$). The combined interaction of these factors also had a significant impact on size-specific growth rate (ANOVA, $F=32.29$, $df=4$, $p \leq 0.01$). At low and medium densities, high food availability significantly increased the growth of 1 mm SH individuals, 0.0101±0.0007 and 0.0076±0.0002 mm day^{-1} respectively, when compared with the same densities with low food supply, 0.0039±0.0005 and 0.0033±0.0005 mm day^{-1} (Table 4.8). Low density and high food availability also resulted in significantly higher growth for the 2mm SH size class when compared with similar densities with low food availability (Table 4.8). This suggested that food limitation (but not its absence) resulted in a decrease in growth rate of smaller individuals only when densities were particularly low. Increasing density itself therefore appeared to elicit the greatest effect on the growth rate of *Hydrobia*. Although a similar trend appeared to be true for larger individuals, growth increments were too small and too sparse to result in significance. Although interaction between food availability and density resulted in variation in growth rate, it should be appreciated that theoretically, the lack of growth in the 'no food' treatment would have occurred regardless of density. It should be noted that the effect of 'Phytoplex' was not examined in isolation. Pseudoreplication between the medium and high food treatments occurred. Both these treatments received phytoplex but the medium food treatment had filtered seawater while the high food treatment did not. Further experiments on the effect of food and density would be required to confirm the above results.

Table 4.8. Mean growth (±S.E.) rate of *Hydrobia* (mm $day^{-1} \times 10^{-3}$) in each size class at low, low and high enrichment status and at densities low ($2,500 m^{-2}$), medium ($7,500 m^{-2}$) and high ($15,000 m^{-2}$).

Enrichment	Density	1mm	2mm	3mm	4mm	5mm	6mm
High	2,500 m^{-2}	10.1±0.7	7.8±0.9	3.4±0.4	2.3±0.1	1.5	-
High	7,500 m^{-2}	7.6±0.2	3.3±0.4	3.4±0.7	1.4	-	-
High	15,000 m^{-2}	3.3±0.3	2.6±0.4	2.1±0.6	1.9	1.3	-
Low	2,500 m^{-2}	3.9±0.5	3.6±0.7	2.4±0.8	1.5	-	-
Low	7,500 m^{-2}	3.3±0.5	2.5±0.3	1.9±0.4	-	-	-
Low	15,000 m^{-2}	4.3±0.5	2.4±0.5	-	-	-	-
None	2,500 m^{-2}	-	-	-	-	-	-
None	7,500 m^{-2}	-	-	-	-	-	-
None	15,000 m^{-2}	-	-	-	-	-	-

4.3.4e Comparing field and laboratory growth rate data-validation

Due to the differences in the way in which laboratory and field-based growth rate estimates were derived, it was not possible to formally compare the results. Field-based estimates were not derived from the repeated measurement of the same individuals, as was the case for laboratory experiments. Indirect comparison between these data where, however, relevant for the purpose of validating growth rate estimates derived.

The main growth phase observed in the field-based *Hydrobia* population, April and June 2001, corresponded to a period when densities ranged between 12,000 and 23,000 individuals m^{-2}. The highest density treatments (15,000 individuals m^{-2}) in the various laboratory growth experiments described above were most comparable with the abundances recorded in the field during the growth phase. Laboratory growth rates were much lower than field rates for the 2 experiments where density exceeded 500 individuals m^{-2} (Tables 4.7 & 4.8). The growth rates recorded for each density with high food availability in experiment 4.3.5d were not significantly different from those recorded in the earlier experiment 4.3.5c, when density was varied with equally high food saturation. Growth rates very similar to those recorded in the field were recorded for 1-2mm SH individuals in experiments 4.3.5a and b, which were conducted with experimental densities less than 500 individuals m^{-2}. Larger sized specimens did not achieve the growth rates predicted using field-based data in any laboratory-based experiment. Environmental water temperatures recorded during the growth season approximated to those in the 16°C laboratory-based experiment 4.3.b and the resultant growth rates obtained were almost identical to those for smaller size classes in the field. As previously stated, the laboratory-based experiment was conducted at much lower densities and, again, the growth rate of larger (>3mm SH) individuals was below that predicted by field data.

4.3.5 Tellina growth estimates-laboratory data

Of the 318 individual *Tellina* used in the 3 different temperature treatments to examine growth, only 7 individuals increased in size by the required 0.1mm (see

4.3.4) to register as 'growth' in the course of 4 months. Five of these individuals were recovered from the 20°C treatment prior to death, 2 from 12°C. In the 20°C treatment a growth rate of 0.0003 mm day^{-1} was recorded in 1 individual with an original SH of 3.9mm. Rates between 0.0004 and 0.0008mm day^{-1} were recorded for the remaining individuals in the size classes 8-12 mm and 12-16 mm. The 12°C treatment tank had a growth rate of 0.0004 mm day^{-1} for two individuals with original SH's of 3.2 and 13.7 mm. These growth rates were lower than rates extrapolated from the field data above in section 4.4.4a, and would not have been expected for individuals with such variable original SH. Mortality was extremely high during the course of the experiment, which further indicated the unreliability of the growth rate estimates (Table 4.9). Survivorship was highest in the low temperature (6°C) with an overall mortality rate of 35%, although no growth occurred. Larger individuals between 8-16mm SH showed the highest survival rate in at this temperature. The 12°C temperature treatment had a mortality rate of 49%, again with most survivors coming from the larger size classes. No individual survived in the 20°C treatment.

Table 4.9. Percentage mortality of *Tellina* in each size class at each temperature.

Size class (mm)	Temperature (°C)	N	% Mortality
All	20	106	100
	12	106	49
	6	106	35
<4	20	5	100
	12	5	100
	6	5	100
4–8	20	20	100
	12	20	65
	6	20	65
8–12	20	35	100
	12	35	50
	6	35	26
12–16	20	42	100
	12	42	38
	6	42	19
>16	20	4	100
	12	4	0
	6	4	75

Further attempts to conduct growth experiments on *Tellina* proved problematic due to rapid and high mortalities in aquaria facilities, even when natural conditions were re-created as closely as possible. Growth did not occur at the low temperatures required to reduce high mortality, and the planned series of experiments similar to those conducted on *Hydrobia* were abandoned.

4.4 Discussion

Despite the inability to age *Hydrobia ulvae* from growth annuli, their high abundance and clear cohort groupings made it possible to model growth using time-series size-frequency data. The use of statistically robust and tested techniques removed any subjectivity (not actual error) associated with the derivation of the estimates. Length-based assessment of growth and mortality has become more widely used in the past few decades as the subjectivity associated with age readings can be less easily resolved in fast growing species (Robinson & Tully, 2000a), or those that inhabit polar or tropical regions where 'seasonality' and resultant band formation is much less pronounced (Sparre & Venema, 1992). Although some previous authors have presented results relating to the aging of *Tellina tenuis*, the studies have often been conducted in the absence of statistically robust methodologies (Wilson, 1995). Blind reading and/or multiple readers and validation data in the form of growth rate data, all of which can be used to quantify the level of subjectivity associated with age-based assessments, were not applied and, therefore, the results should be treated with caution. In particular, a high degree of subjectivity occurs when a single reader conducts age reading, especially for small delicate species such as *Tellina* that are believed to lay down disturbance marks frequently (Barnett & Watson, 1986;Gosling, 2003). Increasing precision in the repeated age readings for a sample group produced by a single reader does not necessarily indicate an increase in accuracy if the reader is 'learning their own mistakes'. Length and age-based assessments are equally valid and robust methodologies when suitable protocols are enforced, however, and the later has been shown to be an effective tool for at least *Hydrobia ulvae* in this study.

Although more labour intensive and time consuming, the use of field-based data with a short interval between sampling events was considered more desirable than using laboratory data alone to derive growth curves. The later often provides growth estimates that are based on near optimal and/or fixed conditions for all but the dependent variable(s) examined. Considering the number of variables that have the potential to regulate population growth in the natural environment, and the rarely described density-dependent and independent combined affects of these, laboratory results can be considered overly-simplistic in isolation. In the absence of field-based data relating to growth phase duration, laboratory trials may also under-estimate

growth rate when the former is constrained to the extent observed for *Hydrobia* in the current study. When used in combination with field data, laboratory studies can be a useful tool for identifying the relative effects of regulatory factors however.

The growth rates and size-at-age estimates derived using cohort analysis for size-frequency time-series and inter-annual data were similar for the spring growth phase when the 60 day duration was applied to the later. In the absence of the growth phase duration data obtained from the time-series analysis, the inter-annual growth increment data would have probably been applied to an assumed growth phase of longer duration, creating much lower growth rate estimates. This was highlighted by the estimates produced by the von Bertalanffy function, the oscillating nature of which assumes relatively even periods of growth and no-growth annually. The prolonged slow growth phases predicted by the model would have been similar to those of the inter-annual estimate had the later been calculated over similar growth periods as described above. The commonly used von Bertalanffy growth model was not suitable for *Hydrobia* data presented in this chapter. This model had been shown previously to be unsuitable for application to species with rapid, truncated growth phases (Robinson & Tully, 2000a). The use of time-series size-frequency data to validate growth rate data, and *visa versa*, in the current study highlighted the need for caution when using a single growth data set alone. Similarly this would be true if aged-based assessment had been used. Again, a regular time-series of age readings would have been required to identify the growth phase duration from the actual period when 'summer' and 'winter' marks were laid down, this being validated by supplemental growth data. Had annuli been counted at one time of year then no reliable information on growth phase duration, and hence seasonal growth rate, could have been obtained. The method would still have application in the production of inter-annual estimates, however, which themselves have application for marine environmental monitoring.

Although the growth rates obtained during laboratory experiments showed that the growth potential of *Hydrobia* was sufficient to match those predicted from field data, at least for smaller size classes, the estimates derived in the laboratory experiments were much lower than predicted when experimental density was considered. Growth rates for individuals <3mm SH only matched field-based estimates when individuals

were maintained at densities many times lower than those observed in the field. High food availability did not reduce the regulatory effect of density as the later increased beyond levels that were comparably low in relation with those occurring in the field. This may suggest that density-dependent regulation of food availability or feeding time is the major determinant of growth for *Hydrobia*. This may have been supported by the fact that the growth phase in the field coincided with the period when chlorophyll *a* concentration was at its lowest. This may suggest that food itself is not limiting, rather access or the duration of access to it. Some authors have postulated that conspecific density restricts food availability either directly due to disruption of feeding activity when physical contact between conspecifics occurs (Blanchard *et al.*, 2000) or indirectly through the inhibitory effects of mucus films or pellet production preventing the consumption of viable food items (Baur & Baur, 1990; Forbes & Lopez, 1986). Constraining the movement within experimental enclosures in the laboratory may have significantly increased contact between individuals or with mucus trails and pellets, as it prevented the expression of avoidance/relocation behaviours such as surface floating that were commonly observed at the site during periods of high abundance. Potential feeding time during laboratory studies was also much higher than could be expected under field conditions due to the lack of tidal exposure in the former. Cessation of feeding generally occurs when *Hydrobia* are exposed by ebbing tides unless surface sediment remains sufficiently fluid (Orvain & Sauriau, 2002), this period extending to the majority of the day when individuals are located on the high shore.

Although many growth estimates derived in the laboratory were less than those derived for field data, the difference between them was less at the smaller sizes ranges. Low/no growth of larger individuals was common under laboratory conditions when compared with the field, however. It was not clear why this decrease in growth rate occurred, but it may have suggested size-specific variation in resource availability in the laboratory affecting only certain groups. Size-specific variation in resource requirements is common for marine invertebrate species (Cobb *et al.*, 1983; Polis, 1984; Byers, 2000; Rossi, 2002; Gosling, 2003). Alternatively, conducting the growth experiments during periods when no growth would have been occurring in the field may have impacted more severely on larger individuals. Providing warmer water, higher food availability or reduced intra-specific competition appeared to

trigger rapid growth of smaller individuals, but only for durations similar to those observed in the field. Growth rates dropped significantly after a period of two months, despite all experimental variables being held constant. This may suggest that the *Hydrobia* population at Bull Island is physiologically adapted to undergo short periods of rapid growth that cannot be sustained for prolonged periods, even when food availability is high and density low. The truncated somatic growth phase observed in the laboratory and field may have been due to the redirection of energies into tissue maintenance, energy storage, reproductive growth or individuals reaching a physiological threshold determined by the stresses associated with the production of new body tissue. In addition to the affects of the various treatments, seasonality in growth potential based on recent growth history may account for some of the differences in size-specific growth rates observed during laboratory growth experiments conducted during different periods of the year. Intra- and inter-annual variability in population parameters could have significantly affected laboratory results and further multi-factorial experimentation should be conducted at the same time of year.

The apparent reduction in the growth rate of larger individuals in captivity may also have been due to a failure to acclimate as readily to laboratory conditions. Many individuals appeared to increase in shell height during growth experiments but not significantly greater than the defined measurement error. This is an important parameter to quantify, particularly prior to growth experiments, as the stated accuracy of modern measuring devices often exceeds that of human error. Individuals >5mm SH should have achieved a growth increment greater than the measurement error during laboratory experiments if they had grown to the extent predicted by field-based data. As no account was taken of changes in the reproductive condition or tissue weight of individuals during laboratory studies, it is also feasible that larger individuals invested proportionally more energies towards these parameters than increasing shell height when compared with smaller individuals. As individuals used for growth experiments were collected outside of the growth phase and after the spring settlement, it is possible that larger animals preferentially invested proportionally more energies into recovering condition than increasing in size. It is a generally accepted that larger individuals invest proportionally more energies into reproductive activity (Wilson, 1975; Polis, 1984; Barnes, 1990, 1996: Cobb *et al.*,

1997; Byers, 2000). Although YOY *Hydrobia* are known to be capable of maturing during the season of settlement (Fish & Fish, 1974) and, therefore, may contribute to future production in that year, they are likely to invest proportionally more energies toward somatic growth when the magnitude and rate of increasing shell height is considered. Further studies should take account of the condition of individuals prior to and after growth experiments.

The merging of cohorts observed for the two *Hydrobia* YOY groups in 2001 was a result of different growth and mortality. Merging of cohorts often occurs for invertebrate species that have a number of settlement events during a reproductive season, have rapid variable growth rate or persist for several years beyond maturity (Barnes, 1996; Robinson & Tully, 2000a). The differential between individual growth rates that occur during the immature phase can become even more pronounced when energies are redirected to reproductive growth. Although the spring *Hydrobia* settlers appeared to grow significantly during the 2 month long population growth period, they were not observed to grow after early June. Previous studies of this species have suggested that early settlers are capable of reaching maturity rapidly and reproducing during the summer of settlement (Fish & Fish, 1974; Barnes, 1990). Although redirection of energies from somatic to reproductive growth may have accounted for the truncated growth phase for this size group, a similarly constrained growth period was observed for previously settled cohorts. As no significant growth was detected in the autumn YOY cohort, its merger with the spring cohort over the winter period must have been the result of differential mortality. Post-settlement recruitment failure for entire settlement cohorts is not uncommon for marine invertebrate species, this being postulated as one of the main advantages of multiple broods/settlement events. Bachelet *et al.* (1987) estimated that at 3.5 months mean survival rate of *Hydrobia ulvae* was 10%. Although environmental conditions may be such that one cohort fails to survive or grow, subsequent cohorts may experience more suitable environs at settlement. Cardoso *et al.* (2002) recorded 4 recruitments during the year for *Hydrobia ulvae* in Portugal. For species that display strong intra-specific competition, settlement cohort success is directly related to that of previously settled groups.

The autumn *Hydrobia* settlers were smaller in mean body size at settlement when compared with the spring settlers. Although this may have been due to variation in the lag time elapsed between settlement and the actual sampling effort for each cohort, the similar sampling frequencies used probably suggest other determining factors. Temperature is known to strongly affect developmental size and stage duration for planktonic invertebrates (Wear, 1974; Robinson & Tully, 2000a). Within the tolerance range of a given species, higher temperatures during development generally result in more rapid development, higher metabolic rate and smaller body size at completion (Gosling, 2003). Although variation in food availability and quality during the planktonic phase may also affect development, deficits normally result in mortality rather than lowered body size (Rumrill, 1990). As the size of offspring is generally correlated to parental body size for marine invertebrates, the mixing of gametes from individuals from the spring cohort with larger previously settled individuals may also have contributed to the smaller mean body size of the autumn YOY cohort if the former did in fact mature as rapidly after settlement as is suggested in the literature (Fish & Fish, 1974). No attempts to assess size-at-maturity were made in the current study, and geographic and site-specific environmentally and/or genetically driven variation is likely to occur.

The growth phase observed for *Hydrobia* in the field was relatively short in duration, appearing to last for approximately 2 months only, this is shorter than growth phases recorded previously for other *Hydrobia* populations (Bachelet *et al.*, 1987; Sola, 1996; Lillebo *et al.*, 1999; Cardosa *et al.*, 2002). Although certain species enter phases where somatic growth is suspended due to the physiological processes associated with reproduction, the former is more often regulated by limiting factors in the surrounding environment. The laboratory experiments conducted during periods of no growth in the field suggested that *Hydrobia* density, temperature and food availability significantly affected growth to varying degrees. The former appeared to elicit particularly strong regulation of growth under laboratory conditions, although it was clear interactions occurred between this and other regulatory factors such as food availability. For example, growth was observed in the smaller size classes at high density and high/low extremes of temperature when food was supplied in abundance. Although the April-May growth phase observed in the field corresponded to the period of lowest chlorophyll *a* concentrations, the beginning of the growth phase also

corresponded to relatively low *Hydrobia* density and the period when water temperatures began to rise for the first time since the over wintering period. Fluctuations in temperature, in addition to environmental temperature itself, are also known to influence certain biological processes in the marine environment. Although the autumn settlement coincided with relatively high water temperature similar to that that resulted in greatest growth in the laboratory (16°C), this cohort failed to grow before the year's end. Despite relatively high chlorophyll *a* concentration at this time, *Hydrobia* density was also at the maximum levels observed. Although the effect of temperature, density and food availability were examined in the current study, other unspecified factors may also regulate growth of *Hydrobia*. It appears that a complex combination of interacting factors result in strong regulation of *Hydrobia* population growth at Bull Island, and further investigation is warranted. As limitations in equipment availability prevented all factors with the potential to regulate growth being examined simultaneously, a direct statistical comparison of the results from each experiment in terms of interaction between processes was not conducted here.

Fish & Fish (1974) estimated shell height of 1.5-2.5mm of 1 year old *Hydrobia* with a longevity of approximately 2.5 years in a population in Wales, agrees closely with the findings of the current study. Very similar results were reported from a population in France where individuals attained a SH of 2mm by the first winter and 4mm by the end of the second (Bachelet *et al.*, 1987). A much shorter longevity of 13 months was predicted for a population of the conger species *Hydrobia ventrosa* in Spain (Drake & Arias, 1995). Cardoso *et al.* (2002) reported that the life span of *Hydrobia* was between 16 and 20 months depending on time of year when recruited in Portugal. Growth rates appeared to be far higher than other locations in this study as 12 months after benthic recruitment a maximum shell height of 4-4.5mm was achieved. Similarly high growth rates and shortened longevity were also observed in another study in Portugal (Lillebo *et al.*, 1999) that cited longevity of 18-24months. Growth was continuous but higher during spring and early summer. After 1 year shell height was between 3.8-4.8mm, increasing to a mean of 5.8mm after 21 months. Sola (1996) estimated a life span of 12-15 months in a Spanish *Hydrobia* population, with increases in shell height between May and September averaging 2.4mm.

It would appear that the high mortality and negligible growth rates observed for *Tellina* in the laboratory was a direct result of the artificial nature of the close-circulation holding facilities. The higher survival rates obtained at low temperature were probably indicative of lowered metabolic rate, which in turn resulted in the cessation of any significant growth. Although attempts to recreate as natural conditions as possible were made, relatively effectively for *Hydrobia*, it was not possible to recreate periodic tidal action and/or the cues associated with it. Despite speculation as to the exact feeding strategy utilised by *Tellina*, many authors believe that filter feeding directly from the water column constitutes a significant proportion of feeding behaviour for the species (Trevallion, 1971; Wilson, 1995). Due to the relatively slow flow-through water supply in the holding facilities available it is unlikely that the food added stayed in suspension for long periods or was re-suspended at a later time. This may have significantly reduced the feeding capacity of *Tellina* to a level where the individuals could not be sustained. Alternatively, the food source used may have been unsuitable for primarily filter feeding species. If food suitability and/or availability were not directly affected in the holding facility, then suppression of the feeding response of this infaunal species due to lack of environmental cues associated with tidal action may have been occurring. If starvation was assumed to be the cause of the high mortalities observed then the metabolic rate and pre-experimental condition of each individual would have determined the time until death.

The failure of cohort analysis and age readings for *Tellina*, combined with lack of significant growth in the laboratory, resulted in the production of an inter-annual estimate for the growth of this species alone. Inter-annual variation of post-settlement growth can significantly affect annual growth rate estimates derived in this way however (Bowman & Lewis, 1977; Roughgarden *et al.*, 1988). Considering the arbitrary size grouping used to divide the population and lack of other data sources for validation, the estimate was treated with extreme caution. The linear nature of the estimate derived was also unusual in showing no sign of reaching asymptote, which would be expected for populations where individuals were persisting to medium/late stages of the natural lifespan. The size-at-age estimates derived were similar to those produced from age reading conducted on a population in Scotland however (Barnett

& Watson, 1986). These authors also concluded that reading age annuli was too subjective and instead aged *Tellina* by use of length frequency plots with a contracted frequency scale that visibly enhanced year groups allowing approximate length of year groups to be read by eye from growth curves fitted to data. Due to blending of cohorts with increasing age Barnett & Watson (1986) only estimated size-at-age up to year 2+ however Wilson (1996) aged *Tellina* by reading annuli up to year 8+. Site-specific spatial variations in size-frequency distribution that occurred at the current study site (see Chapter 2) may indicate that differential growth rates occurred at the Blackrock site in response to one or more environmental factors. Failure to construct statistically significant size-at-age curves prevented examination of growth rates from various shore levels in the current study however. Future studies monitoring the growth and/or size-distribution of this species should account for this phenomenon and adjust sampling protocols accordingly.

The size-frequency distributions for *Hydrobia* would suggest that YOY individuals settle directly into Bull Island from the plankton each year. Clear settlement events were detected when large numbers of individuals much smaller than those observed in previous samples joined the benthic population. These peaks occurred at specific periods during the year, suggesting a synchronised biannual reproductive cycle for this species. Overall population size-frequency was skewed strongly toward high numbers of small individuals at times of settlement, indicating that the juvenile settlement and nursery habitat was identical to that of the adults. Although post-settlement abundance of YOY cohorts often decreased rapidly, the cohort remained distinguishable throughout the rest of the year. This did not occur for *Tellina* at the Blackrock site. Although small pulses of settlement were detected occasionally, these rarely persisted, and never constituted a significant part of the population. This would suggest that, overall, the population has a lower reproductive output compared with *Hydrobia*, initial post-settlement mortality is extremely high and rapid, or that initial settlement does not occur in the adult habitat sampled. Regular recruitment failure has been described for a number of *Tellina* populations, although the causative mechanisms were not described (Stephen 1928; McIntyre, 1970; Barnett & Watson, 1986; Wilson, 1996; Dekker & Beukema, 1999). Settlement into nursery habitats with a subsequent ontogenetic shift to adult habitats is not uncommon for marine

invertebrates. Several species of bivalve mollusc are known to display such behaviour, including the scallop *Pecten* and the mussel *Mytilus*. The former often settles into shallow waters than the adults of the species, the later into algal filaments removed from the adult beds from which they later disperse. Although a similar immigration of small benthic phase *Tellina* into adult populations has been postulated, no evidence for this process exists (Wilson, 1996; Dekker & Beukema, 1999).

The fluctuating abundance of *Hydrobia* and the normally distributed population size-frequency distribution of *Tellina* made it particularly difficult to derive anything but the crudest of mortality estimates for the former and totally negated possible estimates for the later. It is particularly difficult to derive accurate field-based mortality estimates for species that are mobile and/or patchily distributed (Hassell, 1987). Although the size-frequency data suggested that mortality of YOY individuals was high, which is common for many marine invertebrate species (Gosselin & Qian, 1997), it was not possible to accurately quantify the parameter. Losses from the population cannot be attributed solely to mortality for open populations where net reductions in abundance can occur through temporary relocation or permanent migration (Caley *et al.*, 1996). Even when a benthic population is closed, high spatial heterogeneity in the distribution of species and the processes affecting them can make calculation of mortality problematic (Hassel, 1987; Roughgarden *et al.*, 1988). The merging of cohorts, as seen for *Tellina* in the current study, can further complicate calculation of population mortality. Annual cohort/age-specific data that has been collected at the same time during subsequent years with due consideration of spatial variability in size-frequency distribution is often considered a good indication of population mortality however, and is widely used in commercial fisheries management (Fogarty *et al.*, 1991). As variation in inter-annual recruitment and post-settlement processes are difficult to quantify, they are generally considered to be negligible for the purpose of estimating population mortality (Robinson & Tully, 2000a). The reliability of the estimates produced can therefore only be ascertained when a sufficient time-series of data has been accumulated. Although it was not possible to accurately determine mortality for either species in the current study, the data provide a baseline for future time-series analysis.

A combination of methods were used to examine the growth and mortality of *Hydrobia* and *Tellina* with a varying degree of success. It appears that although a complex suite of interacting parameters regulates growth, and presumably population size, of the former, population density elicits the strongest affect on this highly abundant species. The apparent lack of growth restriction during the period when food availability was assumed to be lowest for this species would suggest that 'bottom up' regulation of growth and population size does not occur at the *Hydrobia* densities observed. Due to the relatively sedentary nature and much lower abundance of *Tellina* it is probable that population density plays a less important role in regulating the growth and population size for this species, although this cannot be verified from the current study due to poor results. The habits and habitats of species, and variations in these within-population, will define the parameters that control their growth, mortality and population biomass.

Chapter 5 Examining the relationship between biological and physical sediment properties and the distribution of *Hydrobia ulvae.*

5.1 Introduction

The spatial and temporal distribution and abundance of benthic marine invertebrates varies in response to the habitat requirements of the particular species (West *et al.*, 1978; Austen & Warwick, 1989; Kalejta & Hockey, 1991; Lillebo, 1999; Van der Meer *et al.*, 2001). Each species will be capable of persisting within a specific range of environmental conditions, although generally an optimum level exists at which the species may operate at their most efficient (Wolff, 1983). Geographically widespread and opportunistic species generally have broader ranges of environmental tolerances than specialists, which may have extremely specific and rigid habitat requirements. These requirements may alter numerous times during the lifecycle of species in response to changing physiology and/or body size (Lopez & Levinton, 1987). The ability of a species to adapt to fluctuations in the physical and biological environment will define the range of habitats it can inhabit. The physical and biological parameters that affect distribution and abundance are species-specific, and may vary between populations or individuals within-populations for that species. Due to inter- and intraspecific competition for resources, including space, and the variable nature of the marine benthos, many individuals may be forced to endure sub-optimal conditions for much of their lifecycle.

The physical and biological properties of sediments are an important determinant of the distribution and abundance of both infaunal and epifaunal benthic species (Kalejta & Hockey, 1991). For soft sediment types, grain size is probably the most influential parameter in defining the resident flora and fauna (Hughes, 1970). Organisms burrow into, bind with or reside on the surface of soft sediments, which may in some cases be quite mobile. Physical disturbance and regular tidal action causes the continual deposition and re-suspension of soft sediments in the marine environment, particularly in shallow areas such as the intertidal zone where its influence is greatest (Baillie & Welsh, 1980). As fine sediments are only deposited in areas with low physical disturbance and water velocity (Thornton *et al.*, 1995), they generally only

occur in sheltered, low energy locales. This generally ensures that sediments within habitats such as mudflats are relatively immobile, and suitable for colonisation by epifauna, although the commonly resulting reduction in the depth of the oxic layer may exclude some species of burrowing infauna. In contrast coarser materials, ranging from sands to shale, are more readily deposited even in physically exposed and dynamic areas, resulting in a high degree of surface sediment mobility. These less stable habitats are only suitable for burrowing infaunal species, or those that are highly mobile and able to retreat to deeper waters when unfavourable physical conditions prevail (Wolff, 1983). They lack sufficient shelter from abrasion and physical transportation for small epibenthic species. Additionally, finer substrates retain greater water content during periods of aerial exposure by ebbing tides, this providing epibionts with additional protection from desiccation and thermal exposure. The variability in the suitability of a habitat for colonisation by a particular species will determine not only its distribution, but also the population carrying capacity (Bianchi, 1988).

Sediment primary production accounts for a significant proportion of productivity in temperate marine environments (MacIntyre *et al.*, 1996). A vast number of species constitute the organisms responsible for this production, collectively termed the microphytobenthos. Epipsammic species adhere to the sediment particles, while the epipelic inhabit the interstitial spaces between grains (Admiraal, 1984). In conjunction with other factors such as aspect, nutrient supply and environmental temperature, grain size also determines the productivity of various sediment types. Finer, less mobile/abrasive sediments generally have a greater surface area available for colonisation, less physical disturbance and due to this, generally higher organic matter content (Elliott *et al.*, 1998).

For many marine invertebrates, active substrate selection at the end of the dispersive planktonic development stage, before taking up the benthic phase of the lifecycle, is known to play an important role in mitigating subsequent post-settlement mortality (Van der Meer *et al.*, 2001). Settlement into sub-optimal habitats will result in poor survival and growth unless migration to another more suitable habitat can be achieved. A wide range of physical, biological and chemical cues are known to

influence the selection of a suitable habitat at settlement, or for later adult dispersive phases (Roughgarden *et al.*, 1988). These include substrate hardness and complexity (which together affect the ability to shelter), food availability and the presence or absence of predators, competitors or conspecifics. Particle grain size may elicit a particularly strong influence on the habitat selection of deposit feeding species, as they must feed as well as live upon the sediment. Deposit feeding species generally ingest sediment particles within specific size ranges, this varying with the size or life history stage of the individual (Lopez & Levinton, 1987). Although individuals of certain species can also graze the epipsammic microphytobenthos from the surface of sediment particles, this is not true of all deposit feeders. This may suggest that certain species, and individuals of varying size within a population, are confined to particular substrate types.

The mud snail *Hydrobia ulvae* is a relatively small (1-8mm shell height) surface dwelling deposit feeder that occurs commonly on intertidal mudflats within Europe. This primary consumer utilises microphytobenthos as a food source by ingesting whole sediment particles or grazing it from their surface (Lopez & Levinton, 1987). Due to their ability to be transported by water currents by floating at the surface on mucus strands and their relatively fast surface crawling speed (Forbes & Lopez, 1986; Orvain & Sauriau, 2002) they have the ability to relocate from sub-optimal habitats and select a novel substrate on which to settle and feed. Although some authors have suggested that the former behaviour is a response to high density (Lopez-Figueroa & Neill, 1987), some controversy exists, with others claiming that this behaviour is accidental (Barnes, 1981b) or an artificial behaviour expressed only in captivity (Little & Nix, 1976). During a previous study by the current author, this behaviour was commonly observed in the field. Additionally, the species spatial distribution was not uniform across the site, and varied among size classes. Physical sediment properties and chlorophyll *a* concentration were also distributed patchily across the site, although the high degree of variability prevented any relationship between these variables being identified. The aim of this chapter was to determine whether biological and physical sediment properties have any effect on the spatial distribution of *Hydrobia ulvae* at different life-history stages.

5.2 Materials and Methods

Hydrobia were found to be present in a wide range of substrate types at Bull Island during the sampling conducted in Chapter 2 (see 2.2.1 for site information). Sediments where *Hydrobia* were present ranged from fine mud to coarse sand, with some fairly stony sections also occurring in patches on the high shore. Sediment grain size, and the surface layer available for colonisation by microphytobenthic organisms, therefore, varied greatly across the mudflat. Although surface sediment chlorophyll *a*, a measure of microphytobenthic primary production, also varied across the site, it was not possible to determine a link with sediment grain size.

5.2.1 Examination of sediment grain size

Spatial differences in grain size composition were examined at Bull Island. Ten cores with a sampling area of 70.9cm^2 were taken to a depth of 15cm at each shore level. Samples were taken on the upper shore level 20m from the seawall, by the creek (lower), and midway between the seawall and the creek, (see Figure 2.5). A further set of midshore and upper shore samples were taken on the far side of the creek, as there were effectively two high shores at this site due to the tide ebbing and flooding via the bisecting, central creek. All cores were placed in labelled plastic bags and returned to the laboratory. Samples were dried to constant weight at 100°C, and passed through a series of sieves with mesh sizes 2.000, 1.000, 0.500, 0.250, 0.125, and 0.063mm. The fraction retained in each sieve was weighed to an accuracy of 0.5g.

5.2.2 Substrate preference-Field

The substrate preference of *Hydrobia* was tested in the field by manipulating sediments within small enclosures. Sixteen enclosures each with an area of 177cm^2 were installed at the study site. The enclosures were positioned in a 4x4 arrangement, separated by 25cm and protruding above the mud flat surface by at least 15cm. All sediment and animals within the enclosures were carefully removed and discarded. Four enclosures were filled with each of mud, coarse sand, mearl (~1.5mm diameter) or stone (~5mm diameter) substrates that contained no fauna (Table 5.1). All of these

substrate types had been removed from the Bull Island site during a previous visit, and had contained variable quantities of *Hydrobia*. The sand, mearl and stones had been interspersed or mixed with mud, but were separated during sieving and offered in isolated form. No lid was placed on the enclosures to allow free entry and exit. The enclosures were installed at station C8 at the study site (Figure 2.5).

Table 5.1. Arrangement of substrates within experimental plot at Bull Island.

Mud	Stones	Sand	Mearl
Mearl	Mud	Stones	Sand
Sand	Mearl	Mud	Stones
Stones	Sand	Mearl	Mud

After a period of 15 days, each core was removed from the mudflat and the contents placed in plastic bags. Samples were sieved in the laboratory and *Hydrobia* placed in field trays filled with seawater. Live animals, identified by the presence of an opercular plate or by actual movement, were separated manually from the retained component using forceps and placed in 70% alcohol. Shell height (SH) was measured from posterior to anterior shell tips using Mitutoyo Absolute Digimatic callipers to an accuracy of 0.1mm (see Chapter 4).

5.2.3 Substrate preference-Laboratory

Although *Hydrobia* appeared to select finer substrata preferentially during the experiment described above in Section 5.2.2, a range of these sediments were present at the study site, and elucidating preferences between them was impractical in field-based conditions. The wide difference between grain sizes tested in the previous field experiment were not affected strongly by tidal deposition of fine substrates into the enclosures during the study period. This deposition would have affected the results of a more refined experiment incorporating finer variation in grain size. For this reason, further experimentation was conducted in the laboratory.

An experimental enclosure with an area of 506cm^2 was divided evenly into 54 cells, so that the addition of a single *Hydrobia* to each cell yielded a low density of 1,067 individuals m^{-2}. Each cell was open so that it could be filled to the top with substrate. The thin (1mm) Perspex sheeting that laterally divided the cells prevented the mixing

of substrates while maintaining almost continuous, unbroken coverage. Perspex sheets with slots covered with mesh were affixed to the sides of the container to allow water to enter the enclosure while preventing *Hydrobia* from escaping. The enclosure was placed in a temperature controlled seawater tank maintained at 12°C. Water height was kept above the level of the substrates, as *Hydrobia* is known to be more active when covered by water (Barnes, 1986; Orvain & Sauriau, 2002).

5.2.3.a Medium-scale grain size preference

Examination of the finer sediments at Bull Island revealed that mud, fine sand or a combination of these sediments occurred at all locations across the flat. Each of these 3 sediment types was placed in 18 of the enclosure cells such that no adjacent cells contained the same substrate. A single *Hydrobia* was placed in each cell, and positions recorded after duration of 1 hour. It was assumed individuals could have tested all substrate types during this time as *Hydrobia* have been recorded as having crawling rates of between 12–22cm h^{-1} (Forbes & Lopez, 1986). This process was repeated 10 times with fresh, randomly selected animals used on each occasion. The top portion of substrate in each cell was replaced with fresh sediment after each experimental run to remove traces of mucus trails and any pellets egested. Food concentration was kept in excess by the addition of Phytoplex™ aquaria brand marine phytoplankton to the circulating seawater.

5.2.3.b Fine-scale grain size preference

Six relatively small sediment grain sizes of >2.000, 1.000, 0.500, 0.250, 0.125 and 0.063mm were isolated from mud previously collected from Bull Island by sieving. Sediment of each grain size was placed in 9 cells dispersed throughout the enclosure such that no adjacent cells contained the same substrate. Four freshly collected *Hydrobia* with SH of 3-5mm were placed within each cell. The position of individuals was recorded after 1 hour. Food concentration was kept in excess by the addition of Phytoplex™ aquaria brand marine phytoplankton to the circulating seawater.

The addition of 4 *Hydrobia* to each cell was equivalent to a density of 4,267 individuals m^{-2}. Although five separate 1hour studies were undertaken, a large proportion of individuals did not remain in any of the substrates and were found either floating or climbing the container walls. It was assumed that this was a response to density, as these behaviours were not observed at lower storage densities. Density was therefore reduced to 1 snail $cell^{-1}$ (1,067m^{-2}), significantly reducing 'avoidance' behaviours. A total of 10 experimental durations of 1 hour were then conducted. Different *Hydrobia* were selected randomly from stock for each experimental run. The top portion of substrate in each cell was replaced with fresh sediment after each experimental run to remove traces of mucus trails and egested pellets.

5.2.3.c Grain size and food availability

Substrate preference was tested as described in 5.2.3a above but with varying food availability. Quantities of the three sediment types mud, mud/sand mix and sand were either left unaltered (N), enriched (E) with Phytoplex™, or autoclaved (A) to remove all chlorophyll *a*. Each of these substrates was placed in the cells of the experimental enclosure such that no adjacent cells contained the same substrate. No Phytoplex™ was added to the circulating seawater, and no transfer of dietary items between cells assumed. A single *Hydrobia* was placed in each cell, and positions recorded after duration of 1 hour. This process was repeated 15 times with fresh, randomly selected animals used on each occasion. The top portion of substrate in each cell was replaced with fresh sediment after each experimental run to remove traces of mucus trails and egested pellets.

5.3 Results

5.3.1 Sediment grain size

Differences in sediment grain size composition were observed between shore levels at Bull Island. The sediment at the upper shore level close to the wall consisted of more numerous large particles when compared with other shore levels. Small stones (9%) were most common at this shore level and did not occur often at other levels (Table 5.2). Although changes with shore level in mud/sand content (the 5 smallest grain

size components) were apparent, differences were not statistically significant (ANOVA, F=2.13, df=4, p=0.21).

Table 5.2. Mean grain size composition (%) of sediment at Bull Island at different shore levels. (N=50).

Shore level	>2mm	1-2mm	0.5-1mm	250-500μm	125-250μm	63-125μm	<63μm
High (wall)	9	4	13	40	29	5	<1
Mid	4	1	1	53	35	4	1
Low	2	1	<1	58	35	2	1.5
Mid	3	2	2	56	32	4	1
High	2	2	8	50	33	4	1

5.3.2 Substrate preference-Field

Mean abundance of *Hydrobia* ranged from 4,754±604 individuals m^{-2} (±S.E.) in mud to 198m^{-2}±71 in sand (Figure 5.1). Only 14 individuals were recovered from the sand treatment compared with 336, 197 and 80 in mud, stone and mearl, respectively. There were significantly less *Hydrobia* m^{-2} (ANOVA, F=19.0, df=3, p≤0.01) in sand compared with mud and stone (Table 5.3). Mud also supported significantly higher densities of *Hydrobia* than mearl.

Table 5.3. Scheffe post-hoc tests of between-substrate comparison of abundance of *Hydrobia* m^{-2}. * indicates differences significant at <0.05 level.

Substrate	Stone	Sand	Mearl
Mud	0.07	≤0.01*	≤0.01*
Stone		0.01*	0.14
Sand			0.57

The size-frequency distributions observed in each substrate type varied significantly (Figure 5.2). The mud substrate contained individuals with a size distribution identical to that recorded at the site during sampling described in Chapter 2. In the mud substrate a large number (70%) of individuals had a SH of <1.6mm, which was believed to represent individuals that had settled earlier the same year. Individuals larger than 1.6mm SH were assumed to have settled in previous seasons. Although the sand, mearl and stone substrates did not support high numbers of individuals <1.6mm SH, the stone and mearl did contain some larger individuals.

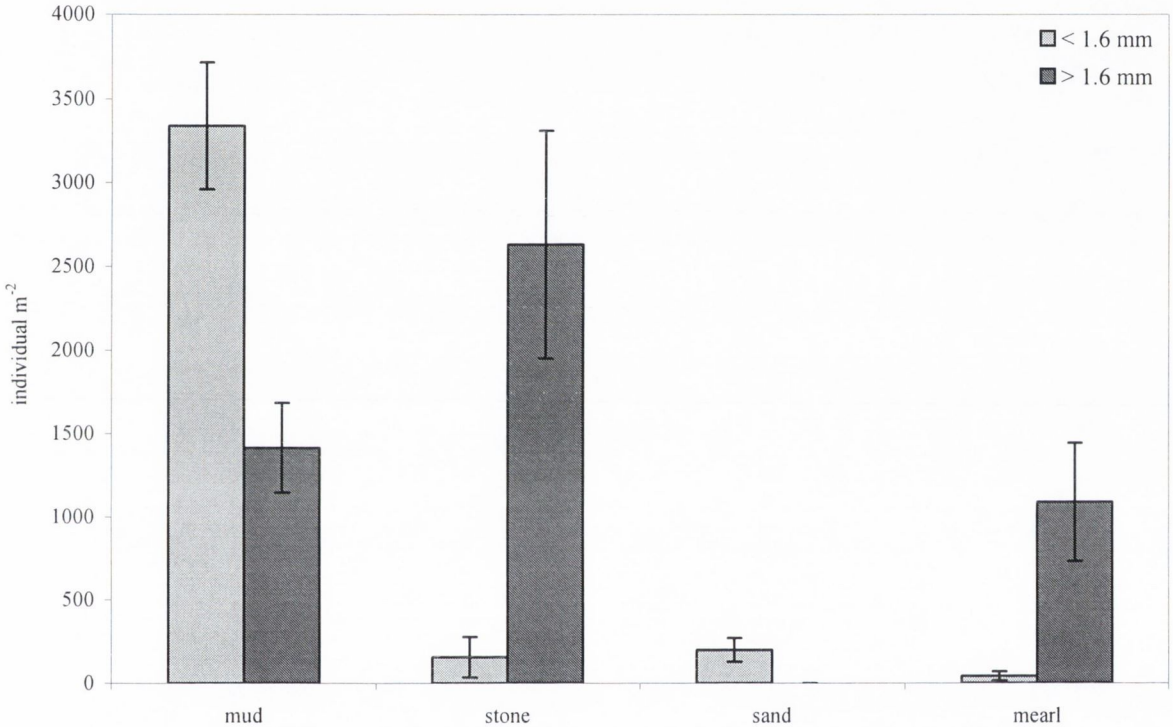


Figure 5.1. Density of *Hydrobia* m^{-2} <1.6mm (SH) and >1.6mm (SH) in experimental enclosures containing varying substrate type, Bull Island. N=617.

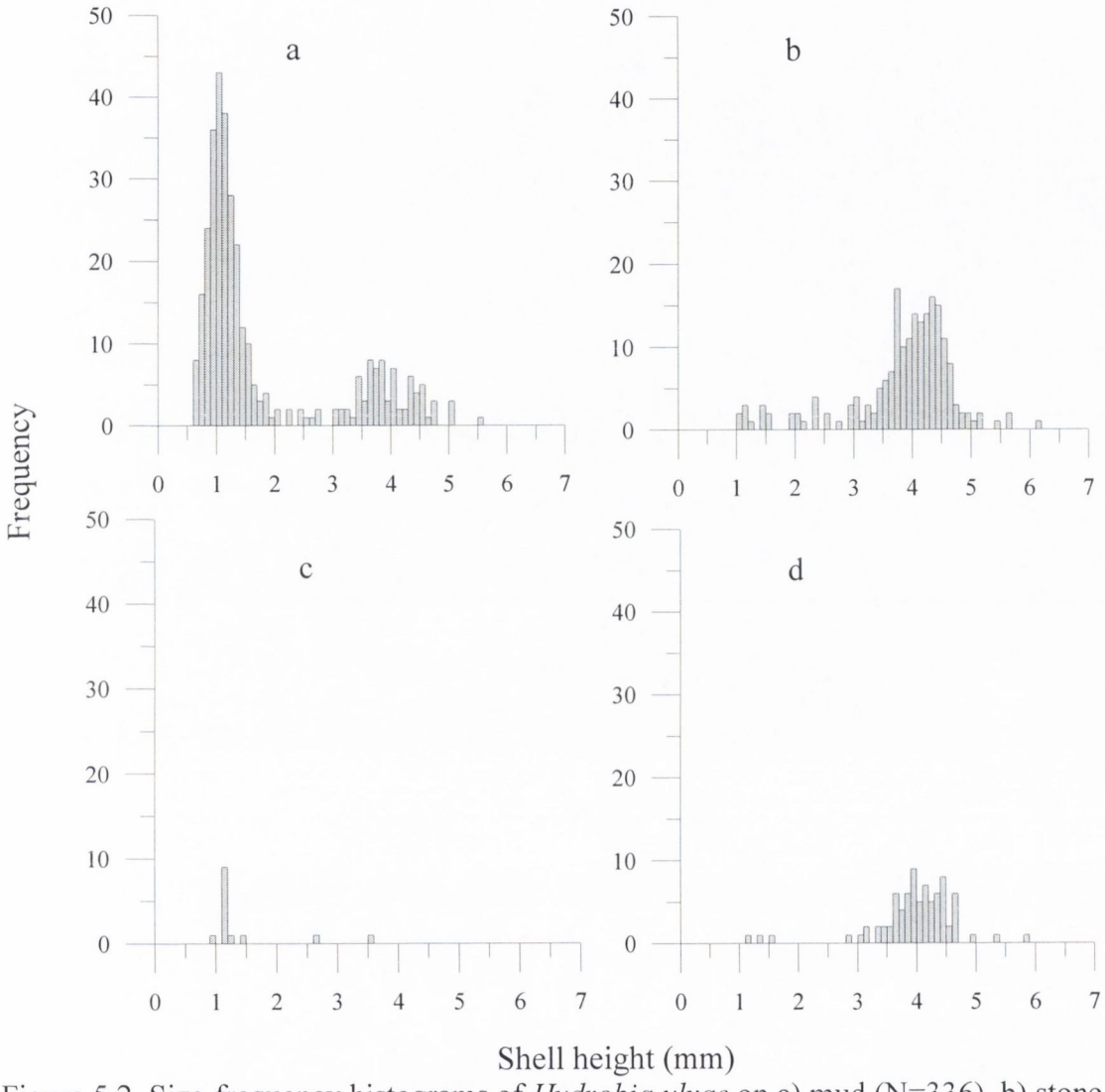


Figure 5.2. Size-frequency histograms of *Hydrobia ulvae* on a) mud (N=336), b) stone (N=197), c) sand (N=14) and d) mearl (N=80) substrates within experimental enclosures, Bull Island.

5.3.3 Laboratory - Medium-scale grain size preference

Significant differences were observed in the densities occurring in the different treatments (ANOVA, F=12.6, df=2, $p \leq 0.01$). Scheffe post-hoc tests revealed that there were significantly higher densities of *Hydrobia* observed in the mud substrate when compared with the sand and mud/sand mix (Table 5.4). No significant differences existed between the sand or mud/sand mix however. An average of 81% of *Hydrobia* remained on the sediment surface during this experiment.

Table 5.4. Mean *Hydrobia* ± S.E. per substrate type at a density of 1,067 individuals m^{-2} (N= 54).

Substrate type	Mean *Hydrobia* per substrate ± S.E.
Mud	14±1.2
Mud/sand mix	9±1.3
Sand	6±0.8

5.3.4 Laboratory - Fine-scale grain size preference

Although the least number of *Hydrobia* were present on the largest grain size of >1,000μm (Table 5.5), no statistically significant differences in density were observed between any of the treatments (ANOVA, F=2.6, df=5, p=0.08). An average of 75% of *Hydrobia* remained on the sediment surface during this experiment.

Table 5.5. Mean *Hydrobia* ± S.E. per substrate at a density of 1,067 individuals m^{-2} (N= 54).

Grain size (μm)	Mean *Hydrobia* per substrate ± S.E.
>1000	5±0.7
500-999	7±0.8
250-499	5±0.8
125-249	8±1.4
63-124	6±0.9
63	6±0.7

5.3.5 Laboratory-Grain size and food availability

The mean number of *Hydrobia* recorded in each treatment varied significantly (ANOVA, F = 48.5, df=7, $p \leq 0.01$). Post-hoc tests (Table 5.6) revealed that the mud substrate enriched in chlorophyll *a* contained significantly more *Hydrobia* than any other substrate type (Figure 5.3). The normal mud substrate also contained higher

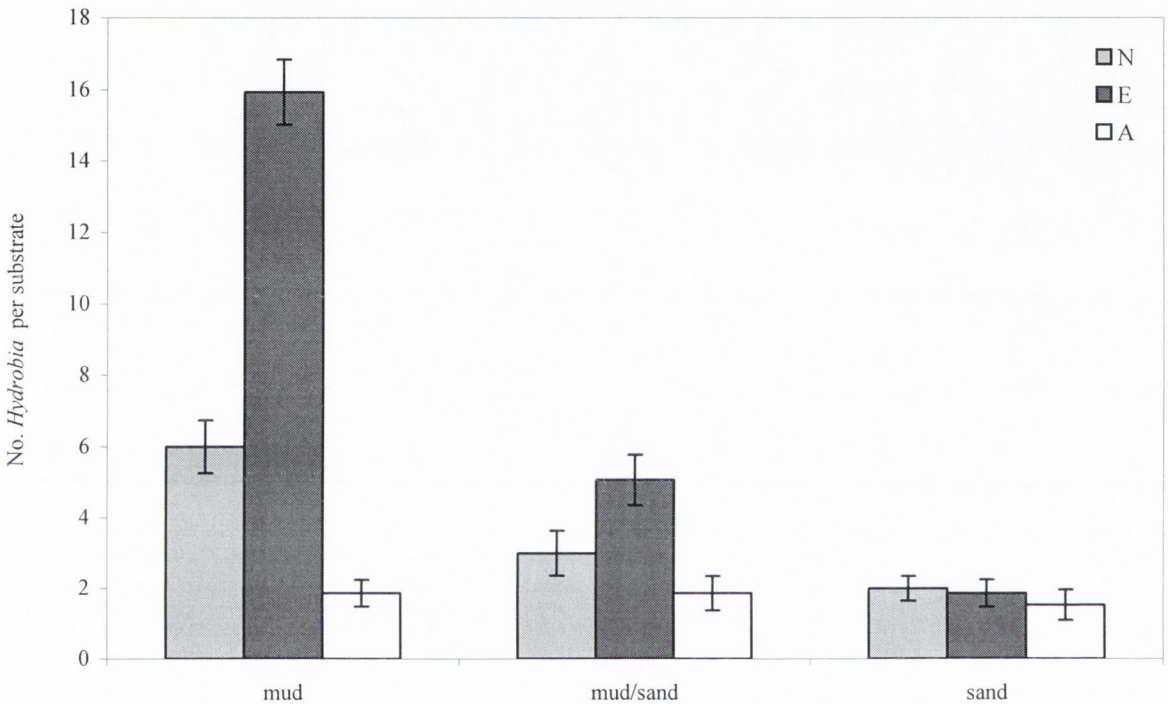


Figure 5.3. Mean (± S.E.) *Hydrobia* per substrate type at a density of 1,067m^{-2}, N = normal chlorophyll *a* content, E = enriched in chlorophyll *a* and A = autoclaved sediment with no chlorophyll *a*. (N=54, replicates=15)

numbers than all treatments except the enriched mud/sand mix. The sand substrate contained the least number of individuals regardless of treatment. Autoclaved sediments, regardless of type, contained the least individuals within- and between-substrate types. An average of 76% of *Hydrobia* remained on the sediment surface during this experiment.

Table 5.6. Scheffe post-hoc tests of between-substrate comparison of distribution of *Hydrobia*. * indicates differences significant at < 0.05 level.

Substrate	Mud (e)	Mud (a)	Mud/sand (n)	Mud/sand (e)	Mud/sand (a)	Sand (n)	Sand (e)	Sand (a)
Mud (n)	≤0.01*	≤0.01*	0.12	0.99	≤0.01*	≤0.01*	≤0.01*	≤0.01*
Mud (e)		≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*	≤0.01*
Mud (a)			0.98	0.07	0.99	0.99	0.99	0.99
Mud/sand (n)				0.63	0.98	0.99	0.98	0.92
Mud/sand (e)					0.07	0.10	0.07	0.03*
Mud/sand (a)						0.99	0.99	0.99
Sand (n)							0.99	0.99
Sand (e)								0.99

5.4 Discussion

Although sediment grain size varied with shore level at Bull Island more significant differences could have been expected from visual examination of the site alone. The upper shore by the seawall was quite stony, and mud near the creek generally had higher sand content. Stones did not, however, cover the entire surface where they occurred however and were interspersed and underlain with mud. Although isolated sediment components were offered in the field-based experiment, in reality the non-mud media did not occur at the study site in isolation. Mud was a component of the sediment in all areas of the flat in varying proportions. Deposition of mud into the experimental enclosures with each ebbing tide would have progressively increased the mud content of the isolated experimental media, altering them over time to be more similar to the mixed sediments found at the site.

Hydrobia displayed a preference for pure mud substrate when held at low density in the laboratory. Habitats that contained lower mud content could therefore be considered sub-optimal for the species. There was however, no clear relationship between *Hydrobia* abundance and grain size at the site (see Chapter 2), as individuals were observed in mud, muddy-sand, mud-mearl, and stone substrates. With the exception of the central channel made by the creek, *Hydrobia* occurred in large

numbers regardless of shore level or substrate type (see Chapter 2). When density was increased in laboratory conditions, a large number of individuals displayed dispersive or avoidance behaviours. This density was relatively low compared with those that occurred in the field (see Chapter 3). This suggests strongly that *Hydrobia* spread across the entire mudflat and inhabit all available substrates in response to density-dependent inter-specific interaction/competition (Blanchard *et al.*, 2000). Dispersal to sub-optimal habitats that still contain some degree of mud content probably assists in mitigating the overall regulatory affect. The causative factor(s) triggering the density-dependent avoidance response of conspecifics remains unclear. Blanchard *et al.*, (2000) suggested that direct physical contact between individuals inhibited feeding behaviour however, while other authors claimed that mucus trails on the sediment surface restricted access to the underlying microphytobenthic food source (Baur & Baur, 1990; Forbes & Lopez, 1986).

The avoidance of substrates containing high sand content in the laboratory may have been a response to increased sediment mobility (Elliott *et al.*, 1998), a preference for smaller grained particles suitable for ingestion (Lopez & Kofoed, 1980) or due to the fact that finer substrates generally support higher concentrations of primary producers (food items) in natural environments (Hopkins, 1963; MacIntyre *et al.*, 1996; Barranguet *et al.*, 1997; Elliott *et al.*, 1998). On average, mud or muddy-sand sediments attracted more individuals than sand containing much higher food content. This suggested, at least at low density, that grain size was relatively more important than food availability in defining distribution. Increasing density and intra-specific interaction may result in a change in the relative importance of these factors, either gradually or when some unidentified threshold density is reached. As the density that elicited avoidance behaviour in the laboratory was lower than that recorded at the study site on any occasion (see Chapter 3), responses to density-dependent intra-specific interaction probably determine the spatial distribution of *Hydrobia* during most of the year (Blanchard *et al.*, 2000). This effect would be particularly strong during settlement events when density is at its highest. If the growth and survival of recently settled individuals with low energy reserves are strongly affected by density-dependent regulation of food availability, then recruitment success will be defined by existing population size during the early benthic life history (Gosselin & Qian, 1997).

Although *Hydrobia* preferentially selected substrates that contained a mud component, they did not display a preference at finer scales. Mud at Bull Island consisted of particles of varying grain size mixed together. Particle ingestion constitutes the main feeding strategy for *Hydrobia*, but particles too large to ingest (>200μm), which represented a significant component of natural mud composition at Bull Island, are surface grazed (Lopez & Kofoed, 1980). As the range of grain sizes that an individual can ingest will be size-specific and increase with body size (Hentschel, 1998), ingestion of particles may be a less regular form of feeding for small individuals (Lopez & Kofoed, 1980). It is not clear whether individuals inhabiting substrates containing media other than mud particles at Bull Island feed on the surface of the larger particles or solely on the mud component itself. If the later is true then a higher availability of ingestible particles will be available in purely mud substrates. If these also contain proportionally more microphytobenthos, as is expected for finer substrata, then food availability would be higher than in mixed sediments when densities were equal (Hopkins, 1963; MacIntyre *et al.*, 1996; Barranguet *et al.*, 1997; Elliott *et al.*, 1998). Higher densities of deposit-feeders are believed to occur in fine-grained sediments as a result of greater food availability associated with small particles with high surface to volume ratio (Lopez & Levinton, 1987; Forbes, 1989). If food availability, or feeding capacity, does vary between sediment types, then growth and survival may also vary over relatively small spatial scales at the study site, although the continual re-location of the relatively mobile *Hydrobia* population may reduce this effect. Although a higher rate of growth of *Hydrobia* has been recorded in muddy substrata when compared with sand sediments (Morrisey, 1990), it has been postulated that this is not due to food limitation (Forbes & Lopez, 1989) but other factors such as disturbance by waves limiting foraging time and/or burial (Chandrasekara & Frid, 1998).

Young of the year (YOY) individuals exhibited stronger substrate preference between experimental media compared with previously settled individuals in the field. This supported the theory that YOY individuals may have stricter habitat requirements due to their smaller body size, which may be linked to sediment mobility, food concentration and/or the grain size range that they are capable of ingesting. It should be appreciated that the experimental media do not occur in isolation naturally at Bull Island however, and similarly to previously settled individuals, YOY were found in

high abundance on all areas of the mudflat during the settlement season. Byers (2000) observed that juvenile individuals of two epifaunal grazing snails *Cerithidea californica* and *Batillaria attramentaria* dispersed at relatively higher rates than adults.

Forbes & Lopez (1986) reported that there were no significant differences in feeding rate between enriched and unenriched sediments but crawling rate decreased on sediment with higher food content and smaller grain size. If the substrates containing a high proportion of non-mud components in the current study contained less food items compared with pure mud substrates, then density-dependent regulation of feeding could be expected to increase in the former. Reduced crawling rate on finer substrates may be in part due to the different feeding strategies employed, as deposit feeding required less locomotory activity (Forbes & Lopez, 1989). This may have contributed to the preferential selection of sediments enriched in chlorophyll *a* during the present study, as slower crawling speeds would have reduced the probability of *Hydrobia* reaching the boundaries of cells containing mud. Although the avoidance of autoclaved sediments may have been due to the lack of food content, Barnes & Greenwood (1978) recorded a repellent effect when the organic status of sediments were altered.

Floating behaviour by *Hydrobia* was frequently observed at the Bull Island site in the current study but its occurrence was not quantified. Little & Nix (1976) recorded negligible numbers of *Hydrobia* displaying floating behaviour in natural conditions. In the laboratory studies of the current study approximately 25% of snails were recorded rejecting all sediments offered in any single experiment either by floating, climbing container walls or remaining inactive, which was a large proportion considering densities were relatively low (~1,000m^{-2}). Barnes & Greenwood (1978) recorded <1% of *Hydrobia* rejected natural substrates under laboratory conditions at a density of ~5,000individuals m^{-2}. Additionally, Barnes (1981a,b) did not consider climbing to be a distinct behavioural response but merely crawling/browsing carried out in a vertical plane unrelated to density. Little & Nix (1976) also concluded that the high incidence of floating in laboratory conditions (between 10-80%) was an artificial behaviour that was not expressed in natural populations. Lopez-Figueroa & Neill (1987) considered inactive snails and other avoidance behaviours to be a

naturally occurring density-dependent occurrence however. These authors reported that the number of inactive individuals recorded increased at higher population densities and migration from sediment by up to 40% of the population when densities were >10,000individuls m^{-2}. A similar level of avoidance behaviour was observed at approximately half this density in the current study.

Although sediment grain size, population density and food availability were shown to influence the spatial distribution of *Hydrobia* in the current study, other factors that were not examined may also elicit an influence on the various size classes within the population. Tidal immersion/emersion regime, salinity, sediment moisture content at low tide, aspect, inter-specific competition, predation and localised hydrodynamics all have the potential to affect spatial distribution (Gosling, 2003). Higher shore levels will require physiological tolerance of aerial exposure and the associated fluctuations in temperature, salinity and humidity (Boaden & Seed, 1993). Biological stress, in the form of competition and predation generally increase at lower shore levels as the spatial refuge from marine competitors and predators is diminished. Significant predation probably occurs on all areas of the mudflat at low tide however, as Bull Island represents an important feeding ground for resident and migratory waterfowl. Although the occurrence of a mudflat at the site suggests a low energy environment, strong tidal currents in the central creek during ebbing and flooding tides probably account for the lack of *Hydrobia* in this area. On a smaller scale, hydrodynamic scouring at the base of the few large boulders scattered across the site also resulted in an absence of *Hydrobia* in the immediate area.

Density-dependent interaction between conspecifics appears to be the major determinant of the spatial distribution and abundance of *Hydrobia* in natural conditions at the Bull Island site. This may be considered unsurprising considering the very high abundance of this species. Although sediment preference was exhibited at low abundance in the laboratory, densities were rarely as low in the field. Smaller individuals appeared to display higher substrate specificity than previously settled individuals, they were, however, also tolerant of the range of sediment types at the study site. This higher substrate specificity of smaller individuals could be due to higher food resource requirements of juveniles or size-specific settlement requirments (Roughgarden *et al.*, 1988). As low physical energy environments are required for the

formation of mudflats, they tend to be relatively infrequent and isolated habitats within the intertidal zone depending on local hydrography. The ability of *Hydrobia* to inhabit a relatively broad spectrum of mud-based sediment types within these habitats probably contributes significantly to the species often being the dominant primary consumer where it occurs.

Chapter 6. Determining the food sources of two intertidal mollusc species and estimating potential ingestion rates.

6.1 Introduction

A wide variety of potential sedimentary food resources, which include microalgae (benthic and planktonic), bacteria, meiofauna and non-living organic matter (Lopez & Levinton, 1987) are available for utilisation by marine/estuarine primary consumers. Although this makes it difficult to define and quantify species-specific food sources and feeding rates, and hence their impact on primary producers, most species feed on a particular size range and type of food. The feeding strategy employed by consumer species may give some indication of the food sources utilised. Suspension feeders generally lack the ability to deposit feed, extracting food items directly from the water column, the converse being true for deposit feeders (Wolff, 1983; Kamermans, 1994). The situation is complicated by the ability of some species to display either of these strategies depending on the prevailing environmental conditions however (Hummel, 1985). The regular passive deposition and re-suspension of food sources between sediments and the water column, by physical disturbance and tidal action (Baillie & Welsh, 1980; Brotas & Catarino, 1995, Lucas *et al.*, 2000, 2001), further complicates the use of feeding strategy alone to determine the type of organisms consumed. Even when food sources have been ingested, they may be rejected internally and egested as pseudo-faeces, or pass through the gut unaltered (Hughes, 1975; Admiraal, 1984; Hummel, 1985).

Although certain laboratory methods have been employed in an attempt identify the food sources of herbivorous molluscan species, the results are the subject of much ongoing controversy (Jorgensen, 1996; Cranford, 2001; Bayne, 2004). Much of this is due to the size range and quality of food items within the feeding cultures used, and the methodologies used to measure their depletion. In natural circumstances species will encounter a very broad size and quality range of food items, of which only a selection will be suitable for consumption (Levinton, 1989). Those selected will depend on factors related to the environment, such as food availability, and the size/physiology of the consumer. Ontogenetic changes in diet type and size occur for many species during development (Hentschel, 1998). For example, it is generally

thought that deposit-feeders alter their feeding behaviour with increasing body size, from ingesting small-sized, high quality food resources when juvenile to larger nutritionally poor material as adults (Lopez & Levinton, 1987). As most previously published data relating to the food sources of consumer species is based on laboratory experimentation with specific and narrow size ranges of individuals feeding on artificial diets with a similarly constrained size composition, their results must be viewed with caution.

In more recent times, stable isotope ratio analysis has proved to be a useful way to determine food sources of aquatic animals (Peterson & Fry 1987, 1999; Jacob *et al.*, 2005). This is based on the fact that the $\delta^{13}C$ and $\delta^{15}N$ values of consumers reflect those of their diets. Stable isotopes can be used to trace organic matter through food chains once the isotope ratios of the diet organisms are isotopically distinct, as certain isotopes fractionate in predictable ways as the elements travel through food webs. A small consistent degree of fractionation of 1‰ in $\delta^{13}C$ isotopic ratios (DeNiro & Epstein, 1978; Fry & Sherr, 1984; Peterson and Fry, 1987; Kang *et al.*, 2003) and a 3 ‰ shift in $\delta^{15}N$ ratios (DeNiro & Epstein; 1981; Peterson and Fry, 1987; Currin *et al.*, 1995; Yokoyama & Ishihi, 2003) occurs between the isotopic signal of the diet organism and that of the consumer. For example, it is generally recognised that microphytobenthos have $\delta^{13}C$ values that are significantly less negative than those of microphytoplankton, terrestrial vascular and saltmarsh plants (Currin *et al.*, 1995; Riera *et al.*, 1999a, b; Kang *et al.*, 1999; Kang *et al.*, 2003). Therefore, if microphytobenthos were the main food source for a consumer species its $\delta^{13}C$ values would be less negative than if they were mainly consuming other food sources.

A wide variety of factors are known to affect feeding rate, duration (ingestion) and subsequent nutrient uptake (assimilation). Controlling factors include moisture content/water coverage (Barnes, 1986; Orvain & Sauriau, 2002), food concentration (Kofoed, 1975; Lopez & Cheng, 1983; Bianchi & Levinton, 1984; Forbes & Lopez, 1986; Morrisey, 1988a; Sprung & Rose, 1988; Barnes & de Villiers, 2000; Orvain & Sauriau, 2002; Rossi, 2002; Navarro & Velasco, 2003), consumer population density (Levinton & Bianchi, 1981; López-Figueroa & Neill, 1987; Morrisey, 1987, 1988a; Peterson & Black, 1987; Kamermans *et al.*, 1992; Blanchard *et al.*, 2000; Roberts *et al.*, 2000; Barnes, 2001), light intensity (Bianchi & Levinton, 1984; Barnes, 1986;

Orvain & Sauriau, 2002), environmental temperature (Hylleberg, 1975; Barnett, 1985; Hagerthey *et al.,* 2002), interspecific competition (Bianchi & Levinton, 1981; Bianchi *et al.*, 1988; Morrisey, 1988; Page *et al.*, 1992; Smith *et al.*, 1996; Gaudy *et al.*, 2000), digestive efficiency (Trevallion, 1971; Foster-Smith, 1975; Hughes, 1975; Lopez & Cheng, 1983; Hummel, 1985; Cammen, 1989; Jorgensen, 1996; Iglesias *et al.*, 1998; Roberts *et al.*, 2000), current velocity for filter feeders (Winter, 1978; Judge *et al.*, 1992; Underwood & Krompkamp, 1999; Gosling, 2003) and sediment grain size for deposit feeders (Barnes & Greenwood, 1978; Lopez & Kofoed, 1980; Forbes & Lopez, 1989; Grudemo & Bohlin, 2000). The relationship between feeding and each of these factors was generally studied under laboratory conditions that isolated or strictly controlled other regulatory mechanisms however. Results were generally obtained after relatively short periods of experimentation (<1hr), and extrapolated to give daily estimates of feeding activity or the relative affect of treatment. For these reasons, most laboratory derived feeding rate and assimilation estimates are probably in excess of those that occur under natural conditions (Ruesink, 2000).

Chlorophyll *a* concentration is considered to represent a good index of potential primary production, as it is an easily quantifiable parameter that is a common component to all primary producers in shallow water (Heip *et al.*, 1995; MacIntyre *et al.*, 1996). For this reason its depletion under experimental conditions is considered a good approximation to ingestion rate as a range of sizes and qualities of food sources, representative of that in natural conditions, can be supplied. This permits the consumer to preferentially select items from its natural dietary range. The affect of deposit feeding on sediment chlorophyll *a* has been examined successfully in the surface deposit feeders, *Macoma nasula*, (Page *et al.*, 1992) *Macoma balthica* (Bianchi *et al.*, 1988; Webb, 1993) and the sub-surface deposit feeders *Yolida limatula* (Ingalls *et al.*, 2000) and *Leitoscoloplos fragilis* (Bianchi *et al.*, 1988). Determination of food type and feeding rate are desirable, as these can be combined with population parameters to estimate the probable impact of a consumer population on the primary productivity within the ecosystem to which it belongs. For most consumer species, there is some speculation as to whether 'top down' or 'bottom up' regulation occurs between primary producers and consumers, or whether there is no link between their relative abundances. Primary consumers are an integral part of the food chain as they facilitate the transfer of nutrients to higher trophic levels that often

contain species that cannot utilise primary productivity directly to the same extent (Heip *et al.*, 1995; Rossi, 2002). The aim of this chapter was to identify the food sources of two herbivorous intertidal mollusc species and compare potential ingestion rates under field and laboratory conditions. In order to determine potential feeding rates size specific feeding behaviour was examined. Food intake was also measured using natural sediment as a food source and using artificial/manipulated food sources.

6.2 Materials and methods

6.2.1 Study sites and species

Two soft sediment study sites in Dublin Bay, described in previous chapters, were examined. The mudflat at Bull Island contains high densities of the gastropod *Hydrobia ulvae*, while the sandflat at Blackrock on the opposite side of the bay supports a population of the infaunal bivalve *Tellina tenuis*. These two species have different feeding modes. *Hydrobia*, a surface deposit feeder, can feed either by ingesting sediment and epipelic diatoms or grazing on epipsammic microphytobenthos attached to sediment particles. Sediment covered with water or with a very high water content is required to support feeding of this species, as it must crawl to ingest food items, a behaviour that is restricted at lower moisture content (Lopez & Kofoed 1980). In contrast, the sedentary *Tellina* is considered to be a deposit feeder that mainly obtains epipelic microphytobenthos from the sand-water interface, although can also filter feed from the water column (Trevallion, 1971).

6.2.2 Stable isotope analysis- defining probable food sources

To determine the food sources of each study species, stable isotope ratio analysis was conducted on individuals collected from the two study sites. As isotopic ratio has been shown to vary between seasons for some species in response to dietary variation, samples that represented seasonal extremes were analysed. *Hydrobia ulvae* of various sizes were collected from the mudflat at Bull Island on 1/7/01 and 9/1/02 to represent summer and winter samples respectively. Samples of *Tellina tenuis* were collected from the sandflat at Blackrock on the same days. All specimens were frozen (-18^{o}C) until analysis. Animals were defrosted and shell height (SH) measured from posterior to anterior shell tips using Mitutoyo Absolute Digimatic callipers to 0.1mm. The soft

body tissue of *Tellina* was removed from the shell valves. The stomach and contents were separated from the remaining flesh and discarded along with the shell valves. The remaining tissue was oven dried and ground with a mortar and pestle. Isotopic variation exists among different tissues within individual animals (Peterson and Fry, 1987) as their various functions result in different rates and action of diet assimilation. Stomach contents were removed as they represent only the recent diet of the specimens whereas body-tissue composition integrates isotope signatures over a longer period of time (Herman *et al.*, 2000), and are therefore more indicative of medium to long-term diet. The tissues from a number of individuals were generally pooled prior to analysis, as this has been found to be advantageous in populations where small-scale temporal variation in population parameters can occur (Lancaster & Waldren, 2001). Intraspecific variation in population parameters are often more common in populations that are densely aggregated and highly abundant. In order to lower sources of variation within pooled samples, replicate animals of a similar size class were used when possible (Table 6.1). Although replication was low, it was comparable with the majority of isotope studies as high analysis costs restricts use of technique. The recommendations of Lancaster & Waldren (2001) and Jacobs *et al.* 2005) were followed in order to decrease any effects due to small sample size. Due to their small body size, it was not practical to remove the stomach from *Hydrobia* specimens. Therefore, individuals selected for isotope analysis were held in filtered seawater for a period of 3days prior to being frozen. It was assumed that this process would result in the complete emptying of the stomach. *Hydrobia* tissue was removed from the shell under a dissecting microscope and was oven dried and ground with a mortar and pestle. Dried tissue of both species (ca.1.5mg) in standard weight tin capsules were analysed for stable carbon and nitrogen isotope ratios using a CE Instruments 1112 Flash elemental analyser coupled via a Conflo III to a Thermo Delta^{plus} Continuous Flow Isotope Ration Mass Spectrometer (CF-IRMS).

Table 6.1. Composition of samples of *Hydrobia* and *Tellina* for stable isotope ratios analysis. Identical samples to those listed were analysed for each of the summer (1/7/01) and winter (9/1/02) periods.

Species	No. individuals	SH (mm)
Hydrobia	4	3-6
	5	3-4
	5	4-5
	5	5-6
	6	2-4
	10	3-4
Tellina	1	12
	1	13
	1	16
	2	11
	2	11-13
	4	11-12
	4	12-13
	4	13-14
	7	3-8

Data were expressed in the standard unit notation where:

$$\delta X = [(R_{sample}/R_{reference}) - 1] \times 10^3,$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Results were reported relative to air for nitrogen isotopes and the Vienna Pee Dee Belemnite standard (VPDB) for carbon isotopes. Standard external precision based on replicate analysis of calibrated in-house standards was <0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$

6.2.3 Field experiments-Hydrobia feeding rate

Attempts were made to determine chlorophyll *a* ingestion rates for *Hydrobia* while *in situ* at the study site. This was considered the most likely way in which to determine the actual ingestion rates achieved in the natural environment. A large amount of surface sediment was collected from the mudflat at Bull Island. This sediment was sieved in the laboratory using sieve size 0.5mm. All *Hydrobia* were collected, while algae and other fauna were discarded. The sieved sediment was homogenised, covered with seawater and aerated in a cold room at a temperature of 10°C. *Hydrobia* were stored in buckets of aerated seawater at the same temperature until required.

Twenty circular pipe enclosures 38cm in length and with a surface area of 176.7cm^2 were pushed into the sediment at Bull Island so that only 10cm protruded above the mud. Each enclosure was fitted with a rigid screw-top lid with 2mm diameter holes drilled 2cm from the top of the pipe. These holes permitted the free flow of water through the enclosure. Ten of the lids were solid while the remainder consisted of a 1mm mesh panel the same diameter as the pipe enclosure. While both lid types prevented the entry and exit of macrofauna, only the later permitted the entry of light.

Enclosures were positioned on the far side of the creek 25cm apart, arranged from upper to lower mudflat (Table 6.2). All sediment to a depth of 20cm below the surface was removed from the enclosures and replaced with the sieved (0.5mm) sediment. Three syringe cores with an area of 2cm^2 were removed from each core to establish initial photopigment concentration per core as described in section 2.2.4. Chlorophyll *a* and phaeopigment concentration of the sediment in each core was not significantly different, (ANOVA, F=0.08, df=19, p=0.96) negating the possibility that initial concentration affected results observed (Table 6.3). A density of 16,977 individuals m^{-2} was established in 10 enclosures by adding 300 previously collected *Hydrobia* in the size range 3-5mm SH. Half of these were capped with a solid lid, the remainder with a mesh lid. This was repeated for the rest of the enclosures, although no *Hydrobia* were added. This resulted in 5 replicates for each of the four treatments, light/dark and with/without *Hydrobia*.

Table 6.2. The arrangement of enclosures at Bull Island showing the layout of dark and light treatments and the cores that contained *Hydrobia* (H).

Dark (H)	Light (H)	Dark	Light
Light	Dark	Light (H)	Dark (H)
Dark (H)	Light (H)	Dark	Light
Light	Dark	Light (H)	Dark (H)
Dark (H)	Light (H)	Dark	Light

Table 6.3. Initial mean chlorophyll *a* concentration for each treatment in each core (N=3).

Light		Dark	
Hydrobia Present	*Hydrobia* Absent	*Hydrobia* Present	*Hydrobia* Absent
94.4	101.6	104.9	95.1
101.6	84.1	106.8	86.7
97.9	93.8	107.7	90.7
90.7	99.7	96.6	89.4
89.2	103.4	88.4	104.9

After a period of 15 days lids were removed from enclosures and three syringe cores for photopigment analysis were extracted and analysed according to section 2.2.4. The surface sediment in each core was removed and returned to the laboratory in order to confirm the presence/absence of *Hydrobia* in each core.

Due to the high level of public access to the Blackrock sandflat, it was not possible to conduct a similar *in situ* experiment for *Tellina* at this site. Attempts to leave equipment at this site were hampered by vandalism.

6.2.4 Laboratory experiments-Hydrobia

Due to difficulties in obtaining feeding rate estimates in field-based experiments, further methods were conducted under laboratory conditions.

6.2.4a Ash free dry weight

Ten containers with a base area of 30cm^2 were filled with freshly collected mud from the Bull Island site. *Hydrobia* in the size range 3-5mm SH were added to correspond to a density of 10,000 individuals m^{-2}. Ten further containers were prepared in the same way, but no *Hydrobia* added. Five sediment samples of approximately 5g were removed from each of the 20 containers and placed in foil containers. These were placed into an oven at 100°C for 48hr, removed and weighed to an accuracy of 0.0001g to determine dry weight, and then transferred to a muffle furnace and incinerated at 550°C for 4hr. Samples were then re-weighted to determine ash free dry weight, the difference between dry and ash free dry weight corresponding to organic content. This process was repeated after 1, 3, 5, 7 and 14 days.

6.2.4b Changes in chlorophyll a-fixed density

Sieved sediment from Bull Island was placed in a thick layer in 40 petri-dishes (area 19.6cm^2). Initial chlorophyll *a* content was determined using the methodology described in section 2.2.4. Dishes were placed in a constant temperature room (15°C), and half retained in darkness and half in light. Twenty *Hydrobia* of SH 3-5mm were placed in 10 petri-dishes within each light/dark treatment, corresponding to a density of 10,188m^{-2}. The remaining dishes were divided between light and dark treatments, but no *Hydrobia* were added. Sediment was kept damp by adding microfiltered (Whatman membrane filter, pore size 0.2μm) seawater to each dish every day as *Hydrobia* become inactive on dry sediments (Barnes, 1986). Three samples per dish were taken after 21 days to determine chlorophyll *a* concentration.

6.2.4c Changes in chlorophyll a-variable density

As it was not clear whether the relatively high density used in the previous experiment had affected the grazing behaviour of *Hydrobia*, the experiment was repeated with varying densities and under 12hr light/dark regime. The appropriate numbers of *Hydrobia* (3-5mm SH) were placed in each dish according to the densities shown in Table 6.4 with 10 replicates per treatment. Three samples to determine photopigment concentration were taken at the beginning of the experiment and again after 7,14 and 21 days.

Table 6.4. Number of *Hydrobia* placed in each treatment (10 replicates) and the corresponding density.

No. *Hydrobia*	Approximate Density (m^{-2})
0	0
10	5,000
15	7,500
20	10,000
25	12,500

6.2.5 Feeding experiments-Tellina tenuis

6.2.5a Ash free dry weight

Ten containers with a base area of 30cm^2 were filled with freshly collected sand from the Blackrock site. *Tellina* in the size range 8-16mm SH were added to correspond to a density of 2000 individuals m^{-2}. Ten further containers were prepared in the same way, but no *Tellina* added. Five sediment samples of approximately 5g were removed from each of the 20 containers and placed in foil containers. These were placed into an oven at 100°C for 48hr, removed and weighed to an accuracy of 0.0001g to determine dry weight, and then transferred to a muffle furnace and incinerated at 550°C for 4hr. Samples were then re-weighted to determine ash free dry weight, the difference between dry and ash free dry weight corresponding to organic content. Microfiltered seawater (Whatman membrane filter, pore size 0.2μm) was added to each beaker, placed in a constant temperature room (15°C) in the dark and each container was aerated. This process was repeated after 1, 3, 5, 7 and 14 days.

6.2.5b Changes in sediment chlorophyll a

Ten containers with a base area of 30cm^2 were filled with freshly collected sand from the Blackrock site. *Tellina* in the size range 8-16mm SH were added to correspond to a density of 2000 individuals m^{-2}. Ten further containers were prepared in the same way, but no *Tellina* added. Five syringe cores with an area of 2cm^2 were removed from each enclosure and initial chlorophyll *a* content was determined using the methodology described in section 2.2.4. Microfiltered seawater (Whatman membrane filter, pore size 0.2μm) was added to each beaker, placed in a constant temperature room (15°C) in the dark and each container was aerated. After a period of 21 days a further 5 syringe cores for chlorophyll *a* analysis.

6.2.5c Clearance rate

In order to determine the feeding rate of *Tellina* when filtering suspended photopigments from the water column it was necessary to obtain a clearance rate

(number of cells retained animal^{-1} h^{-1}). Two methods were used to try to ascertain the clearance rate.

Method 1-chlorophyll *a* concentration

Concentration of chlorophyll *a* in suspension can be measured routinely using a light absorption relationship (Crisp, 1984). Seawater and *Tellina tenuis* of various sizes were collected at Blackrock, returned to the laboratory and stored in the dark at 4°C. *Tellina* were cleaned, rinsed in seawater, measured and assigned to an appropriate size group. *Tellina* were then removed and placed in an aerated holding tank for 24h. On the following day of use specimens were placed in microfiltered seawater (Whatman membrane filter, pore size 0.2μm) for 1-2h prior to experimentation. Groups of individuals were placed in a static system (beakers in a temperature controlled bath) filled with 0.5l seawater at the test temperature of 15°C in the combinations described in Table 6.5. A further group of control beakers were treated in the same manner in the absence of *Tellina*. Initial chlorophyll *a* concentration at time zero (t_0) was measured from 0.5l of the same stored seawater stock used in the experiment (N=10 per experiment). This initial chlorophyll *a* concentration was quite low (1.9-3.5 ugl^{-1}) but was thought that natural seawater concentrations would allow *Tellina* to feed normally and as a result realistic feeding rates determined. All beakers were aerated during the experiment to supply oxygen and to ensure an evenly distributed food concentration. The experiment was carried out in the dark to slow down the degradation of chlorophyll *a* by light (Marker *et al.*, 1980; Rai, 1980). The beakers were left undisturbed for 30min after which all *Tellina* were removed quickly and returned to a holding tank. On one occasion, experimental duration was increased to 1hr. The seawater from each beaker was quickly filtered in the dark to collect chlorophyll *a*. Concentration was determined by the methodology described in section 2.2.4.

Table 6.5. Experimental design for *Tellina* clearance rate experiments.

Experiment	Duration (min)	*Tellina* size range (shell height mm)	Individuals per chamber	Experimental & control replicates
1	30	10-18	1	20
2	30	8-18	1	20
3	30	8-18	2	20
4	30	8-18	5	20
5	30	8-18	7	20
6	30	10-18	1	20
7	30	4-8	1	20
8	60	10-18	1	20

Method 2-particle counter

Tellina of various sizes were collected from Blackrock and placed in filtered seawater (Whatman GF/C). They were acclimatised for 10-14 days to the experimental temperature of 15°C, during which time they were fed the experimental food item. A mixture of aquarium brand marine phytoplankton (*Nannochloropsis, Tetraselmis, Isochrysis sp. tahaitian*) called "Phytoplex" supplied by Kent Marine Quality ™ was used as the food item. Calibration measurement of "Phytoplex" with a Sysmex CDA–500-particle counter analyser showed a size range of 1.5–8.48μm. An average of 98.5% of the algae was in the size range 1.5–3.5μm with a mean size of 1.7μm. The correction of the initial concentration with the baseline reading revealed that very few cells (10-20 cells ml^{-1}) were available at a size >5μm in comparison to 20,000 cells ml^{-1} <5μm. Therefore particles >5μm were disregarded as dietary items and the mean particle size of the food was defined as 1.7±0.3μm. A food concentration range between 16,000–20,000cells ml^{-1} was chosen to prevent the production of pseudofaeces and to ensure maximum possible filtration rate. This concentration was close to that determined by Sprung and Rose (1988) as the incipient limiting concentration for *Dreissena polymorpha* and was also used successfully in clearance rate experiments for *Sphaerium corneum, Musculium lacustre* and *Pisidium subtruncatum* (O'Toole, 2001).

Twenty-four hours prior to experimentation, *Tellina* were placed in micro-filtered seawater (Whatman membrane filter, pore size 0.2μm) to flush out their guts. A stock solution of micro-filtered seawater (Whatman membrane filter, pore size 0.2μm) containing Phytoplex with an initial algae concentration of 16,000–20,000 cells ml^{-1} was stirred vigorously and 110ml poured into each 120ml experimental chamber. The

chamber was aerated using an air pump to ensure suspension of the food items. A 5ml sample was taken to measure the initial food concentration per chamber. The required number of *Tellina* (Table 6.5), were placed individually into the chambers, placed in the dark and allowed 5min to open their valves and extend their siphons. *Tellina* were removed from the chambers after a further 30min. On one occasion, experimental duration was increased to 1 hr. Chambers were closed and shaken to ensure even suspension before a 5ml sample was taken. This process was repeated for controls that contained no *Tellina*. The particle concentration and size range was measured with the Sysmex CDA–500-particle counter analyser and the data obtained for t_0 and t_1 corrected for a blank. The clearance rate from experimental chambers was compared with that in controls using paired t-test.

6.3 Results

6.3.1 Stable isotopes

Due to equipment error, the stable isotope analyser failed to determine the $\delta^{13}C$ for 10 samples and $\delta^{15}N$ for 1 sample. The single value obtained for the alternative isotope in each case was considered accurate and included in the following analyses. Due to the failure of these samples it was not possible to examine size-specific variation in isotope signatures in the current study.

The $\delta^{13}C$ values for *Hydrobia* ranged from -17.8 to -20.9‰ (Figure 6.1). The winter *Hydrobia* specimens were more $\delta^{13}C$ enriched than the summer samples with one exception, and differences were non-significant. The $\delta^{15}N$ values for *Hydrobia* in the summer had a relatively narrow range of 4.7 to 5.5‰ compared with 5.3 to 7.9‰ during the winter (Figure 6.1). Although the $\delta^{15}N$ values for *Hydrobia* in winter were enriched compared with the summer isotope ratios with one exception, these differences were not statistically significant.

The $\delta^{13}C$ values for the *Tellina* samples ranged from -18.0 to -19.5‰ with the summer samples generally more depleted than the winter specimens (Figure 6.1). Although the *Tellina* summer samples also had a wider range in values than the winter specimens, there were no statistically significant differences between seasons. In

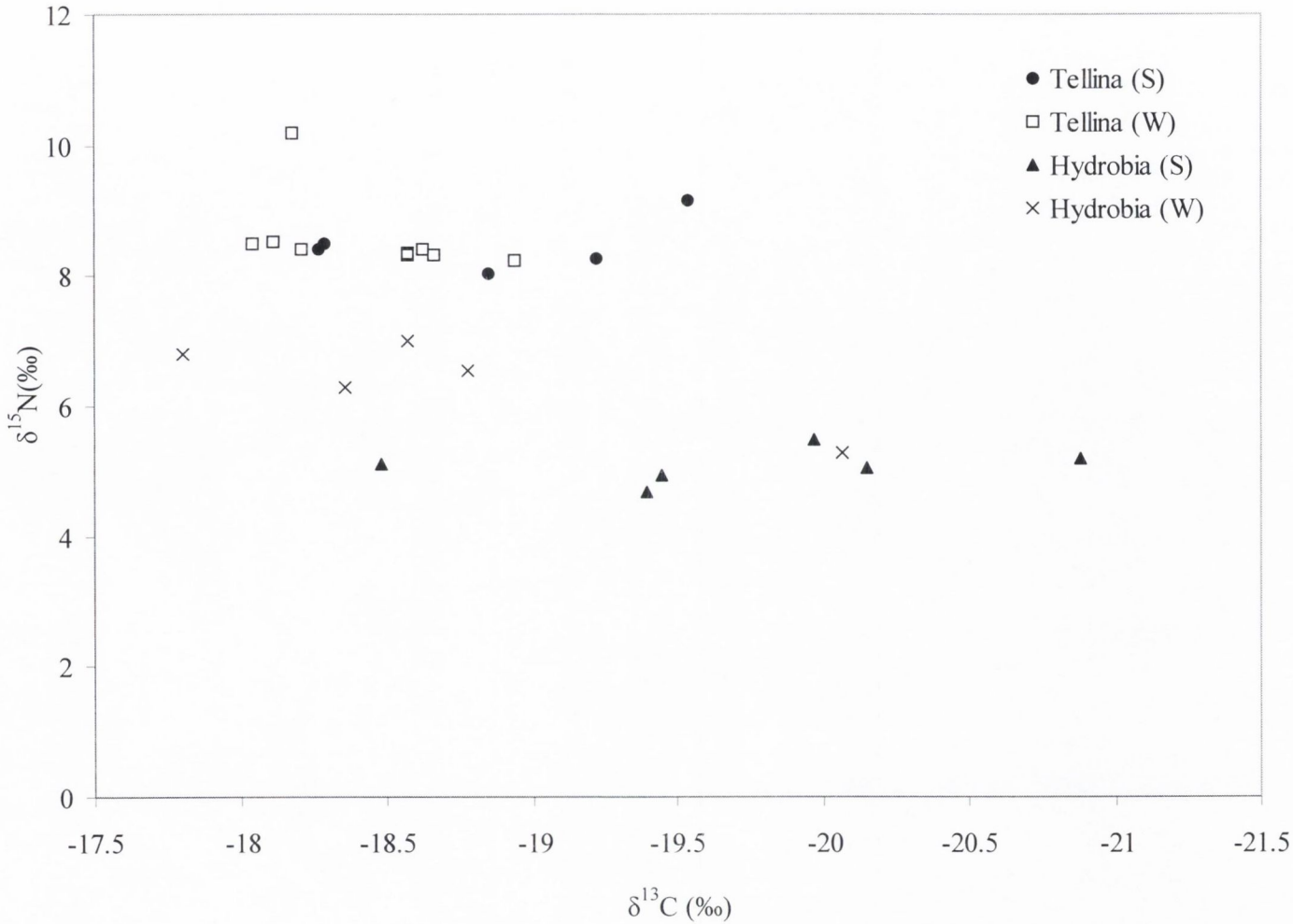


Figure 6.1. $\delta^{13}C$ and $\delta^{15}N$ values for *Hydrobia* and *Tellina* in summer (S) and winter (W) in Dublin Bay. The 11 samples where one isotope value was not resolved were omitted for clarity.

summer, the $\delta^{15}N$ values for *Tellina* ranged from 7.9 to 9.2‰ and from 8.1 to 10.2‰ in winter. These differences were not significant, however.

The *Hydrobia* isotope ratios had a wider $\delta^{13}C$ range than *Tellina*. *Hydrobia* summer isotope ratios were significantly more negative than the signatures of *Tellina* in both summer and winter (ANOVA, $F = 7.8$, df=3, $p \leq 0.01$), Table 6.6. *Tellina* was significantly more $\delta^{15}N$ enriched than *Hydrobia* in both summer and winter (ANOVA, $F = 31.3$, df=3, $p \leq 0.01$) (Table 6.6).

Table 6.6. Comparison of $\delta^{13}C$ isotope ratios of *Hydrobia* and *Tellina* in summer and winter. * indicates differences significant at < 0.05 level.

	Hydrobia (W)	*Tellina* (S)	*Tellina* (W)
Hydrobia (S)	0.08	0.03*	≤ 0.01*
Hydrobia (W)		0.99	0.79
Tellina (S)			0.65

Table 6.7. Comparison of $\delta^{15}N$ isotope ratios of *Hydrobia* and *Tellina* in summer and winter. * indicates differences significant at < 0.05 level.

	Hydrobia (W)	*Tellina* (S)	*Tellina* (W)
Hydrobia (S)	0.13	≤ 0.01*	≤ 0.01*
Hydrobia (W)		≤ 0.01*	≤ 0.01*
Tellina (S)			0.89

Carbon:nitrogen ratios were low for both species and varied little in range (Table 6.8). *Hydrobia* had a lower C:N ratio in summer than in winter, the opposite pattern observed for *Tellina*. Significant differences were observed between the carbon:nitrogen ratios (Kruskal Wallis, $\chi^2=9.4$, df=3, p=0.02). Dunn post-hoc tests revealed that the summer ratio was significantly less than the winter ratio in the *Hydrobia* samples (p=0.04). No difference was observed with season for the *Tellina* samples but a difference was observed between *Hydrobia* and *Tellina* in summer (p=0.02).

Table 6.8. Mean (±S.E.) carbon/nitrogen ratio for *Hydrobia ulvae* and *Tellina tenuis* in two seasons.

Season	*Hydrobia*	*Tellina*
Summer	3.7 ± 0.07	4.1 ± 0.15
Winter	4.1 ± 0.08	3.8 ± 0.02

Assuming a presumptive 1.0‰ trophic shift in $\delta^{13}C$ and a 3‰ shift in $\delta^{15}N$, the expected values of the diet items of *Tellina* and *Hydrobia* would be in the range shown in Table 6.9. The range of $\delta^{13}C$ and $\delta^{15}N$ values for food sources of *Tellina* (S.D.= 0.4) displayed less variation between summer and winter samples compared with *Hydrobia* (S.D.=1.0).

Table 6.9. The range of isotopic ratio values of dietary food for *Tellina* and *Hydrobia* given a 1.0‰ trophic shift in $\delta^{13}C$ and a 3‰ shift in $\delta^{15}N$ isotope ratios.

Species	Season	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Hydrobia	Summer	-21.0 to -21.9	2.1 to 2.5
Hydrobia	Winter	-18.8 to -21.1	2.3 to 4.0
Tellina	Summer	-19.3 to -20.5	5.1 to 6.2
Tellina	Winter	-19.1 to -19.6	5.3 to 7.2

6.3.2 Field experiment-Hydrobia

Mean chlorophyll *a* increased from 91.2±3.8mg m^{-2} (S.E.) to 138.3±21.7mg m^{-2} in the light control treatment with no *Hydrobia* present over the 15 day experiment (Figure 6.2). Mean chlorophyll *a* also increased from an initial level of 93.3±8.8mg m^{-2} to 101.7±5.1mg m^{-2} in the light treatment with *Hydrobia* present. These increases from initial to final concentration under light conditions were not significant in either the control (Paired t-test, t=0.77, df=14, p=0.07) or the experimental cores (Paired t-test, t=-1.39, df=14, p=0.18). Mean chlorophyll *a* decreased in the dark treatment without *Hydrobia* present from 99.1±7.6 to 90.6 mg m^{-2}±5.6, while the dark treatment with *Hydrobia* also decreased from 95.1±5.6 to 91.4±9.2 mg m^{-2}. Again there were no significant differences between initial and final concentrations in the control (Paired t-test, t=0.58, df=14, p=0.57) and experimental (Paired t-test, t=-0.99, df=14, p=0.33) treatments. Although chlorophyll a concentration increased in the light treatment and control and decreased in the dark treatment and control these differences were not statistically significant (ANOVA, F=1.8, df=3, p=0.09).

Phaeopigment concentration showed an increase in the light experimental cores and a decrease in the cores kept in the dark (Figure 6.2). The light *Hydrobia* treatment increased significantly from an initial concentration of 64.5±4.8 to 96.1±8.4 mg m^{-2}

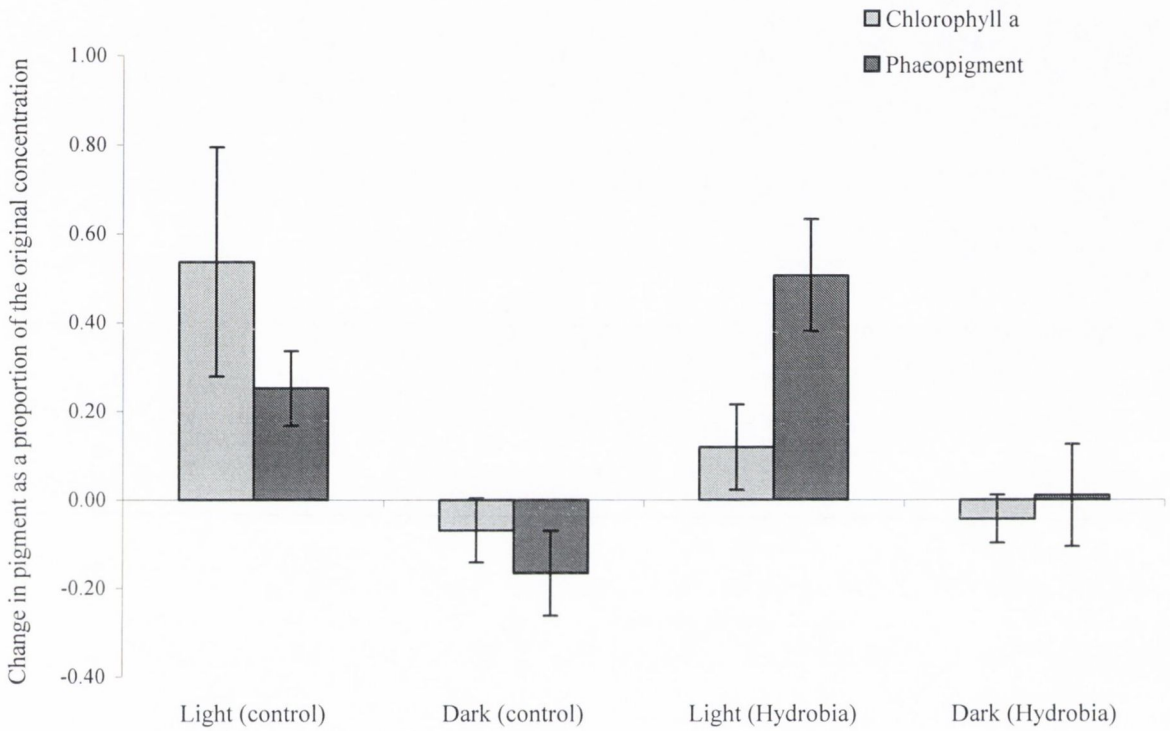


Figure 6.2. Change in sediment chlorophyll *a* and phaeopigment content (mg m^{-2} ± S.E.) as a proportion of the initial concentration within each of 4 treatments. N=60.

(Paired t-test, t=-2.16, df=14, p=0.04). The light control did not show any differences between initial and final concentrations (Paired t-test, t=-0.05, df=14, p=0.96). Significant differences between the initial and final phaeopigment concentrations occurred in both the control (Paired t-test, t=3.46, df=14, $p \leq 0.01$) and experimental (Paired t-test, t=2.48, df=14, p=0.02) dark treatments. Phaeopigment concentration did show significant differences between treatments (ANOVA, F=6.42, df=3, $p \leq 0.01$). The light experimental cores containing *Hydrobia* contained significantly higher phaeopigment concentration when compared with the dark experimental cores (Scheffe post-hoc tests, p=0.03). The light experimental cores containing *Hydrobia* were also significantly different to the dark control cores (Scheffe post-hoc tests, $p \leq 0.01$). Significant differences were not observed between the two light treatments (Scheffe post-hoc tests, p=0.35) or the two dark treatments (Scheffe post-hoc tests, p=0.82).

6.3.3 Laboratory experiments-Hydrobia

6.3.3a Ash free dry weight

Mean organic content did not decrease significantly over any of the time periods sampled (Table 6.10). In some cases organic content was estimated to have increased during certain periods of the experimental duration. This trend was also observed in controls containing no *Hydrobia*. It was not possible to estimate *Hydrobia* grazing rate from this experiment.

Table 6.10. Mean (±S.E.) organic content (mg) per 5g of sediment with and without (control) *Hydrobia* present over various time periods. N=20

Treatment	Time 0	1 days	3 days	5 days	7 days	14 days
Hydrobia	22.2±4.3	25.1±4.1	24.0±6.2	28.9±2.2	30.7±6.6	26.2±4.1
Control	25.3±3.9	25.6±3.4	28.0±4.1	24.1±4.5	22.2±2.3	24.3±4.5

6.3.3b Changes in chlorophyll a-fixed density

Mean chlorophyll *a* concentration decreased in all treatments over the 21 day duration (Table 6.11). Although the decrease was greater in treatments with *Hydrobia* present when compared with controls, differences were not statistically significant at the 5%

level (ANOVA, F=1.24, df=3, p=0.52). Sediment phaeopigment content followed a similar trend, particularly in the dark treatment. There were no significant differences between light and dark treatments for phaeopigment (ANOVA, F=2.33, df=3, p=0.18).

Table 6.11. Mean (±S.E.) decrease in chlorophyll *a* and phaeopigment (mg m^{-2}) over 21 days in light and dark treatments, with and without (control) *Hydrobia* present. (N=30 per treatment).

	Treatment	Mean chlorophyll a (mg m^{-2})	Mean phaeopigment (mg m^{-2})
Light	Hydrobia	38.4±8.4	33.8±4.3
	Control	24.0±7.2	32.9±5.6
Dark	Hydrobia	39.8±6.9	43.2±6.3
	Control	33.3±9.1	34.2±4.9

Had the differences in mean chlorophyll *a* concentration been significant, then the reduction between treatments in the dark, 6.5mg m^{-2}, would have represented grazing by *Hydrobia*. If all individuals were assumed to have fed equally and continuously, then a grazing rate of 0.016mg $individual^{-1}$ day^{-1} was suggested. The difference between the light and dark control, 9.3mg m^{-2} could therefore be assumed to represent the productivity of the standing micro-algae stock over this period. This was 10.8% of the mean standing concentration at the start of the experiment.

6.3.3c Changes in chlorophyll a -variable density

Mean sediment chlorophyll *a* content decreased in all treatments after 7, 14 and 21 days (Figures 6.3, 6.4, 6.5, respectively). In the first week (Figure 6.3), the highest density treatment (12,500 individuals m^{-2}) displayed the greatest decrease in mean chlorophyll *a* concentration, but this was not significantly lower than any other density treatment or the control (ANOVA, F=0.61, df=4, p=0.66). In subsequent weeks the magnitude of the decrease in chlorophyll *a* concentration increased. Although no significant differences occurred between treatments (ANOVA, F=0.14, df=4, p=0.96), the greatest decreases occurred in the control treatment for weeks 2 and 3 (Figures 6.4 and 6.5, respectively). Therefore, it was not possible to estimate the grazing rate of *Hydrobia* from this experiment, or detect significant variation in feeding activity with density.

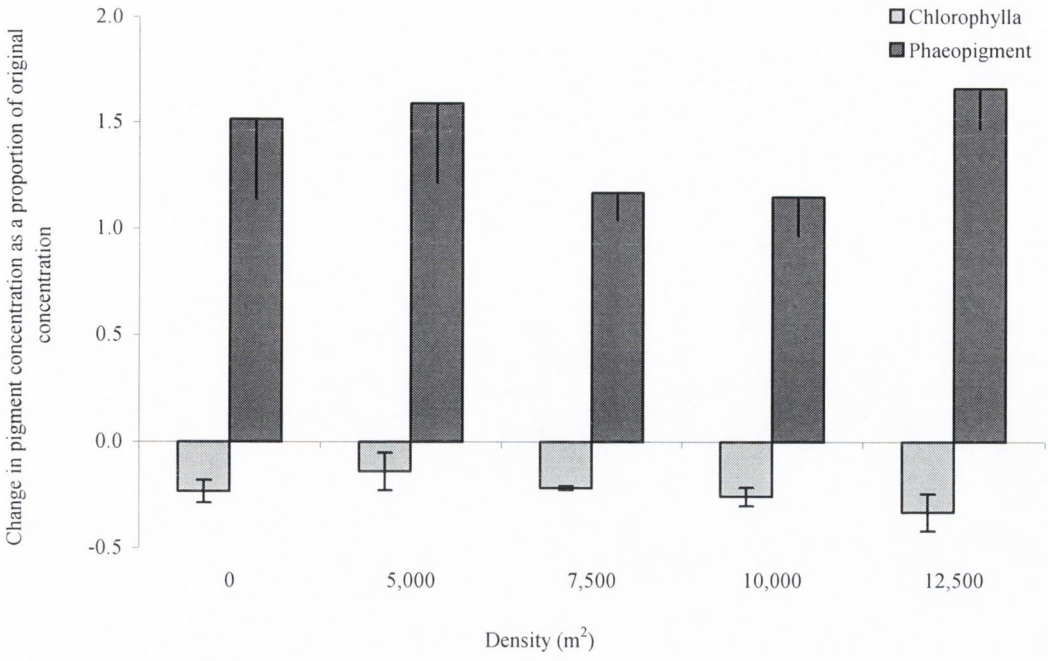


Figure 6.3. Change in sediment chlorophyll *a* and phaeopigment content (mg m^{-2} ± S.E.) over one week as a proportion of the initial concentration within each of 5 density treatments in the laboratory. N=150.

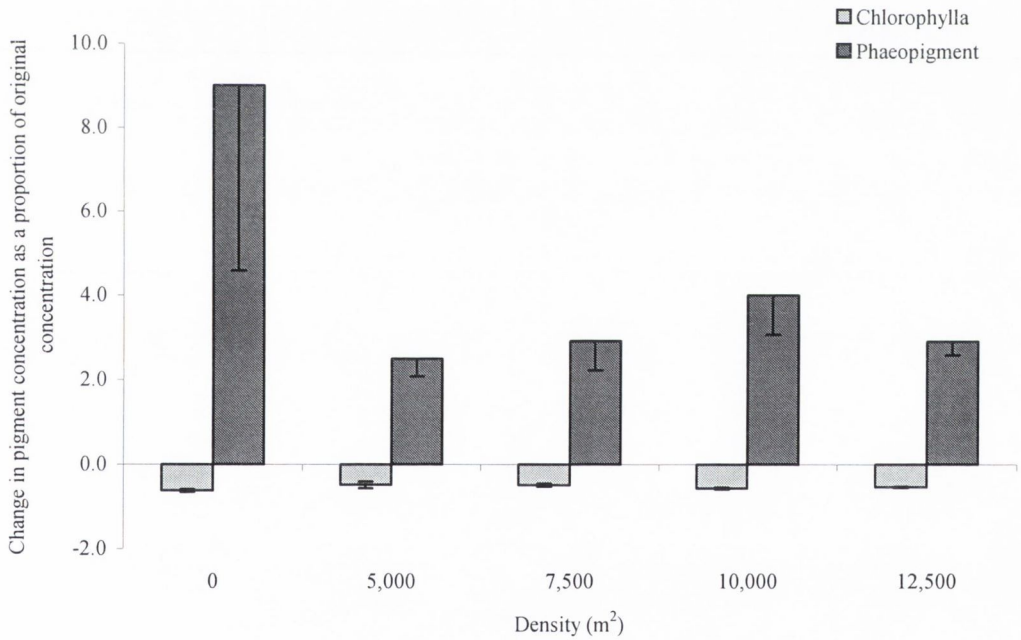


Figure 6.4. Change in sediment chlorophyll *a* and phaeopigment content (mg m^{-2} ± S.E.) in the second week (see Fig. 6.3) as a proportion of the initial concentration within each of 5 density treatments in the laboratory. N=150.

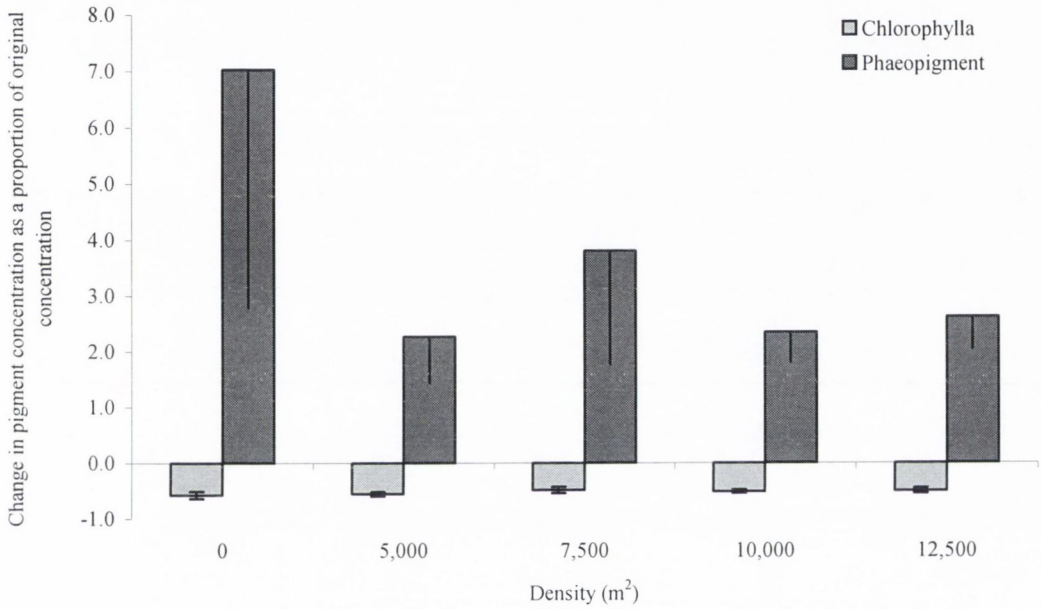


Figure 6.5. Change in sediment chlorophyll *a* and phaeopigment content (mg m^{-2} ± S.E.) during the third week (see Fig. 6.4) as a proportion of the initial concentration within each 5 density treatments densities in the laboratory. N=150.

Average phaeopigment concentration increased in all treatments in all weeks (Figures 6.3, 6.4, 6.5). In the first week, the highest increase occurred in the highest density (12,500 m^{-2}) treatment, and in the control treatment during weeks 2 and 3. No significant differences were recorded between any of the density treatments or the control (ANOVA, F=0.37, df=4, p=0.48).

6.3.4 Laboratory experiments-Tellina

6.3.4a Ash free dry weight

Mean organic content did not decrease consistently over any of the time periods sampled (Table 6.12). In some cases organic content was estimated to have increased during certain periods of the experimental duration. This trend was also observed in controls containing no *Tellina*. It was not possible to estimate *Tellina* grazing rate from this experiment.

Table 6.12. Mean (±S.E.) organic content (mg) for sediment with and without (control) *Tellina* present over various time periods. (N=10)

Treatment	Time 0	1 days	3 days	5 days	7 days	14 days
Tellina	12.2±2.4	14.2±4.5	14.6±4.2	12.2±3.3	12.2±3.9	13.3±4.0
Control	10.0±3.6	9.3±3.1	8.9±4.7	9.7±5.5	11.0±4.1	11.2±4.2

6.3.4b Changes in sediment chlorophyll a

Although mean sediment chlorophyll *a* decreased in the experimental containers containing *Tellina,* while controls increased slightly (Table 6.13), the differences were non-significant at the 5% level (2-sample t-test, t=0.09, df=9, p=0.19). This was mainly due to high variability in mean estimates as some replicates recorded the opposite trend to those recorded for the majority. Mean phaeopigment decreased over the experiment in both experimental and control treatments but these changes were not statistically significantly (2-sample t-test, t=-1.52, df=9, p=0.66). As a result it was not possible to estimate *Tellina* grazing rate from this experiment.

Table 6.13. Mean (±S.E.) change in chlorophyll *a* and phaeopigment (mg m^{-2}) over 21 days, with and without (control) *Tellina* present. (N=10)

Treatment	Mean chlorophyll *a* (mg m^{-2})	Mean phaeopigment (mg m^{-2})
Tellina	-5.6±7.4	-19.3±8.1
Control	+12.9±10.2	-16.4±12.9

6.3.4c Clearance rate

Method 1 - chlorophyll *a* concentration

The chlorophyll *a* concentration of the experimental containers did not decrease consistently in the presence of *Tellina* compared with controls that had no animals present. Some experiments actually showed a higher decrease in chlorophyll *a* in the controls than in the experimental containers where *Tellina* were present (Table 6.14). Experimental runs that increased animal numbers per chamber in an attempt to increase the feeding effect of the *Tellina* did not give consistant results. The number of *Tellina* per chamber was also reduced to minimise intra-specific interactions and hence result in an increased feeding rate. Despite numerous attempts and modifications such as increasing animal number per container and increasing the duration of the experiments a suitable protocol could not be established. Hence it was not possible to estimate *Tellina* clearance rate from this experiment.

Method 2 – particle counter

Estimating clearance rate by counting the change in particle numbers before and after feeding also yielded non-consistent results. No significant differences were observed between control and experimental chambers (Table 6.14). Numerous modifications, including changing time period, chamber size, *Tellina* size and N per chamber, were attempted in order to establish a successful protocol for obtaining a feeding rate. A reason for the large decrease in particle numbers in the control treatments could not be established however, and as a result significant differences between experimental and control treatments were not determined.

Table 6.14. Mean (±S.E.) clearance rate of chlorophyll *a* concentration and food particles for *Tellina tenuis* feeding in laboratory conditions with varying density and size. Results analysed using paired T-test of initial versus final concentrations, p-values shown.

Exp	Method	Time (min)	*Tellina* size range (shell height mm)	N in chamber	Mean decrease in control ($\mu g\ l^{-1}$)	Mean decrease in treatment ($\mu g\ l^{-1}$)	p-value
1	Chlorophyll conc.	30	11-18	1	0.50±0.15	0.83±0.22	0.42
2	Chlorophyll conc.	30	8-17	1	0.16±0.03	0.29±0.06	0.09
3	Chlorophyll conc.	30	8-17	2	0.19±0.05	0.45±0.19	0.35
4	Chlorophyll conc.	30	8-17	5	2.36±0.57	1.52±0.40	0.22
5	Chlorophyll conc.	30	8-17	7	0.62±0.17	1.21±0.31	0.10
6	Chlorophyll conc.	30	10-18	1	0.19±0.11	0.52±0.09	0.06
7	Chlorophyll conc.	30	4-8	1	0.32±0.04	0.45±0.05	0.17
8	Chlorophyll conc.	60	11-17	1	1.20±0.26	1.43±0.41	0.64
Exp	Method	Time (min)	*Tellina* size range (shell height mm)	N in chamber	Mean decrease in control (cells hr^{-1})	Mean decrease in treatment (cells hr^{-1})	p-value
1	Particle counter	30	11-18	1	3025±161	3110±130	0.68
2	Particle counter	30	8-17	1	1900±636	4024±886	0.06
3	Particle counter	30	8-17	2	2620±726	4637±685	0.07
4	Particle counter	30	8-17	5	4182±725	6206±881	0.08
5	Particle counter	30	8-17	7	3205±267	3135±218	0.84
6	Particle counter	30	10-18	1	3556±874	4339±926	0.54
7	Particle counter	30	4-8	1	1878±638	2775±774	0.15
8	Particle counter	60	11-17	1	2935±298	3145±274	0.61

6.4 Discussion

An extensive review of previously published literature revealed that the isotopic ratios determined for *Hydrobia* and *Tellina* in the current study in Dublin Bay were within the range of values recorded for many herbivorous intertidal macrofaunal species in other geographic locations. These data are summarised in Appendix 6.1. The $\delta^{13}C$ values observed in the literature for various macrofauna were in a relatively narrow range from -15 to -23‰. In comparison, the $\delta^{15}N$ signatures showed a relatively wide range in values from 2.2 to 19.7‰. With the exception of estuaries with strong anthropogenic influences the majority of $\delta^{15}N$ values were <10‰. Some of these values were for groups of different species with similar feeding habitats, while others examined individual species. The range in isotopic signatures of individuals species varied, with some such as *Crassostrea gigas* (Ruesink *et al.*, 2003) displaying relatively broad ranges and others such as *Ovatella bidentata* (Creach *et al.*, 1998) being much more constrained (Appendix 6.1). This may be in part due to the different feeding strategies employed by the species, species-specific variation in the type/size of food sources consumed or variation in the range of primary producers available for consumption. Some individuals studied were only collected during one period of the year, or information regarding the timing of sampling was not stated at all. Therefore, in addition to the actual utilisation of different food sources between species, the variation in ranges observed may be in part due to failure to account for seasonal changes in diet for some studies.

Isotopic $\delta^{13}C$ signatures of *Hydrobia ulvae* were reported previously from other geographic locations in Europe. Ratios of -15.1±0.2‰ (mean±S.D.) were stated for a population in France (Riera *et al.*, 1998) and between -15 to -15.5‰ in the Netherlands (Herman *et al.*, 2000) (Appendix 6.1). These values were less negative than the isotopic signatures of *Hydrobia* determined in the current study (-19.3±0.9‰). This may suggest geographic variation in the food sources utilised by these spatially distinct populations. Additionally, the greater range in values for *Hydrobia* at Bull Island may indicate a greater variety of food sources are utilised by this population. Herman *et al.*, (2000) recorded $\delta^{15}N$ signatures between 14 and 15.8‰ for *Hydrobia ulvae* in the Netherlands, which were highly enriched compared with the Dublin Bay population in the current study (5.9±1.0‰).

Although there are no published isotopic ratios for *Tellina tenuis*, a number of studies have focused on *Cerastoderma edule,* a bivalve found in similar habitats to *Tellina* (Appendix 6.1). These species co-occur at the Blackrock site. The $\delta^{13}C$ values for *C. edule* ranged from -18 to -22‰ (Kang *et al.*, 1999) and from -19.8 to -20.1‰ (Herman *et al.*, 2000) in France and the Netherlands, respectively. In the current study *Tellina* had $\delta^{13}C$ ratios within the ranges observed in both of these studies. *Scrobicularia plana,* a deposit-feeding bivalve, had $\delta^{13}C$ ratios in the range -15.9 to -19.2‰ in a population studied in Portugal (Costa *et al.*, 2000), also within the range determined for *Tellina* in the current study (-18.6±0.4‰). Recorded $\delta^{15}N$ signatures for C. *edule* in France were 8.0‰ (Kang *et al.*, 1999) and in the Netherlands 14.1 to 15.4‰ (Herman *et al.*, 2000) and 10.4 to 18.7‰ (Riera *et al.*, 2000). The $\delta^{15}N$ signatures in France had comparable isotope signatures to *Tellina* in Dublin Bay (8.1 to 10.2‰). However, both studies in the Netherlands had much higher $\delta^{15}N$ ratios compared with this study. It should be appreciated that these values may be inappropriate for comparison with *Tellina* however. The co-occurrence of *Tellina* and *Cerastoderma* at the Blackrock site may be possible due to limited niche overlap with regard to food sources utilised, and further investigation is warranted.

Changes in isotope signatures with season have been recorded in some estuarine macrobenthic species. Two filter-feeding species in Portugal had significantly lower $\delta^{13}C$ values in summer compared with winter (Machas *et al.*, 2003). Another study showed lower $\delta^{13}C$ and $\delta^{15}N$ in intertidal bivalves in early summer, the period that corresponded with a phytoplankton bloom in the estuary (Page & Lastra, 2003). Seasonal variations have also been recorded in *Cerastoderma edule* in France (Kang *et al.*, 1999), with $\delta^{15}N$ ratios more depleted in summer than winter and $\delta^{13}C$ more negative in March than during the summer months. The grazing on phytoplankton during the spring and summer months, when most abundant could have accounted for more depleted $\delta^{13}C$ values at this time (as phytoplankton has very negative ratios). A similar trend was observed in the current study for both *Tellina* and *Hydrobia* with slightly more depleted $\delta^{13}C$ and $\delta^{15}N$ signals in summer than in winter, although ratios were not significantly different. Phytoplankton is not readily available in temporate waters during the very late autumn and winter months (Soetaert & Herman, 1995; Goto *et al.*, 1998).

The relative importance of microphytobenthos/phytoplankton derived food sources can vary depending on feeding type. Suspension feeders have $\delta^{13}C$ values close to pelagic algae while benthic grazers have $\delta^{13}C$ values closer to the microphytobenthos (Herman *et al.*, 2000). Differences in the isotopic signatures between pelagic and benthic feeders were recorded by Kang *et al.*, (2003) (Appendix 6.1). Mixing models to identify the relative contribution of the two food sources to consumer diets can be used if there is sufficient contrast in their isotope signature (Fry & Sherr, 1984; Kang *et al.*, 2003). Percentage contributions of microalgae and phytoplankton to different feeding groups can then be quantified. The range of isotopic values derived for the probable food sources of *Hydrobia* and *Tellina* were used for comparison with the isotopic signatures of reference primary producers. Average isotope signatures from a number of sources for intertidal microphytobenthos, phytoplankton and suspended particulate matter (SPM) from previous studies (Appendix 6.2) were used for comparison with the data from the current study. The average $\delta^{13}C$ isotope ratios of microphytobenthos and phytoplankton were -16.5‰ and -21.5‰, respectively. The values for the probable food sources of *Hydrobia* in summer were similar to the average values for phytoplankton, and the winter samples were midway between the average ratios for phytoplankton and microphytobenthos (Table 6.10). The range in $\delta^{15}N$ values for *Hydrobia* food sources in Dublin Bay both in summer and winter were more depleted than the average literature values of 5.5‰ and 7.3‰ for microphytobenthos and phytoplankton respectively. However, they were within the range of values described in the literature (Appendix 6.2). This would suggest that *Hydrobia* utilised proportionally more phytoplankton during the summer, probably as this was the period when it would have been most abundant, but the main constituent of its diet was phytobenthos. As *Hydrobia* cannot remove phytoplankton directly from the water column it must have been present on the sediment surface when consumed. The low energy environment at Bull Island probably results in the regular deposition of phytoplankton at the site, which is grazed from the surface along with the phytobenthos. Constant flux of microphyto-organisms between the benthos and overlying water column commonly occurs (Palmer & Round, 1965; Admiraal, 1984). Although mixing models can be used to estimate the relative proportions of phytoplankton and phytobenthos in a species' diet, they were not applied in the current study as the isotope ratios of primary producers in the area were not determined.

Although *Hydrobia* is a surface deposit feeder, the main feeding mode of *Tellina* (filter or deposit feeder) is a topic of some controversy. The range of $\delta^{13}C$ values recorded for the latter species lay within those recorded for *Hydrobia* in the current study however. Although the $\delta^{13}C$ values for the dietary components of *Tellina* were intermediate between those for microphytoplankton and microphytobenthos, the range of $\delta^{15}N$ values were very similar to those for the latter in both summer and winter samples. This would suggest that despite some claims that this species filter feeds directly from the water column, microphytobenthos actually account for the majority of the diet. The more exposed and higher energy environment at Blackrock may result in much lower deposition of phytoplankton on to the sediment surface, further reducing its availability as a food source at this site. Chanton & Lewis (2002) divided macrofauna in an American estuary into water-column feeders and benthic feeders, with reported $\delta^{13}C$ ratios of -21.4 to -23.2‰ and -19.6 to -22.3‰ respectively. Although application of these figures to European populations without knowledge of geographic variability in the food sources available at the 2 sites may lack sufficient caution, it would suggest that *Tellina* and *Hydrobia* both utilise microphytobenthic food sources in Dublin Bay to some extent. The relatively depleted $\delta^{13}C$ values for water-column feeders reported by Chanton & Lewis (2002) do not, however, appear to be in agreement with all studies that examined species with this feeding strategy (e.g. Machas *et al.*, 2003, Appendix 6.1)

As previously stated, any comparison between the isotope signatures recorded in the current study with literature values from different estuaries must be treated with caution. Isotopic signatures have been shown to vary within an estuary (Thornton & McManus, 1994; Middleburg & Nieuwenhuize, 1998; Ruesink *et al.*, 2003; Martineau *et al.*, 2004), between closely positioned estuaries (Riera *et al.* 2000), with different estuary types (Middelburg *et al.*, 1997; Kang *et al.*, 2003) and with season (Kang *et al.*, 1999; Machas *et al.*, 2003; Page & Lastra, 2003). In addition to the fact that more than two food sources could be utilised by consumers at any time it must be recognised that in complex estuarine systems isotopically intermediate food sources can also exist (Hentschel, 1998). Isotopic signatures may differ considerably between sub-habitats within the same system. Significant $\delta^{15}N$ differences have been found in primary producers within the same lake, but in the different zones of the littoral,

pelagic and profundal habitats (Vander Zanden & Rasmussen, 1999). Vertical and horizontal mixing of these components could further complicate accurate determination of food sources.

A possible reason for very enriched $\delta^{15}N$ values for primary consumers in some estuaries is the effect of anthropogenic inputs (Herman *et al.*, 2000; Riera *et al.*, 2000; Machas *et al.*, 2003). The $\delta^{15}N$ composition of sewage has been observed as 2.3‰ (Thornton & McManus, 1994), however nitrification and denitrification processes in wastewater treatment plants cause isotopic fractionation leading to ^{15}N enriched discharges (Owens, 1987; Riera *et al.*, 2000). If ^{15}N enriched wastewater nitrate enters an estuary it tends to increase $\delta^{15}N$ in the estuarine DIN (dissolved inorganic nitrogen) pool and hence in primary producers. This heavy N load can then be passed on to consumers causing enriched $\delta^{15}N$ values (Riera *et al.*, 2000). Nitrogen discharge from the Dublin Bay sewage plant does not appear to be having this effect on the $\delta^{15}N$ composition of the two intertidal species tested, as relatively low average values of 8.6±0.2‰ and 5.9±0.3‰ were observed in *Tellina* and *Hydrobia,* respectively. This is probably due to the overall pattern of currents and dispersion in Dublin Bay that has been shown to be effective in reducing the impact of the large wastewater discharge at Ringsend on the shoreline of the bay (ERU, 1992). Although prevailing currents generally transport discharge directly out of the Bay, recent improvements to the sewage system in Dublin allow much of the tertiary treated effluent to be pumped to outfall pipes located outside and to the north of Dublin Bay.

In addition to using isotopic composition to examine seasonal variations in consumer feeding type, ontogenetic changes in diet have also been elucidated using this technique. These processes are poorly understood and difficult to study unless a technique such as isotope analysis is used. Hentschel (1998) used body-size-dependent variations in $\delta^{13}C$ as a diet tracer for four species of surface-deposit-feeding polychaetes. Ontogenetic dietary changes were observed with the smallest juveniles mostly feeding on benthic diatoms compared with adults feeding on detritus from macroalgae or saltmarsh grasses. Differences in the $\delta^{13}C$ value of juvenile and adult *Cerastoderma edule* were observed in France (Kang *et al.*, 1999) with juveniles more dependent on microphytobenthos than adults. A higher selectivity for benthic microalgae with increasing carapace width was observed in two species of grapsid

crab in an intertidal mangrove habitat (Bouillon *et al.*, 2002). Although it is possible that ontogenetic changes in diet occur in the two intertidal species studied in Dublin Bay, the failure to identify isotope values for all samples in the current study hampered further investigation of size-specific variations in food source utilisation.

To quantify the feeding rates of primary consumers is not straightforward. No standard, universally accepted methods exist and published data is often conflicting. In theory measuring feeding rates of filter feeding organisms should be easier than for deposit feeders as the food source (phytoplankton) is not mixed with or bound to inert sediment particles that may contain detritus and micro-organisms in difficult to quantify proportions as is the case for deposit feeders. However there is controversy about the reliability of measuring filter-feeding rates for suspension feeding bivalves using any method (Jorgensen, 1996; Cranford, 2001; Riisgard, 2001; Bayne, 2004; Petersen, 2004; Riisgard, 2004). There is a general view that suspension feeding is physiologically regulated in response to variations in quantity and quality of suspended matter (Bayne *et al.*, 1989). A review of feeding by bivalve mollusc species by Jorgensen (1996) concluded that no convincing evidence for sorting of suspended particles according to food value existed. Trevallion (1971) stated that *Tellina tenuis* was a relatively unselective feeder with the size of the mouth and functioning of the gill cilia determining the particles ingested, and not quality of food. The clearance rate of *Tellina tenuis,* obtained by the indirect method of counting the decrease in particle concentration over time in a closed container, could not be determined in the current study. Although the method gave reliable rates comparable with the flow-through and bio-deposition methods (Petersen *et al.*, 2004), and have been widely used to derived clearance rate estimates for numerous species, the concern surrounding reliability of results obtained in this way was supported by the current study. Lopez & Levinton (1987) stated that many tellinid bivalves display the entire range from deposit- to suspension-feeding among morphological similar species, and that many probably feed simultaneously both ways, as evidenced by *Scrobicularia plana* (Hughes, 1970). Indications from the stable isotope analysis in the current study suggested that *Tellina* was reliant on microphytobenthos as a food source, however, which would invalidate filter-feeding methods for determining feeding rate for this species. As it was necessary for animals to be maintained without sediment when using such methods the normal feeding behaviour of *Tellina* was

probably constrained or entirely disrupted. These experiments were conducted prior to the results of the isotope analysis being available, and highlight how knowledge of feeding strategy is required for the formulation of robust experimental design. When the controversy surrounding the use of inappropriate food sources when conducting filtration rate experiments is considered along with the additional complication associated with determining the exact feeding strategy/strategies of a species, and how these may be altered in response to fluctuating environmental conditions, caution in interpreting past and future results using these methods is suggested.

Change in chlorophyll *a* concentration is considered a useful variable for examining feeding rate of primary consumers, as it is a good index of all types of algae present in the sediment and hence available to deposit feeders. The measurement of chlorophyll *a* permits the use of food media containing many different types and sizes of primary producer, negating complication that can occur when monocultures containing inappropriate sizes or qualities of food are used. In essence, a range of food sources more similar to that that is likely to be encountered under natural conditions can be offered. Previous studies have shown that chlorophyll *a* degrades more quickly in the presence of deposit-feeders than in their absence (Bianchi *et al.*, 1988; Page *et al.*, 1992; Ingalls *et al.*, 2000; Cartaxana *et al.*, 2003). A reducing effect of feeding on chlorophyll *a* and phaeopigment concentration has also been determined for copepods (Shuman & Lorenzen, 1975; Penry & Frost, 1991; McLeroy-Etheridge & McManus, 1999). Amphipods in Baltic sediments have also been found to increase the degradation rate of sediment photopigments (Bianchi *et al.*, 2000). Cartaxana *et al.* (2003) reported that *Hydrobia ulvae* grazing caused a significant reduction in chlorophyll *a* compared with controls under experimental conditions. The mudsnail *Ilyanassa* has been shown to remove more than 10% of surface chlorophyll *a* per day (Barnes & Hughes, 1988) in laboratory conditions. Other studies have produced inconclusive results. For example, Webb (1993) found no identifiable effects on either sediment chlorophyll *a* or phaeopigments in the presence of the surface deposit feeder *Macoma balthica*, and although at higher density treatments the condition of the animals declined this did not correspond with any change in chlorophyll *a* concentrations in weekly sampling of experimental microcosms. The egestion rate of surface deposit feeding worm *Saccoglossus kowalevskii* increased with increasing

chlorophyll *a*, but gradually declined as the latter continued to increase in concentration (Karrh & Miller, 1994). In the current study, changes in the concentration of chlorophyll *a* or organic matter in the sediment due to grazing were not apparent, although the presence of *Tellina* and *Hydrobia* did appear to increase the degradation of chlorophyll *a* to phaeopigments. Forbes & Lopez, (1989) noted that chlorophyll *a* or other sediment characteristics such as organic matter were not necessarily appropriate parameters to examine deposit feeding as the requirements of the animals themselves may not be based directly on these parameters alone.

Ultimately all methods attempting to determine a feeding rate for either *Hydrobia* or *Tellina* in the current study, both in the field and laboratory were unsuccessful and no significant or reliable feeding rates could be determined. Increases/decreases in chlorophyll *a* and phaeopigment in these experiments also did not necessarily follow consistent trends, indicating that the effect of deposit feeding was variable and unpredictable. The time-scale on which each experiment was conducted may have contributed to the lack of significant results. For example the results presented in section 6.3.3c showed that the highest decrease in chlorophyll *a* (non-significant) occurred in the highest density treatment after 1 week. This trend did not continue in weeks 2 and 3, however, when highest decreases in chlorophyll *a* occurred in controls with no grazers present. There is no standard time-frame in the literature for examining deposit feeding activity, and, additionally, the duration required would probably be species-specific and only determined after significant trial and error. This itself could affect the subsequent interpretation of results. There is evidence to suggest that experiment duration is a key controlling factor in determining the results achieved. For example the experiment of Cartaxana *et al.* (2003) showed a decrease in chlorophyll *a* with *Hydrobia* grazing over a period of 4 days, but Webb (1993) observed no significant differences during periodic sampling over a 42 day grazing period for *Macoma balthica*. Although longer experimental durations may be required to identify the grazing impact of relatively small species held at low density with high food availability, this probably introduces greater probability of variations in primary productivity/degradation or other factors such as the production of faeces or nutrient limitation confusing subsequent results. The necessity to constrain many

variables in the laboratory to determining feeding rate may result in the production of quite artificial results that are not representative of those in the wild.

Another possible reason for the failure of the experiments in this study was insufficient accuracy provided by the spectrophotometric method for determining chlorophyll *a* and phaeopigment concentration. Mixed samples containing chlorophylls and their derivatives cannot be distinguished using spectrophotometric analysis because their absorbance bands overlap (Lorenzen, 1967). Although spectrophotometric analysis is suitable for detailing relative differences in chlorophyll *a* concentrations (Pinckney *et al.*, 1994), it is possible that in feeding rate studies such as those attempted in the current study, more detailed analysis of individual photopigments was required. The fluorometric method (which also does not distinguish all photopigments) has been used successfully to examine the effects that three bivalve species had on the abundance of microalgae during previous studies by Page *et al.* (1992). High performance liquid chromatography (HPLC) provides the most accurate measurement of chlorophyll *a* compared with spectrophotometric and fluorometric methods (Pinckney *et al.*, 1994). The method also provides additional information by separating and quantifying all chlorophyll pigments. Cartaxana *et al.*, (2003) used HPLC analysis and, although recorded a significant difference in chlorophyll *a* between grazed and control sediment, suggested that phaeophorbide a_4 and phaeophytin a_2 were more useful markers of intertidal microphytobenthos grazing by *Hydrobia ulvae*. The processing time required for each sample (~25min) compared with <5min for spectrophotometric methods (Pinckney *et al.*, 1994) is the primary disadvantage of using HPLC, but in the current study the financial constraint of using the more expensive HPLC method was the determining factor for the formers use.

Other methods that have been used in an attempt to examine food type and grazing rate during deposit feeding include observing the change in abundance of specific microalgae. Most studies have emphasised the dependence of *Hydrobia* species on diatoms (Kofoed, 1975a; Levinton & Bianchi, 1981; Bianchi & Levinton, 1984; Morrisey, 1988) with other food sources including bacteria and blue-green algae of less importance (Kofoed, 1975a,b; Levinton & Bianchi, 1981; Bianchi & Levinton, 1984). However a 'pure' monoculture of a particular food source were generally

utilised, so applicability to the natural environment can be difficult to determine. Other studies have stated that deposit feeders do not affect microalgae assemblages/community structures at all (Page *et al.*, 1992; Hagerthey *et al.*, 2002).

Blanchard *et al.* (2000) measured the ingestion rate of *Hydrobia ulvae* experimentally by quantifying grazing of a pre-radiolabelled epipelic microalgal food source in the dark. Results were converted to chlorophyll *a* ingested per snail and revealed a significant decrease in ingestion rate as *Hydrobia* density increased. Each experiment lasted 1 hour, with very few *Hydrobia* examined. Any experiments extrapolating the behaviour of one or a small number of snails must be treated with caution. Conflicting results have been reported for the behaviour of *Hydrobia* in laboratory-based experiments. Egestion rates have been found to be highly variable both between different snails feeding on the same sediment and with the same individuals feeding on different sediments (Forbes & Lopez, 1986). Many authors have either removed and replaced snails which were not feeding (Kofoed, 1975b) or excluded non-feeding snails from the statistical analysis of the results (Barnes, 2001). It is probable that non-feeding or inactive snails also occur in the natural environment however, hence removing snails that do not conform to the desired behaviour is likely to bias and invalidate results. The impact of long-term population feeding on primary production can only be assessed when the range of naturally occurring feeding rates is considered. Ruesink (2000) stated that feeding trials may overestimate community impact due to failure to account for alternative food, search times, resource productivity/growth or interference from other consumers.

The applicability of comparing feeding rates derived in the laboratory with those in field situations is complicated by conflicting evidence with regard to the influence of environmental factors such as light/dark, animal density, sediment nutrient status, temperature, live/dead food material and experimental duration. Whether experiments were conducted in the dark (to minimise photo-oxidative degradation of pigments) or light (allowing natural regeneration of the microalgae population) has a huge affect on results reported. There is conflicting data on the affect of these variables. Barnes, (2003) observed feeding to be maximal at mid-day even though snails were more active in the dark (Barnes, 1986), while feeding activity was not altered with respect to light and dark treatments, (Bianchi & Levinton, 1984). A density-dependent effect

on feeding rate has been observed in a number of studies (Lopez-Figueroa & Neill, 1987; Morrisey, 1987;) although not in others (Hagerthey *et al.*, 2002; Barnes, 2001). The mechanisms of density-dependent effects (if real) remain unclear, but possible limiting factors include food, space and the rate of pellet breakdown (Rhoads & Boyer, 1982; Forbes & Lopez, 1986). Temperature and food concentration can also affect feeding rates (Hymel & Plante, 2000). Due to the lack of standardisation with regard to the execution of grazing rate studies most report feeding/activity/growth rates in units specific to the particular experimental conditions examined, which often negates the direct comparison of results.

Novel data pertaining to the feeding ecology of two herbivorous intertidal mollusc, that are the dominant primary consumers in their respective habitats, have been acquired during this study. Data suggested that *Tellina* and *Hydrobia* utilise primarily microphytobenthos as a food source with phytoplankton, and possibly other food sources, forming a variable proportion of the overall diet seasonally. Isotope analysis of all sources of primary production in the Dublin Bay system, and spatial and temporal variability in their availability in the study sites are required to examine the feeding dynamics of these species in more detail. Although it was not possible to determine feeding rates despite the application of a number of methodologies, there remains some considerable debate as the accuracy of feeding rates derived in laboratory-based studies when compared to those that occur in the field. Further development of protocols to quantify feeding rate must consider temporal and spatial variations in physical and biological parameters that may regulate individuals and overall population feeding rate and/or duration.

Appendix 6.1. $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of intertidal macrofauna from various estuaries.

1° Consumers	$\delta^{13}C$ (‰±S.D.)	$\delta^{15}N$ (‰±S.D.)	Location	Source
Macrofauna		2.2 to 7.5	U.S.A.	Currin *et al.*, (1995)
Corophium volutator (amphipod)	-15.7 ± 0.7	8.8 ±1.4	France	Creach *et al.*, (1997)
Ovatella bidentata (pulmonate)	-21.6 ± 0.7	8.3 to 8.7		
Hydrobia ulvae (gastropod)	-15.1±0.2	7.7 ± 0.3	France	Riera *et al.*, (1998)
Cerastoderma edule (bivalve	-18.2 ± 1.2	8.0 ± 0.9	France	Kang *et al.*, (1999)
Scrobicularia plana (bivalve)	-15.9 to -19.2		Portugal	Costa *et al.*, (2000)
Hydrobia ulvae (gastropod)	-15	14	The Netherlands	Herman *et al.*, (2000)
	-15.5	15.8		
Cerastoderma edule (bivalve)	-19.8	15.4		
	-20.1	14.1		
Cerastoderma edule (bivalve)		10.4 ± 0.5	The Netherlands	Riera *et al.*, (2000)
		18.7 ± 0.7		
Corophium volutator (amphipod)		20.3		
Littorina littorea (gastropod)		12.2 ± 0.8		
		19.7 ± 0.9		
Water column feeders	-21.4 to -23.2		U.S.A.	Chanton & Lewis (2002)
Benthic feeders	-19.6 to -22.3			
Mytilus galloprovincialis	-16.4 ± 0.7	8.2 ± 0.8	Portugal	Machas *et al.*, (2003)
Tapes decussates	-16.6 ± 0.8	8.1 ± 0.8		
Cerastoderma edule (bivalve)	-14.0 to -16.0	8.0 to 10.7	Portugal	Page & Lastra (2003)
Crassostrea gigas (oyster)	-18 to -22		U.S.A.	Ruesink *et al.*, (2003)
Theora lubrica (bivalve)	-16.0 to -17.8	7.5 to 9.4	Japan	Yokoyama & Ishihi (2003)
Nemotodes	-14.0 to -18.5		The Netherlands	Moens *et al.*, 2002
Estuarine macrofauna	-21.0 ± 0.9	7.3 ± 0.8	Canada	Martineau *et al.*, 2004

Appendix 6.2. $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of different primary producers from various estuaries.

1° Producers	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Location	Source
Microphytobenthos	-13.0 (summer)	-0.3	U.S.A.	Currin *et al.*, (1995)
	-17.6 (spring)			
Benthic diatoms	-14.4 ± 0.8	6 ± 1.4	France	Creach *et al.*, (1997)
Microphytobenthos	-14.9		U.S.A	Stribling & Cornwell (1997)
Microphytobenthos	-19.2 to -21.3		U.S.A	Hentschel (1998)
Microphytobenthos	-16.0 ± 0.6	5.3 ± 0.8	France	Kang *et al.*, (1999)
Benthic diatoms		4.1 to 6.9	France	Riera *et al.*, (2000)
Microphytobenthos	-16.0 ± 0.6		France	Sauriau & Kang (2000)
Microphytobenthos		9.1 ± 0.2	The Netherlands	Riera *et al.*, (2000)
Microphytobenthos	-17.3 ± 1.7	1.7 ± 1.7	India	Bouillon *et al.*, (2002)
Microphytobenthos	-24.0 (>10psu)	7.5	U.S.A	Cloern *et al.*, (2002)
	-20.0 (<5psu)	6.6		
Microphytobenthos	-17 to -21		The Netherlands	Moens *et al.*, (2002)
Benthic diatoms	-14.1 ± 0.4	11.0 ± 0.9	Korea	Kang *et al.*, 2003
Microphytobenthos	-10.3 (March)	4.9 (Dec)	Japan	Yokoyama & Ishihi (2003)
	-19.5 (October)	2.5 (October)		
Phytoplankton	-21.1		U.S.A	Currin *et al.*, (1995)
SPM	-20.1 to -28.9	9.5 to 12.0	The Netherlands	Middelburg & Nieuwenhuize (1998)
SPM	-22.2 ± 1.1	5.0 ± 0.9	France	Kang *et al.*, (1999)
Phytoplankton	-21.3 ± 1.1	8.6 ± 1.0	U.S.A	Peterson (1999)
SPM		1.4 ± 1.3	The Netherlands	Riera *et al.*, (2000)
Estuarine plankton	-22 to -29		U.S.A	Chanton & Lewis (2002)
Phytoplankton	-21.5	8.0	U.S.A	Cloern *et al.*, (2002)
Phytoplankton	-20.8 ± 1.1	11.4 ± 0.9	Korea	Kang *et al.*, (2003)
SPM	-18.5 to –21.9	3.9 to 7.4	Japan	Yokoyama & Ishihi (2003)

Chapter 7. Estimating annual consumption of chlorophyll *a* by two populations of herbivorous intertidal mollusc species.

7.1 Introduction

Although most herbivorous intertidal species are relatively small in body size and individually consume only minute quantities of food items, they often occur in such high densities that their combined feeding activity may impact significantly on the abundance of their chosen food sources within the habitats they occupy (Ruesink, 2000; Gosling, 2003). The numerous types of photosynthetic micro-organisms resident in sediments and the water column, which account for the majority of primary production in the marine environment, commonly represent the major component of many such species diets (MacIntyre *et al.*, 1996; Miller *et al.*, 1996; Underwood & Krompkamp, 1999). Some species filter feed living microphytoplankton directly from the water column or water-filled interstitial spaces within substrates, while others remove microphytobenthic organisms bound to sediment particles by surface grazing or whole particle ingestion. Certain species are capable of expressing a combination of these feeding strategies (Hughes, 1970; Hummel, 1985). The vertical migratory behaviour of many of the microphyto-organisms within the marine sediment (Harper, 1977, Palmer & Round, 1965, Admiraal *et al.*, 1982) and the occurrence of almost constant physical transport mechanisms associated with the tide (Baillie & Welsh, 1980; de Jonge & van Beusekom, 1992; Conde *et al.*, 1999) results in a highly dynamic flux in primary production between the benthos and water column. As the level of productivity varies widely within the water column and with sediment properties, microphyto-food sources can be patchily distributed and not equally available within the marine environment (Levinton & Kelaher, 2004). Due to the rapid population turnover rate associated with small, short-lived primary consumers, there is some speculation that their population sizes may be regulated by such variations in spatial and temporal food availability when limiting.

Estimates of primary productivity have been made previously for many of the types of substrate that occur within the intertidal zone (Heip *et al.*, 1995, MacIntyre *et al.*, 1996,

Underwood & Krompkamp, 1999). These estimates vary widely due to a combination of many factors that include sediment grain size and mobility, aspect, nutrient availability, environmental temperature, physical disturbance and geographic location. Sediments with larger grain size and high levels of physical disturbance tend to be less productive relative to smaller grained, sheltered substrates with similar nutrients loads, this generally being associated with the increased sediment surface area available for microphytobenthos colonisation in the smaller grain sizes (Hopkins, 1963; Fielding *et al.*, 1988; MacIntyre *et al.*, 1996; Barranguet *et al.*, 1997; Elliott *et al.*, 1998). Some habitats can support sufficient primary productivity to result in a net surplus of nutrients that may remain at the site or be transported to other areas in the water column. Transport of nutrients between sites may also occur through the exchange of biomass associated with primary producers, consumers or higher trophic organisms. If surplus production in any form is subsequently delivered to a less productive habitat that would otherwise suffer a net deficit in productivity, the donor habitat can be defined a 'source' and the recipient a 'sink' (Roughgarden *et al.*, 1988). Anthropogenic inputs can significantly alter naturally occurring patterns of productivity in the marine environment (Aston, 1980; Hecky & Kilham, 1988; Harrison, 1993; Nienhuis, 1993; Jeffrey *et al.*, 1995; Krompkamp *et al.*, 1995; Malcolm & Sivyer, 1997; de Sousa *et al.*, 1998; McClelland & Valiela, 1998; Wulff *et al.*, 2000; Jassby *et al.*, 2002).

The feeding time of intertidal molluscan herbivores that consume only microphytoplankton that is suspended in the water column is constrained by the periodic removal of these dietary elements by the tide. This holds true for many surface grazing species that feed on microphytobenthos, as these generally become inactive and/or shelter when exposed aerially. Only species that feed primarily below the surface can continue to graze after the tide has receded, and then only when the sediment retains sufficient water content to permit normal physiological function (Gosling, 2003). As the main distribution of phytobenthos generally occurs in a relatively thin layer of the surface sediment due to the requirement for light (Underwood & Paterson, 1993; MacIntyre *et al.*, 1996; Paterson *et al.*, 1998; Oxborough *et al.*, 2000; Wulff *et al.*, 2000; Perkins *et al.*, 2001) sub-surface feeding may be relatively inefficient. A number of additional factors

can also affect feeding rate and/or duration. These include food concentration, environmental temperature, intra- and inter-specific interaction, physical disturbance events and the necessity to express other behaviours as may be required during the lifecycle (Ruesink, 2000; Gosling *et al.*, 2003). Regulation of feeding activity by one or a combination of these factors may be sufficiently large to constrain or cause fluctuations in the population size of grazing species.

Although feeding rate data derived from laboratory experimentation are available for numerous species of herbivorous molluscs, there is some controversy as to the accuracy and reliability of these estimates with regard to their comparison to rates that may occur in the natural environment (Jorgensen, 1996; Ruesink, 2000). Feeding experiments have generally been conducted over relatively short duration and results extrapolated for longer time periods under the overly simplistic assumption that this rate could be expressed indefinitely. Adjustment of the experimental duration has been shown to significantly affect results obtained for several species (see Chapter 6). Additionally, estimates are generally obtained through experimental replication with individuals of similar sizes exposed to relatively constant and rigidly controlled conditions. With the exception of the variable examined, other parameters are typically held at the assumed optimal for the species. As temporal and spatial variability in numerous physical and biological parameters (and interactions between these) probably regulate feeding rate and duration in the wild, multi-factorial studies could be considered more appropriate than single-factorial studies but these have rarely been conducted. Temperature, salinity, light/dark regime, food concentration, food size and food quality have all been shown to influence feeding rate and duration of primary consumers under laboratory conditions (Lopez & Cheng, 1983; Hummel, 1985; Forbes & Lopez, 1986; López-Figueroa & Neill, 1987; Sprung & Rose, 1988; Cammen, 1989; Judge *et al.*, 1992; Kamermans *et al.*, 1992 Page *et al.*, 1992; Smith *et al.*, 1996; Iglesias *et al.*, 1998; Blanchard *et al.*, 2000; Gaudy *et al.*, 2000 Grudemo & Bohlin, 2000; Hagerthey *et al.*, 2002; Orvain & Sauriau, 2002; Rossi, 2002; Gosling, 2003). Direct and indirect density-dependent and independent competition between species or conspecifics is also known to regulate feeding activity. Confusion over the precise feeding strategy employed by a particular species, and

therefore the application of inappropriate experimental design, and a general failure to account for size-specific variation in grazing rate necessitates caution when interpreting previously published results. The lack of universally accepted methods for the determination of feeding potential for primary consumers has lead to the application of numerous protocols that often produce conflicting results. Although many previous studies have been correctly criticised, this has contributed to more rational development toward more suitable and applicable methodologies in recent times (Gosling, 2003).

Despite the availability of some reliable feeding rate estimates for primary consumers, these data have generally not been combined with parameters relating to wild populations to assess their overall potential impact on primary production. As primary consumers are often preyed upon by a wide range of invertebrate and vertebrate predators that cannot utilise microphyto-organisms as food sources themselves, they represent an essential component of intertidal food-webs. The ingestion and assimilation of primary producers to higher trophic levels ensures ecosystem nutrient and energy flux are maintained. It is unclear whether the availability of primary producer food sources limit the population size of primary consumers (bottom-up regulation) and hence the potential for nutrient transfer in various habitat types, or whether the converse is true (top-down regulation). Due to the complex interaction between biological and physical processes regulating both primary productivity and the population size of consumers, there may be no direct relationship between the abundance of either. The aim of this chapter was to estimate the annual population ingestion of chlorophyll *a* for two intertidal mollusc species with varying habits and habitats to determine their importance in the transfer of nutrients between trophic level, and assess whether bottom-up or top-down regulation may be occurring at the sites.

7.2 Materials and Methods

Selected data presented in previous chapters were combined with previously published data by other authors and used as input parameters to estimate the total *Hydrobia ulvae* and *Tellina tenuis* 'population' consumption of chlorophyll *a* at the Bull Island and

Blackrock sites, respectively. Only previously published feeding rate data were utilised due to the failure of the experiments conducted in this study.

7.2.1 Population estimates

The total areas surveyed during Chapter 2 were defined as the study sites. Although some individuals were encountered outside the designated study areas, they generally occurred in low abundance, which decreased more rapidly with further distance from the areas defined. These areas were assumed to be the preferred range of the species studied and to contain the whole population under examination. Total study areas of 27,000 and 69,000m^2 were defined for the Blackrock and Bull Island sites, respectively.

Twelve dates were selected at approximate monthly intervals from those sampled during the time-series monitoring conducted in Chapter 3. The density of *Tellina tenuis* and *Hydrobia ulvae*, and the concentration of chlorophyll *a* recorded on the selected sampling dates (and the associated errors) were used in conjunction with area data for the sites to estimate total 'population' size:

Total Population size (n) = Density estimate (individuals m^{-2}) x site area (m^2)

Associated error estimates were taken as the square root of the summed squared errors from the original density estimates. Although the results of Chapter 2 suggested that the species densities, size-frequency distributions and chlorophyll *a* concentrations recorded within the selected sampling area during Chapter 3 may not have been representative of the whole site, the results obtained were applied to all areas for the purpose of this analysis. Sampling dates were chosen to correspond as closely as possible to those when Dublin City Council recorded monthly seawater temperature readings. Dates when low numbers of individuals were collected were not selected to reduce the error associated with compartmentalising the total population estimate into the various cohorts/groups present. The estimates generated from models corresponded to periods of 370 days for *Tellina* and 385 days for *Hydrobia*.

7.2.2 Cohort and group abundance

To increase the accuracy of chlorophyll *a* consumption estimates each population estimate was divided into cohorts or groups that were each assigned a size-specific grazing rate. The *Hydrobia* population estimate generated for each day was divided into the various cohorts identified previously in Chapter 4. Mean shell height (SH) in each of these cohorts was used to define size-specific grazing rate on each date. As it was not possible to identify *Tellina* cohorts in Chapter 4, the population estimate was divided into the arbitrary size classes (<4, 4-8, 8-12, 12-16, 16+mm SH). Mean SH of 2, 6, 10, 14 and 18mm were used to calculate size-specific grazing rate for each of these size groupings, respectively.

7.2.3 Chlorophyll a concentration and feeding strategies

Estimates for total surface sediment chlorophyll *a* were considered to be indicative of the relative production in the areas at the time of sampling. Although it was not possible to determine the daily flux of chlorophyll *a* through the areas, or between the water column and benthos, it was assumed to be high and far in excess of variations occurring in the populations of the two grazer species. The rapid 'turnover' rate of primary producers, combined with regular passive tidal removal/redistribution/deposition and behavioural migrations into the water column, has the potential to alter sediment chlorophyll *a* concentration on an almost continuous basis. Variability in the 'standing stock' concentration estimates recorded on each sampling occasion was, however, considered a good indication of relative local productivity.

As *Hydrobia* is a deposit-feeding surface grazer it was assumed to impact directly on the surface sediment chlorophyll *a* concentration at the Bull Island site. The inhalant siphon of *Tellina tenuis* is either extended above the sediment surface and directed downward to a position just above the sediment surface or extended directly upwards to the sediment-water interface when feeding. It is not known in what proportion loose food items are filtered as they pass across the surface of the sediment in the water column, from the interstitial spaces of the sediment itself or whether both of these strategies are employed

(Trevallion, 1971). In the former case, *Tellina* would not impact directly on surface sediment chlorophyll *a* concentration and instead affect the quantity in the water column, which may or may not be subsequently deposited to the sediment itself. Although isotope analysis presented in Chapter 6 suggested that the species utilised a high proportion of microphytobenthos as a food source, no direct observations of feeding behaviour were made. For this reason, the chlorophyll *a* consumption estimates for *Tellina* were not assumed to directly impact on the surface sediment chlorophyll *a* concentration at the Blackrock site, although it seems probable that they do to some, undetermined, extent.

7.2.4 Using previously published grazing rates

As the feeding experiments conducted in Chapter 6 failed to yield significant results, previously published feeding rates were used to derive estimates of population chlorophyll *a* consumption. For both species, the previously published feeding rate used was the only known available in the literature in an applicable format. In each case, the rates were specific to certain sizes of individuals held at a single constant temperature. Although some level of inter-population variability in feeding rate/activity may have occurred between the previously studied and current populations, all were located in northern Europe and therefore differences were considered negligible for the purpose of the model.

Trevallion (1971) determined the feeding rate of *Tellina tenuis* from a population on the west coast of Scotland by conducting clearance rate experiments under laboratory conditions. Individuals held at 20°C with a mean dry weigh of 20mg were observed to ingest an average of 200µg of organic carbon a day, of which 90% was subsequently egested as faeces and 10% assumed assimilated. No error was quoted for any of these estimates. Although work presented in Chapter 6 would suggest that the *Tellina* feeding rate estimate derived using clearance rate should be utilised with extreme caution, no other previously published estimates are available for this species or any other relatively closely related congeners. A range of values between 1:25 and 1:100 can be found in the literature for chlorophyll *a*:organic carbon ratio (de Jonge, 1980; Varela & Penas, 1985;

Gould & Gallagher, 1990; Cloern *et al.*, 1995), which essentially estimates the amount of non-photosynthetic organic components also ingested when a microphyto-organism is consumed. Variation in this ratio is probably a result of the numerous species that constitute the primary producers, which differ widely in body size and cellular organisation. A ratio of 1:40 was used in the current study, as this has been previously in European estuaries similar to Dublin Bay (de Jonge, 1980; de Jonge & de Jonge, 1995; Rybarczyk *et al.*, 1996)

Blanchard *et al* (2000) estimated a density-dependent chlorophyll *a* ingestion rate for *Hydrobia ulvae* by pre-radiolabelling an epipelic microalgae food source added to experimental microcosms. The individuals examined were collected from a population located on the Atlantic coast of France. Between experimental densities of 7,000 and 30,000 individuals m^{-2} mean ingestion rate decreased significantly from 26.6±1.1 to 22.4±1.0 ng chlorophyll *a* $snail^{-1}$ hr^{-1}. As the experiment was not repeated at intermediate densities, it was not possible to describe the nature of the relationship between density and feeding rate using actual experimental data. The authors attempted to elucidate the relationship by computer simulation under certain constraint criteria. Although these authors did not calculate assimilation efficiency for *Hydrobia* as results were not extrapolated to this stage, Lopez & Cheng (1983) estimated an efficiency of 40% for a closely related species *Hydrobia totteni* from the east coast of the U.S.A.

7.2.5 Hydrobia chlorophyll grazing: density-dependent models

Two distinct models for estimating density-dependent regulation of grazing rate were utilised to adjust the previously published (Blanchard *et al.*, 2000) estimate for individual *Hydrobia* of 3.5mm (SH) for the densities recorded at Bull Island on each sampling occasion. The daily estimates produced represented the maximum potential population ingestion estimates, as they assumed constant feeding over a 24hr period.

Model 1- Linear relationship

In the absence of ingestion estimates at other experimental densities, the decrease in ingestion rate cited by Blanchard *et al.*, (2000), 26.6±1.1 to 22.4±1.0 ng chlorophyll *a* snail^{-1} hr^{-1} for 7,000 and 30,000individuals m^{-2}, respectively, was assumed to have been linear (Figure 7.1). It was also assumed that the relationship between the 2 points was linear and could be extrapolated to describe the feeding rate for all other densities, and that no threshold or stepwise change in feeding rate occurred. The density-dependent feeding rate of an individual of 3.5mm (SH) was calculated using the densities recorded on each sampling date using the following equation:

Feeding rate (ng hr^{-1}) =-0.0002 x density (individuals m^{-2}) + 27.947

Model 2-Negative power relationship

A random walk simulation modelling exercise was conducted by Blanchard *et al.*, (2000) to examine the hypothesis that physical contact between individuals inhibited feeding activity and these contacts increased with density. Reduction in effective feeding time was predicted to follow a negative power function (Figure 7.2), with a rapid decrease in feeding at lower densities becoming progressively less pronounced at medium to high densities. The negative power function predicted higher feeding time at densities <7,000 individuals m^{-2}, lower feeding time at densities between 7,000 and 30,000 individuals m^{-2}, and higher again at densities >30,000m^{-2} when compared with the linear model (Figure 7.2). For the purposes of the current study the relationship described:

Relative feeding time (as proportion) = 132.61 x density (individuals m^{-2}) $^{-0.67}$

was assumed to be relevant for the density-dependent experimental feeding rates, 26.6±1.1 and 22.4±1.0ng chlorophyll *a* snail^{-1} hr^{-1} for 7,000 and 30,000 individuals m^{-2} respectively (Blanchard *et al.*, 2000). Combining these data suggested that relative feeding activities of 35% and 13% of optimum would have occurred at the 2 experimental densities (Figure 7.2). Back extrapolation of the model suggested that an effective feeding time of 1 (or 100%) would be expressed at a density of 1,475

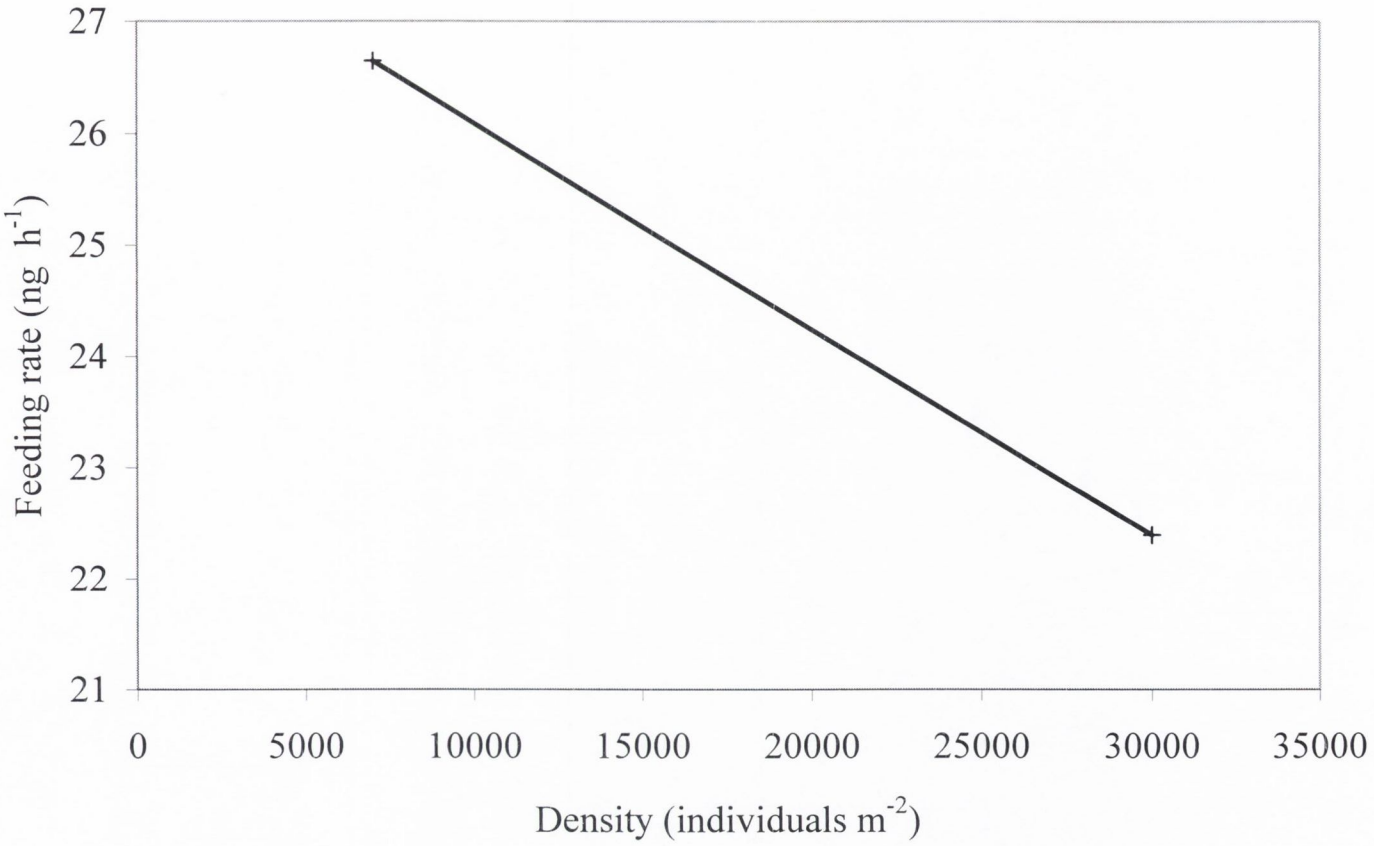


Figure 7.1. Feeding rate of *Hydrobia ulvae* at two densities under laboratory conditions ($y = -0.0002x + 27.947$). Data reproduced from Blanchard *et al.*, (2000).

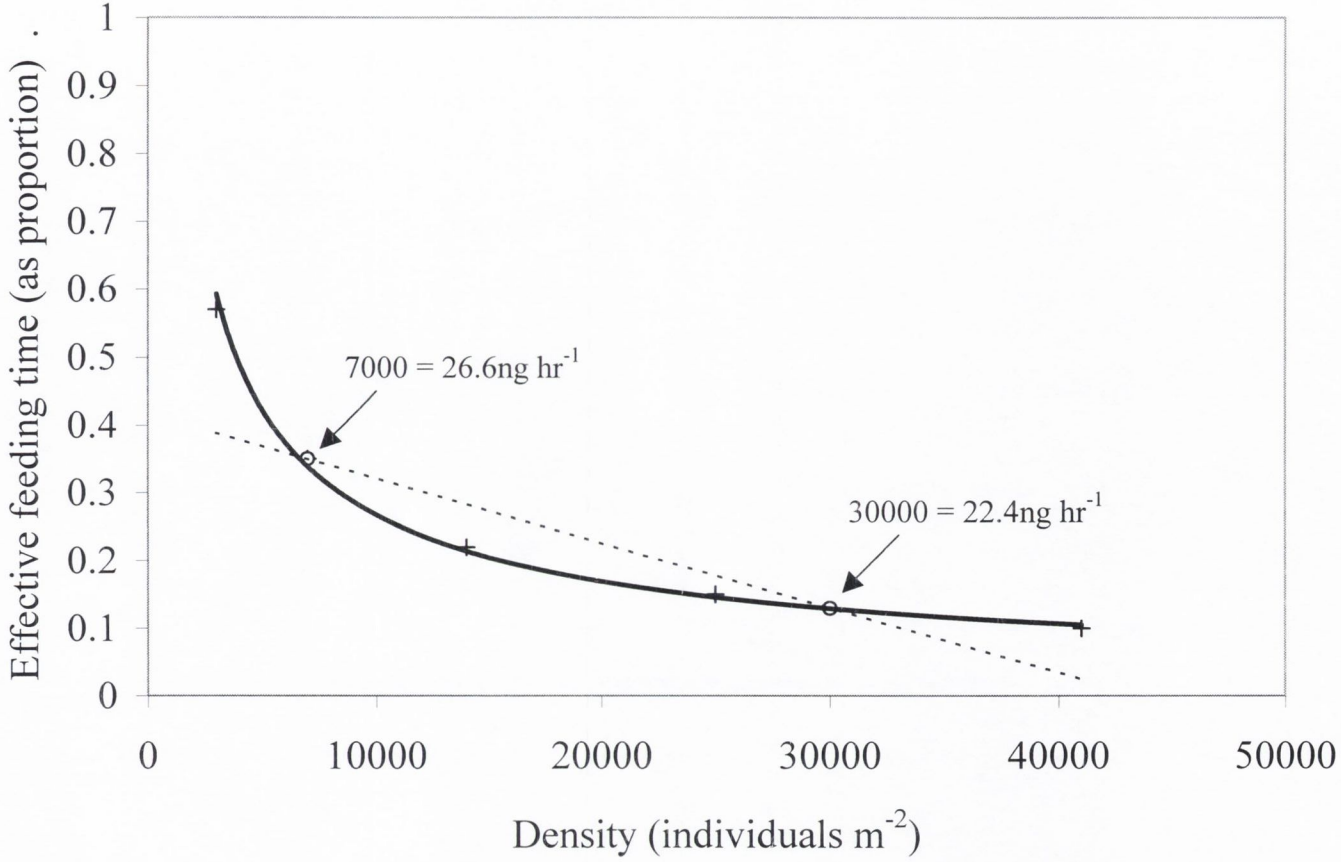


Figure 7.2. Reduction in effective feeding time with increasing density of *Hydrobia* predicted from random walk model assuming contact between individuals temporarily inhibited feeding activity ($y = 132.61x^{-0.67}$, $R^2 = 0.98$). Linear relationship shown for comparison.

indivdiauls m^{-2}, that would peak at a rate of 32ng chlorophyll *a* $snail^{-1}$ hr^{-1} for individuals between 3-4 mm (SH) at 20°C. Although further back extrapolation to the y-axis was possible this exceeded an effective feeding rate of 1 and, therefore, 1475 indivdiauls m^{-2} was assumed to be the threshold density below which no further reduction in density-dependent regulation of feeding activity occurred. The natural log of the two experimental grazing rates at 7,000 and 30,000 individuals m^{-2} were superimposed onto the relationship derived from the natural log of the relative feeding efficiency curve. The exponent of the curve resulted in the density-dependent feeding rate relationship shown in Figure 7.3. This negative power relationship was used to estimate feeding rate for an individual of 3.5mm (SH) at each of the sampling densities.

As it was highly unlikely that the density-dependant ingestion rate estimate derived for an individual of 3.5mm (SH) was applicable to all sizes of individual in the population, size-specific grazing rates were generated for the mean SH in each cohort. As there is some debate in the literature as to the nature of size-specific feeding rates and body size for intertidal mollusc species generally, and no rates specific to *Hydrobia* exist, the estimates from a number of methodologies were compared.

Method 1. Size-weight relationship

Feeding rate was assumed to be a function of body weight. This relationship has been postulated for several species under the assumption that the increasing size of an individual permits it to utilise a greater range of food items while providing a proportionally greater gut volume for storage and processing (Bianchi & Levinton, 1981). A total of 100 *Hydrobia* of various sizes were collected from the Bull Island site, and their wet weight (g, accuracy 0.001, Mettler Toledo AG245 5 point balance) and SH (mm, accuracy 0.1mm, Mitutoyo Absolute Digimatic callipers) recorded (Figure 7.4). A significant size-weight relationship was determined:

$$\text{Wet weight (g)} = 0.0002 \times \text{Shell height (mm)}^{2.74}$$

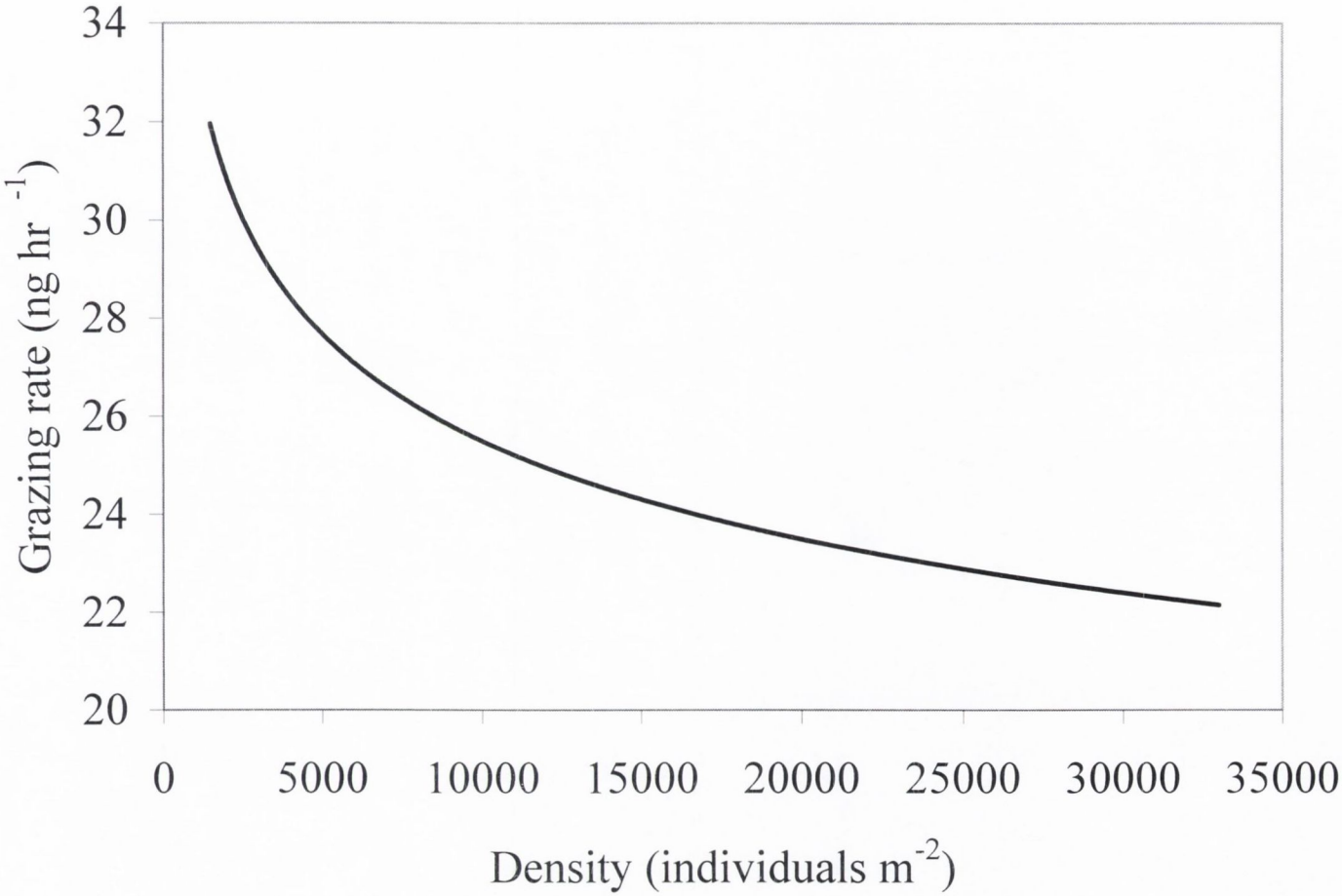


Figure 7.3. Density-dependent grazing rate for *Hydrobia* calculated from the experimental grazing rate data and computer simulation model of Blanchard *et al*. (2000) ($y = 75.67x^{-0.12}$).

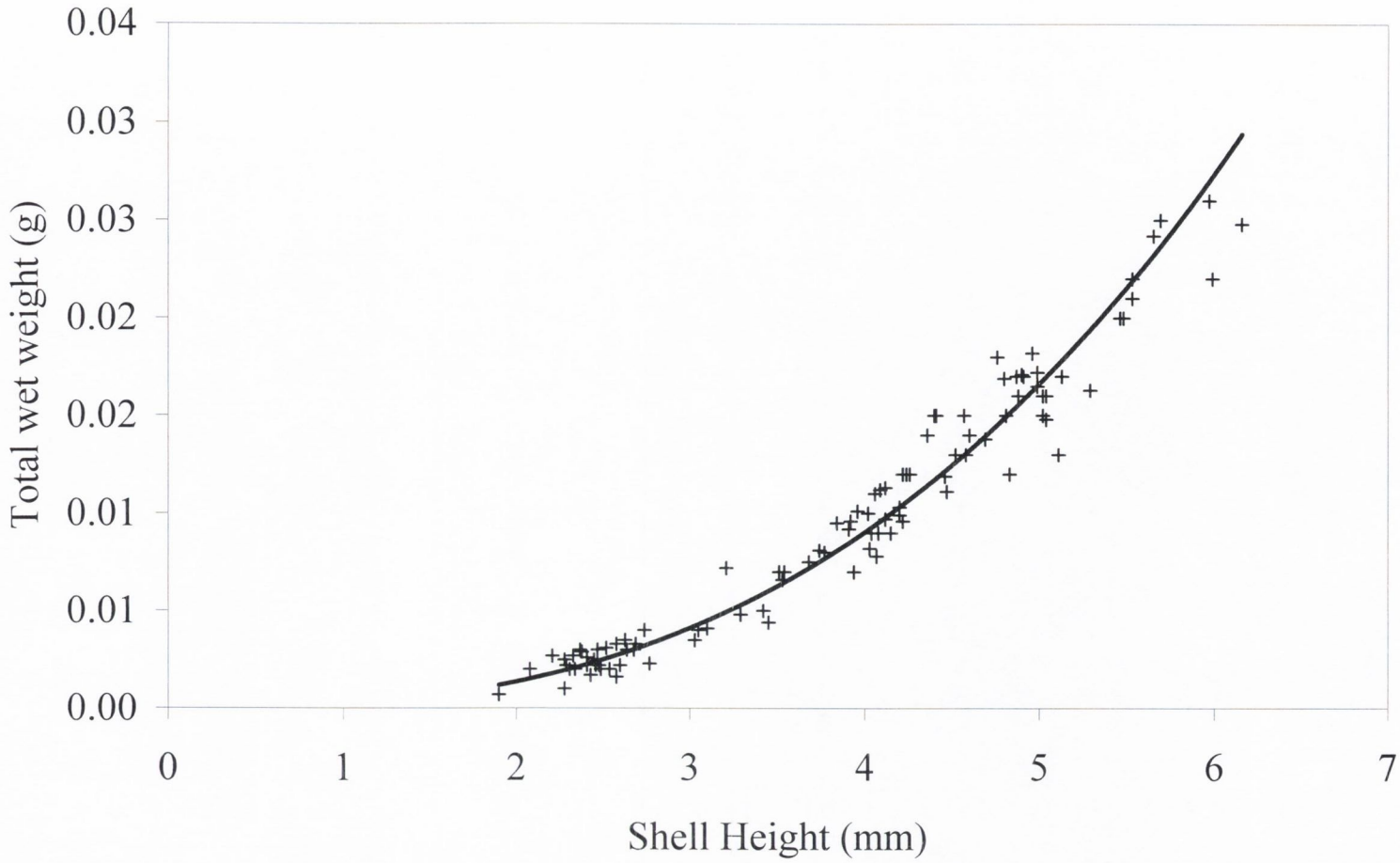


Figure 7.4. Relationship between shell height (mm) and total wet weight (g) for *Hydrobia ulvae*, Bull Island ($y = 0.0002x^{2.74}$, $R^2 = 0.9536$). N=103.

When the density-dependent grazing rate of an individual of 3.5 mm SH had been estimated by each of the models, it was adjusted using the relationship above to be specific for the mean size in each cohort by:

$$GR^{x} = WW^{x} + (GR^{3.5} - WW^{3.5})$$

where

GR^{x} was the grazing rate of cohort x
WW^{x} was the mean wet weight of an individual of mean SH in cohort x
$GR^{3.5}$ was the density-dependent grazing rate at SH 3.5mm
$WW^{3.5}$ was the wet weight of an individual of mean SH 3.5mm

Method 2. Cumulative growth

Feeding rate was assumed to be a function of the cumulative growth rate, as this was indicative of size-specific activity/metabolic rate. A decreasing increment in feeding rate with increasing size has been reported for several deposit feeding intertidal molluscs species (Cammen, 1980). As no size-specific grazing rates were available to estimate the nature of the reduction in feeding with size for *Hydrobia*, the size-specific cumulative growth rate observed in the laboratory experiments conducted in Chapter 4 (section 4.3.5) was assumed to be indicative of relative metabolic rate and activity, and therefore ingestion rate. The cumulative growth rate data for *Hydrobia* maintained at an experimental temperature of 20°C (Figure 7.5), was fitted to statistical significance to an adapted version of the Beverton and Holt (1957) model:

$$\text{Cumulative growth} = (SH / (SH + \alpha * L_{max})) * L_{max}$$

where

SH was Shell Height (mm)
α was a constant associated with the slope of the line

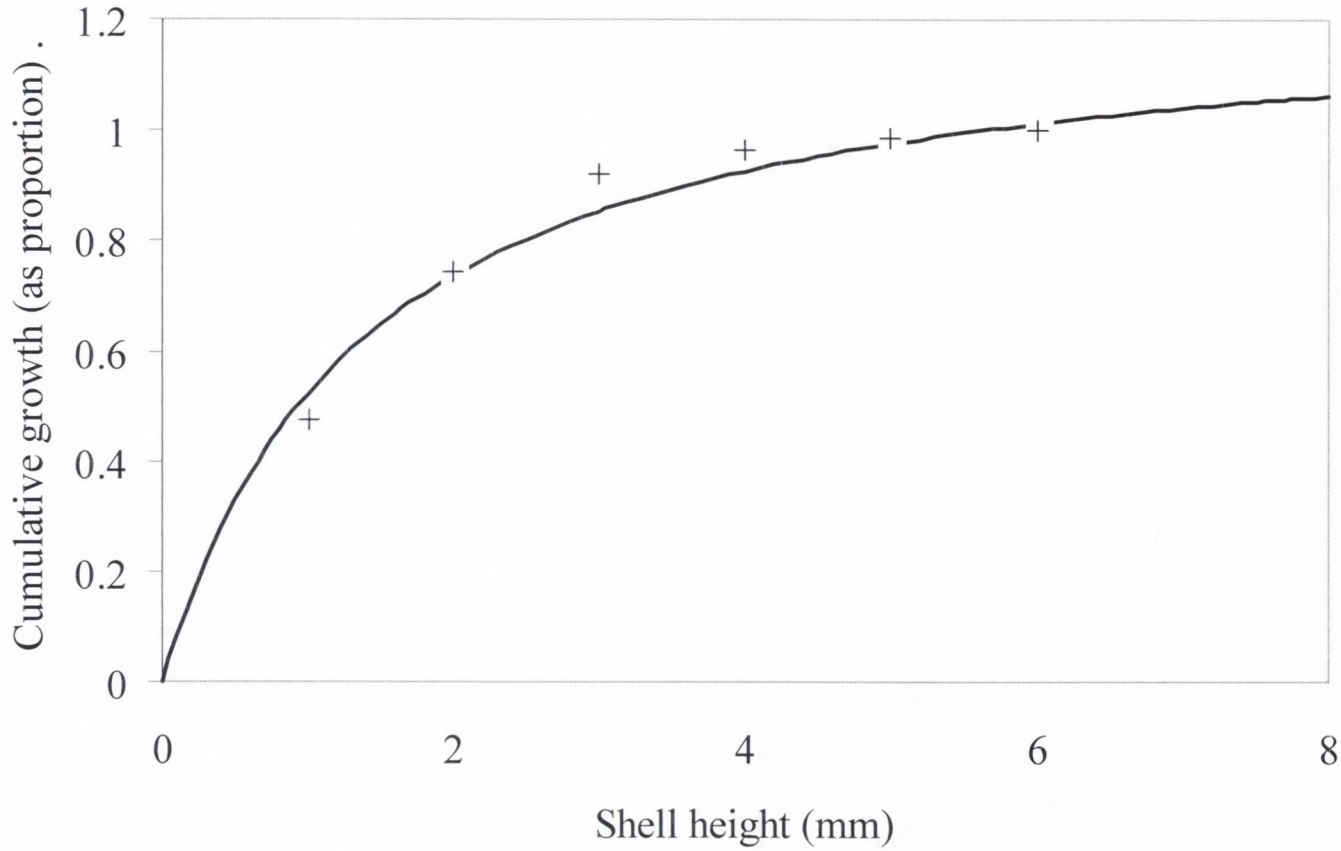


Figure 7.5. An adapted version of the Beverton and Holt (1957) recruitment curve fitted to the cumulative growth rate data for *Hydrobia ulvae* under laboratory conditions (y = (x/(x + 1.096 * 1.246)) * 1.246).

L_{max} was the theoretical cumulative growth at infinite size. (N.B. This value often exceeds 1 as the estimate effectively relates to an infinitely old specimen, a condition that obviously does not occur in nature)

As this model is not commonly used it was necessary to test significance using a chi-square goodness of fit test. Model fit was optimised by minimising the sum of the squared errors associated with the differences between observed (actual growth data) and expected (the fitted line) values at a given size using the 'Solver' function in Excel©. Significance was tested using relevant statistical tables. Size-specific grazing rate was calculated from the density-dependent feeding rate of an individual of a size 3.5mm:

$$GR^x = (GR^{3.5} / CG^{3.5}) * CG^x$$

where

GR^x was the grazing rate of cohort x
CG^x was the cumulative growth (as a proportion) at mean SH in cohort x
$GR^{3.5}$ was the density-dependent grazing rate at SH 3.5mm
$CG^{3.5}$ was the cumulative growth (as a proportion) at mean SH 3.5mm

Method 3. Shell Height

Feeding rate was assumed to be linear and a function of shell height (SH). An approximation to this relationship would result if the assumptions of the previous 2 methods were both applicable to natural populations. Size-specific grazing rate was calculated from the density-dependent feeding rate of an individual of a size 3.5mm:

$$GR^x = (GR^{3.5} / SH^{3.5}) * SH^x$$

where

GR^x was the grazing rate of cohort x

SH^x was the mean SH in cohort x

$GR^{3.5}$ was the density-dependent grazing rate at SH 3.5mm

$SH^{3.5}$ was SH 3.5mm

As each of the methods resulted in the adjustment of density-dependent feeding rate at mean SH 3.5mm, it is at this size grazing rate estimates converged. Figure 7.6 shows the relative effect of utilising the various methods to calculate size-specific grazing rates at one particular density. Alteration of density in the predictive models effected the y-axis position of these relationships, without altering their position relative to one another. An example of the way in which the position of the size-specific feeding curves, in this case the size-weight relationship, altered with density is shown in Figure 7.7. As it was assumed the effects of increasing/decreasing density were equal for all sizes regardless of the densities involved, the shape of the size-specific relationship remained unchanged. Differences in population consumption estimates produced by utilising each of the size-specific methodologies were therefore a result of variation in population size-composition and not population size.

The density-dependent, size-specific chlorophyll *a* ingestion rates calculated by each of the methodologies above were raised by the number of individuals in each cohort. The sum of these estimates was taken as total population chlorophyll *a* ingestion day^{-1}.

7.2.6 Tellina chlorophyll consumption

As *Tellina* is infaunal, sedentary and occurs in relatively low abundance, it was assumed that density had no effect on ingestion rate. Therefore, the basic model applied to estimate total population chlorophyll *a* ingestion for this species utilised size-specific consumption rates that were not adjusted in any way to account for variation in density recorded during each sampling event. The daily estimates produced represented the maximum potential population ingestion estimates, as they assumed constant feeding over a 24 hr period.

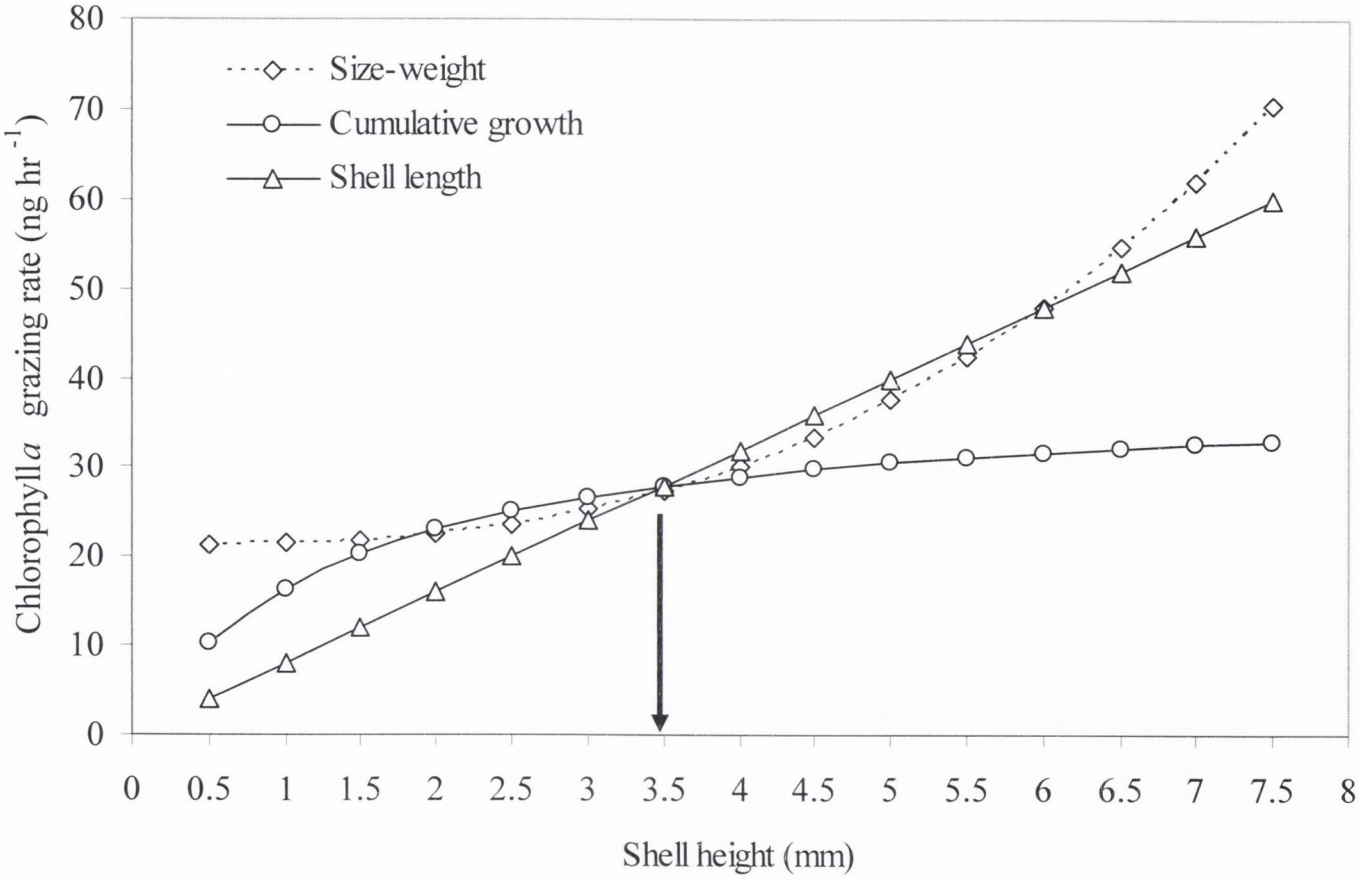


Figure 7.6. Relative affect of applying various methodologies to calculate size-specific grazing rates for *Hydrobia* from a known grazing rate at 3.5mm shell height.

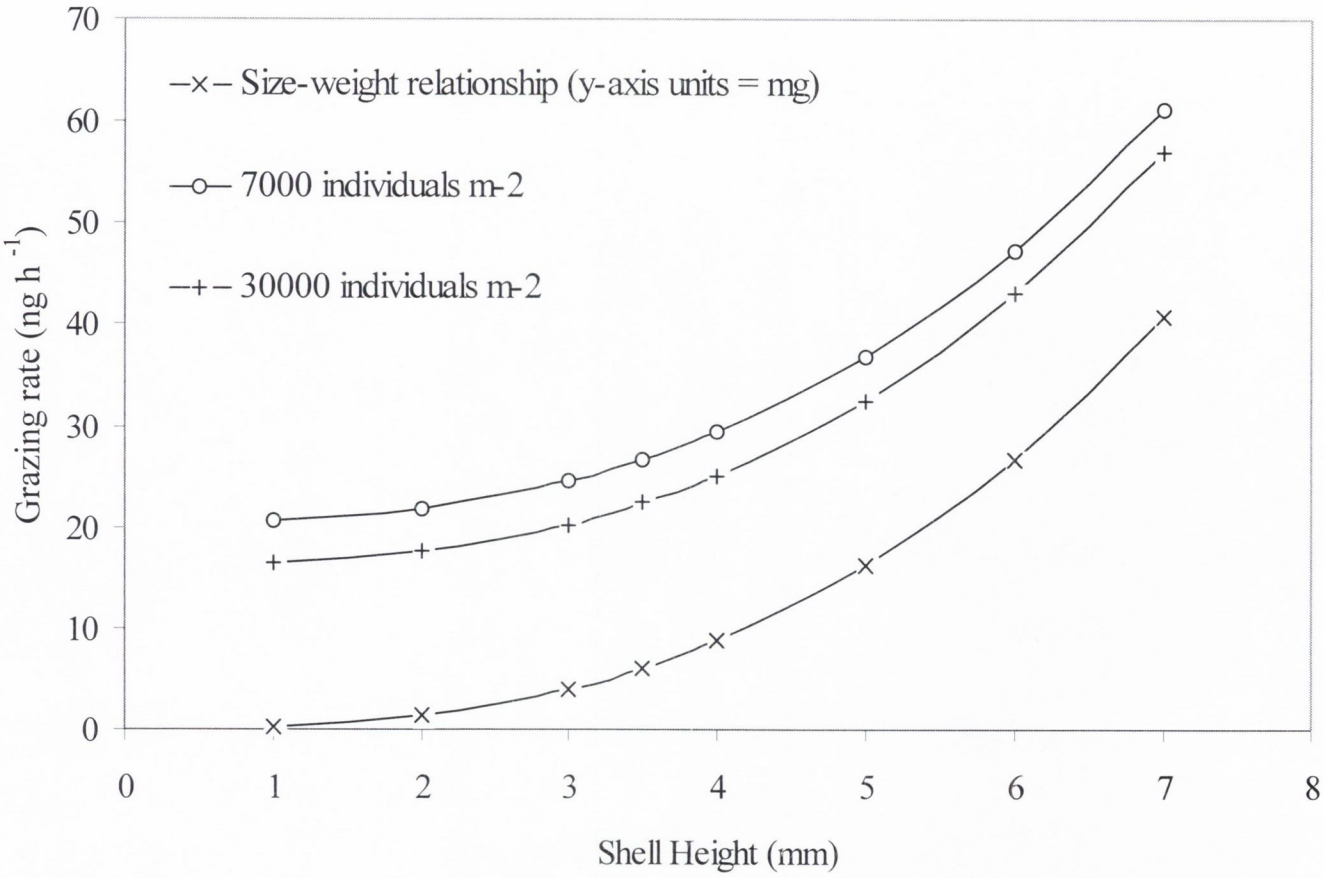


Figure 7.7. Example of how the size-weight relationship of *Hydrobia* was used to calculate size-specific grazing rates for varying density.

Model 1 *Tellina* population chlorophyll *a* consumption: size-specific ingestion rates

The mean clearance rate of 200 μg of organic carbon day^{-1} cited by Trevallion (1971) for individual *Tellina* with a mean dry weight of 20mg was converted to mg chlorophyll *a* ingested $snail^{-1}$ hr^{-1} using the conversion equation cited by Rybarczyk *et al.*, (1996). Assuming a chlorophyll *a*:organic carbon ratio of 1:40:

200μg of organic carbon day^{-1} / 40 = 5 μg chlorophyll *a* day^{-1}

5μg chlorophyll *a* day^{-1} / 24 = 0.2085 μg chlorophyll *a* hr^{-1}

or 0.000208 mg chlorophyll *a* ingested $snail^{-1}$ hr^{-1}

This ingestion rate estimate was adjusted utilising 2 methods to derived size-specific consumption rates for each of the arbitrary size groupings.

Method 1. Size-weight relationship

Ingestion rate was assumed to be proportional to body weight. Trevallion (1971) utilised *Tellina* with a mean dry weight of 20 mg (range 15-25 mg) to determine ingestion rate. A total of 100 *Tellina* of various sizes were collected from the Blackrock study site and returned to the laboratory. The SH (mm) of each individual was recorded using digital callipers to an accuracy of 0.1mm and the soft tissue separated from the shell valves and weighed to an accuracy of 0.001g using a Mettler Toledo AG245 5 point balance. Soft tissue was placed in an oven for 48 hr at 100°C. Tissue was removed, placed in a desiccator and rapidly weighed. Tissue was returned to the oven for a further 24 hr and then re-weighed. If tissue dry weight had decreased from the previous occasion than it was returned to the oven and the drying process repeated. When tissue weight did not decrease between successive readings it was assumed that the dry weight condition was reached. A significant relationship between SH and dry weight was determined (Figure 7.8).

Tissue dry weight (g) = 0.00001 x Shell height (mm) $^{2.59}$

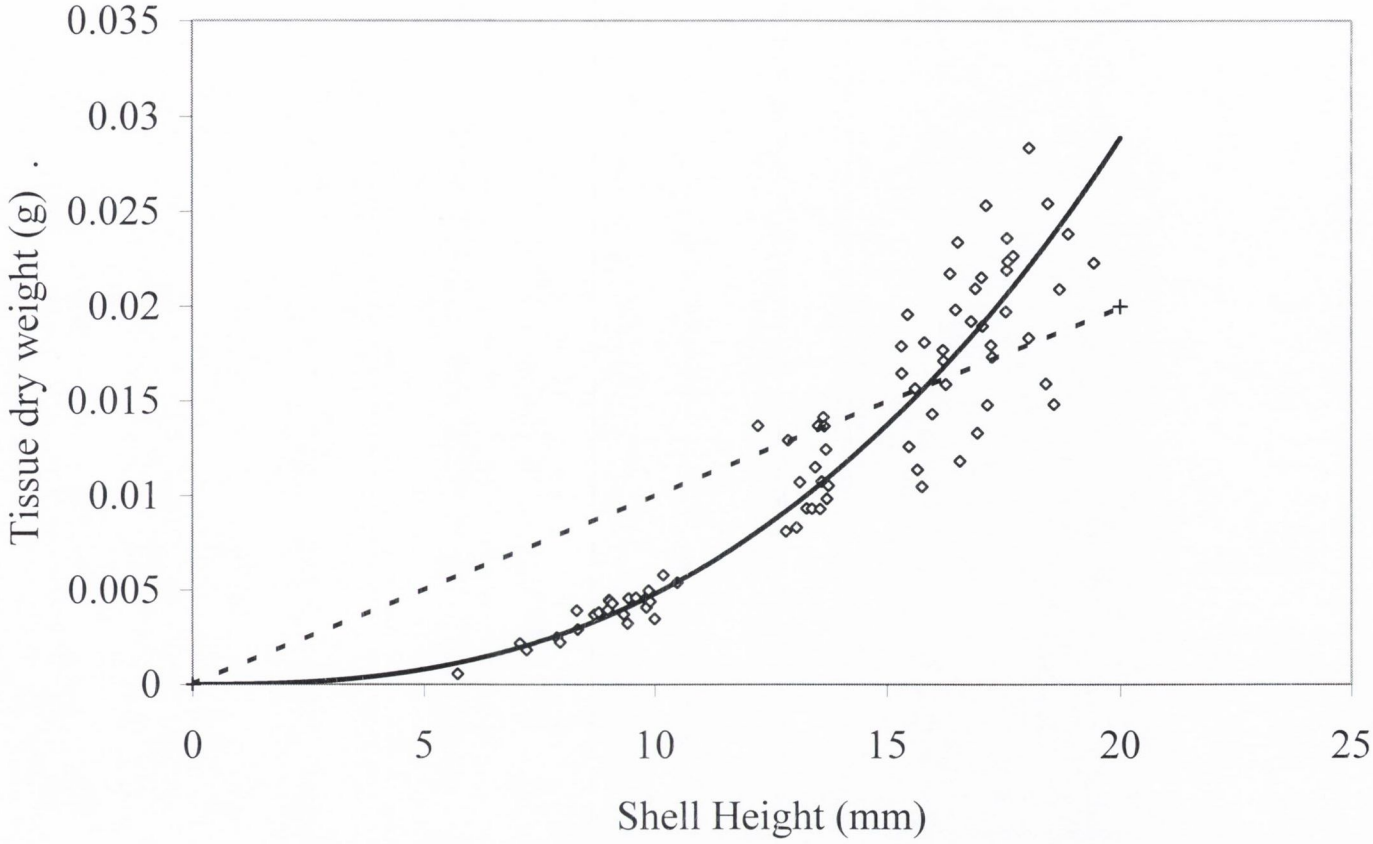


Figure 7.8. Relationship between shell height (mm) and tissue dry weight (g) for *Tellina tenuis*, Blackrock (N=80). The dashed line indicates a theoretical relationship if dry weight/grazing rate was proportional to shell height ($y = 0.00001x^{2.59}$, $R^2 = 0.93$).

A tissue dry weight of 20mg cited by Trevallion (1971) was equivalent to an individual of approximately 19mm SH from the Blackrock site. The published feeding rate was assigned to an individual of this size. The size-weight relationship above was then used to adjust the clearance rate at 20mg dry weight (19 mm SH) to the mean size/dry weight in each arbitrary size grouping.

Method 2. Shell height

Ingestion rate was assumed to be proportional to shell height and therefore linear. As stated above, consumption rate was calculated for an individual of 19 mm (SH). This was adjusted to be proportional to the mean shell height in each arbitrary size group in the same way as described for *Hydrobia* cohorts in method 3 above. The relative effects of the two methods used to adjust for the size-specific grazing rate of *Tellina* are shown in Figure 7.8 by the addition of the dashed line. In the absence of reliable *Tellina* growth rate data, it was not possible to adjust size-specific ingestion rate estimates as was conducted in method 2 for *Hydrobia* above.

Daily estimates of total *Tellina* population chlorophyll *a* ingestion were derived from the sum of each of the size-specific clearance rates raised by the population estimate for that the relevant size grouping.

7.2.7 Estimate error

Each model used to predict total population ingestion of chlorophyll *a* incorporated the same estimates for total population size and the associated error. It was not possible to include additional error to the model to account for variation in grazing rate however. The clearance rate for *Tellina tenuis* previously published by Trevallion (1971) was not presented with any associated error, and therefore it was not possible to incorporate this into the estimates derived. Although error was cited for *Hydrobia* in the work of Blanchard *et al.* (2000), it was only cited for the two experimental densities (not those generated in the computer simulation) and accounted for less than 5% of the ingestion

rate estimate. This was significantly lower than error associated with the population estimates generated for each sampling date. As it was desirable to use the same methodology for each species to allow comparison of results, the error associated with the initial population estimates were applied to the population ingestion estimates without the addition of grazing rate error. Additionally, although it would have been possible to incorporate further error to the estimates based on the variation in mean size within each cohort, its magnitude would have been negligible in comparison to that of the initial population estimates and was discounted.

7.2.8 Summary of general model assumptions for both species

1. That the chlorophyll *a* concentration, species density and cohort/group strength estimates derived during the sampling conducted in Chapters 3 and 4 were representative of the whole site at the time of sampling.

2. That the area defined in Chapter 2 contained the whole population under investigation.

3. The previously published grazing/clearance rates used as inputs to the models were identical to those exhibited in the field at the Bull Island/Blackrock sites.

4. Ingestion rate was size-specific.

5. Individuals within the same cohort/size group had identical feeding rates and durations (no error associated with actual grazing estimates).

7.2.9 Constraining feeding estimates

The daily total population ingestion estimates for each species were based on feeding rates obtained during laboratory experimentation conducted over relatively short (<1hr) time periods (Trevallion, 1971; Blanchard *et al.*, 2000) extrapolated to daily estimates on the assumption of constant feeding at the specified rates. It is, however, highly unlikely

that constant feeding occurs in natural populations. Therefore, the daily total population ingestion estimates produced by each of the models were adjusted by constraining feeding rate or duration to provide results that were considered more realistic or comparable with those that may occur in the studied populations. As each successive constraint was applied without removal of those previously applied, the effect was additive.

7.2.9a Constraints on Hydrobia ingestion estimates

Constraint 1-The effect of tidal immersion/emersion on feeding activity

The ingestion estimates derived from Model 2 were adjusted to account for periods of inactivity during aerial exposure. Although *Hydrobia* were occasionally observed to be active on the surface of the mudflat when exposed by the tide, this was not common and occurred only were small pools of water were present. The majority of individuals appeared to be buried in the sediment and inactive. It was, therefore, assumed that feeding activity was negligible during periods of aerial exposure.

Duration of tidal exposure was dependent on the shore level at which individuals were located when first exposed. Although *Hydrobia* can move relatively long distances by crawling or floating at surface waters, no significant change in horizontal or vertical position is probable once aerial exposure occurs. Individuals located on the high shore are the first to be exposed by an ebbing tide, and the last to be submerged again by the subsequent flooding tide (Figure 7.9). Individuals located on the low shore are exposed for much less time in comparison. Examination of tide table information and observation of the relative exposure times at the various shore levels at the Bull Island study site suggested that approximate exposure times of 4, 12 and 18 hr were on average representative for the low, mid and high shores respectively. It should be appreciated that the spring and neap tide cycle would affect this estimate on any particular day, but the figures quoted were considered to be a reasonable approximation for the purposes of the current study.

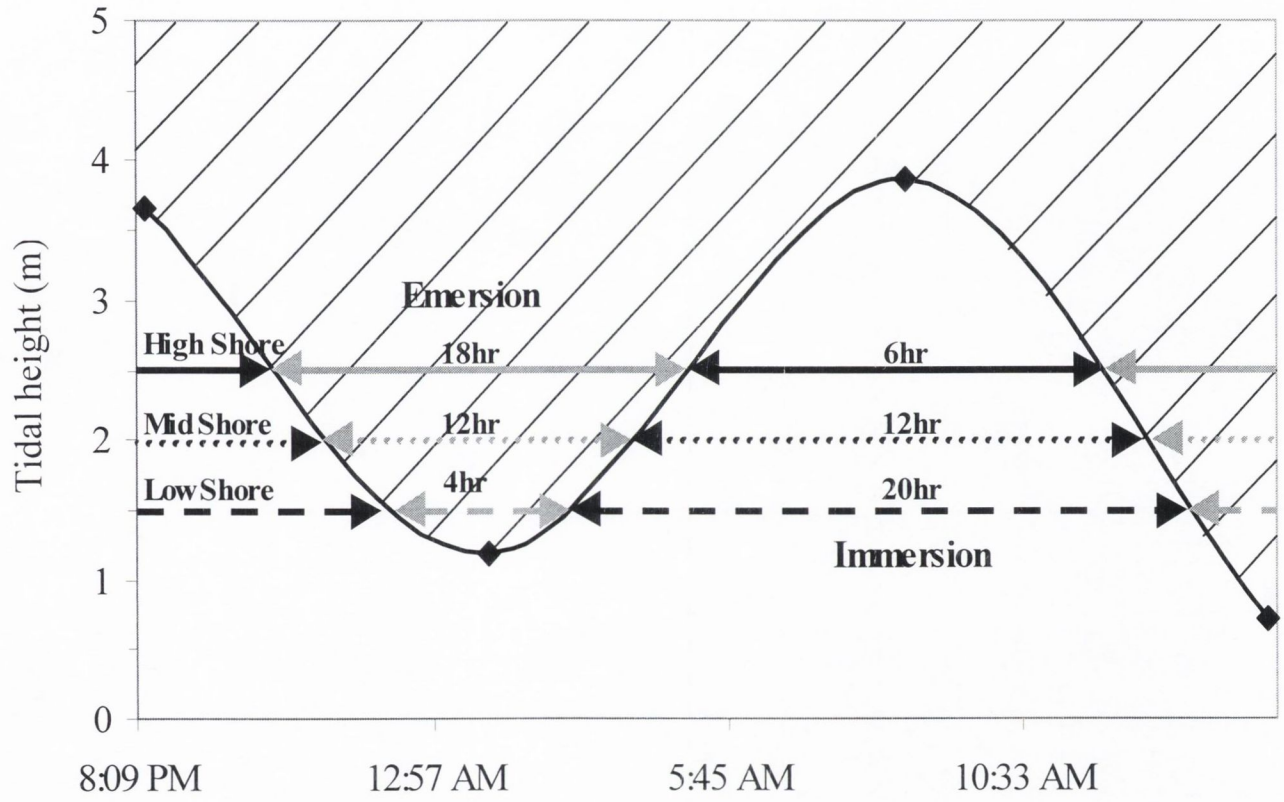


Figure 7.9. Example of the variation in emersion/immersion time with tidal height at the Dublin Bay sample sites.

The results presented in Chapter 2 suggested that the various *Hydrobia* cohorts were not evenly distributed across the Bull Island study site. Although the spatial distribution was only examined on one occasion, it was assumed that the patterns recorded were representative of all other periods. Table 7.1 shows approximate estimates of the proportion of individuals in each cohort present at each of three shore levels. With the exception of cohort 1, which contained the small, recently settled young of the year, a larger proportion of individuals occurred high on the shore. The population estimate for each cohort was divided into these proportions and adjusted from the 24 hr feeding regime expressed in Model 2 to the relevant tidally enforced maximum feeding time of 20, 12 or 6 hrs for the low, mid and high shore levels, respectively.

Table 7.1. Proportion of individuals within each *Hydrobia* cohort present at various shore levels at Bull Island, April 2000.

Tidal level	Cohort 1	Cohort 2	Cohort 3	Cohort 4
High shore	0.33	0.60	0.60	0.60
Mid shore	0.33	0.35	0.35	0.35
Low shore	0.33	0.05	0.05	0.05

Constraint 2-The effect of day/night, variation in behaviour and disturbance on feeding activity

The daily ingestion estimates derived from the application of constraint 1 were further adjusted to account for periods when behaviours other than feeding occurred. Field and laboratory observation (see Chapter 5) suggested that feeding does not occur constantly during periods of immersion, and that other behaviours are commonly expressed. These are distinct from the brief interruption of feeding activity associated with physical contact between conspecifics modelled above (Blanchard *et al.*, 2000), and include burrowing, resting, floating, crawling and mating (Barnes, 1994). Although there are no reliable data available on the relative proportion of time spent expressing each of these behaviours, it is probable that they occupy a significant proportion of an individuals time considering the frequency that these behaviours have been recorded previously (Barnes, 1981a,1981b, 1986, 1994, 2003; Little & Nix, 1976; Lopez-Figueroa & Neill, 1987; Orvain & Sauriau, 2002). Additionally, there are a number of published accounts stating significant variation in *Hydrobia* activity occurs between day and night phases, although they are

often conflicting and no general rule seems to apply. In common with most other shelled mollusc species, *Hydrobia* retract into their shells when affected by external physical disturbance in their vicinity. It is probable that this behaviour is relatively common considering the large amount of waterfowl and juvenile fish present in the Bull Island lagoon. Although individuals emerge from their shells relatively quickly after such disturbances have ceased, these events probably reduce total feeding time available at the site. For this reason, the grazing estimate derived after the application of constraint 1, which assumed feeding occurred at all times during tidal immersion were divided in 2 to act as a more realistic indication of the population chlorophyll *a* ingestion for this species. It should be appreciated that figure was chosen to indicate the relative affect of altering feeding time, and other reductions may be more appropriate with knowledge of overall behaviour/disturbance events.

Constraint 3-The effect of temperature on feeding activity (Q_{10})

The previously published consumption rate used to estimate total population ingestion rate in Models 1 and 2 (Blanchard *et al.,* 2000) was determined for individuals maintained at a constant temperature of 20°C. The activity rate of molluscs is influenced by environmental temperature, however, which in turn directly influences feeding capacity per unit time. Hylleburg (1975) described increasing egestion rates for *Hydrobia* with increasing temperature to a threshold of 30°C. Wilson (1995) described a Q_{10} relationship where a 10°C increase in water temperature resulted in a doubling of the feeding rate for *Tellina tenuis* in Dublin Bay. This relationship was assumed to be applicable for *Hydrobia* in the absence of species-specific data. The grazing estimates derived after the application of constraint 1 and 2 were adjusted using water temperature data from Dublin Bay for the study period (Figure 7.10) by:

$$GR_2 = GR_1 \times 2^{((t2-t1)/10)}$$

where

GR_1 was the experimental grazing rate at 20°C (Blanchard *et al.,* 2000)

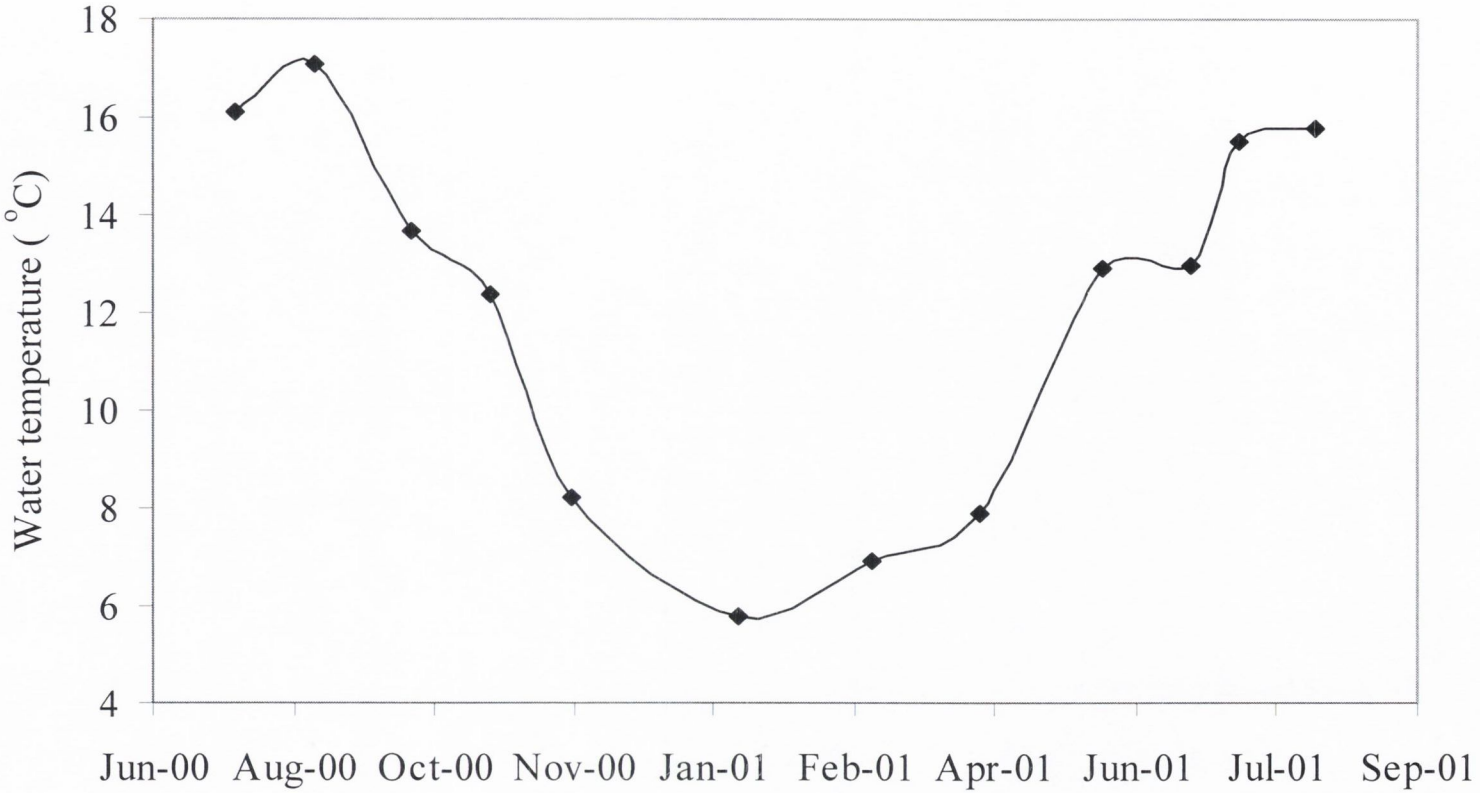


Figure 7.10. Water temperature in Dublin Bay, June 00-August 01. Error estimates not available. Courtesy of Dublin City Council.

GR_2 was the adjusted grazing rate for the temperature recorded in the field

t2 was the temperature recorded in the field

t1 was the experimental temperature when determining GR_1

7.2.9b Constraints on Tellina clearance estimates

Constraint 1-The effect of tidal immersion/emersion on feeding activity

The daily population ingestion rate estimates derived in Model 1 were adjusted for tidal exposure in the same way as previously described for *Hydrobia*. Although it is possible that *Tellina* continues to feed in the interstitial spacing between sediment particles when the sediment surface above is exposed, feeding rate is probably reduced due to sediment drainage and was considered negligible for this exercise. The distribution of the arbitrary size groupings observed at the Blackrock site during the survey conducted in Chapter 2 was assumed to be representative of all other sampling periods. Abundance of the two smallest size groupings decreased with distance up the shore, the opposite being observed for the three groupings containing larger individuals (Table 7.2).

Table 7.2. Proportion of individuals within each *Tellina* arbitrary size grouping at various shore levels at the Blackrock site, April 2000.

Tidal level	Group 1	Group 2	Group 3	Group 4	Group 5
High shore	0.10	0.10	0.50	0.60	0.80
Mid shore	0.30	0.30	0.40	0.30	0.15
Low shore	0.60	0.60	0.10	0.10	0.05

Model 2-The effect of temperature on feeding activity (Q_{10})

The daily population ingestion rate estimates derived after the application of constraint 1 were adjusted for environmental water temperature following the exact methodology described for *Hydrobia*.

7.2.10 Annual population chlorophyll ingestion capacity

The daily estimates of chlorophyll *a* consumption were used to derive estimates of annual feeding capacity. The number of days between each of the successive sampling dates was divided evenly. The population consumption estimate obtained on each date was

then assigned to half of the days between both the previous and subsequent sampling events. The first date's estimate was projected only forward, the last only back. All daily estimates were summed to derive annual chlorophyll *a* consumption estimates for each model. The square root of the sum of the squared errors was calculated. An estimate of the corresponding quantity of organic carbon ingested was obtained using the conversion ratio of Rybarczyk *et al.*, (1996) stated earlier (1:40). This accounted for the fact that non-photosynthetic organic matter is also ingested along with chlorophyll *a* when microphyto-organisms are consumed. The total organic carbon utilised was predicted using previously published assimilation estimates of 10% for *Tellina* (Trevallion, 1971) and 40% for *Hydrobia* (Lopez & Cheng, 1983). The potential number of individual grazing days for the study period was estimated by extrapolating daily total population estimates using the same methods above.

An estimate of total annual microphytobenthic primary production was derived for each area using the extrapolation conversion equation of Colijn & de Jonge (1984):

Primary production (g C m^{-2}) = 0.6705 x Chlorophyll (mg m^{-2}) + 4.01

The mean of all individual chlorophyll *a* samples (mg chlorophyll *a* m^{-2}) taken during all dates in the study period when pooled together was used as the input parameter.

7.3 Results

7.3.1 Population and chlorophyll a estimates

The daily population estimates (±S.E.) for *Hydrobia ulvae* ranged between a low of 2,798±262 during July-00 to 33,831±2,965 during September of the same year (Table 7.3). Standard error estimates ranged between 4.1 and 13.5% of the population estimate on any given day. The number of individuals and mean shell height for each cohort are presented in Appendix 7.1. A peak value of 100.9±5.9 mg m^{-2} of chlorophyll *a* was estimated during July-00, the lowest occurring during April-01 (Table 7.3).

Table 7.3. Daily population estimates (±S.E.) for *Hydrobia ulvae* and surface sediment chlorophyll *a* (mg m^{-2}) Bull Island.

Date	Population (m^{-2})	±S.E.	% error	Chlorophyll a (mg m^{-2})	±S.E.
22-Jul-00	2798	262	9.4	100.9	5.9
20-Aug-00	10983	1091	9.9	78.1	5.3
23-Sep-00	33831	2965	8.8	81.7	9.3
21-Oct-00	23417	2565	11.0	74.5	6.3
19-Nov-00	8600	518	6.0	43.1	2.6
18-Jan-01	7875	703	8.9	67.4	4.6
06-Mar-01	8963	1164	13.5	71.1	4.2
14-Apr-01	12330	990	8.0	10.3	1.9
27-May-01	19622	800	4.1	32.7	2.6
27-Jun-01	5803	698	5.7	35.6	3.4
14-Jul-01	18651	1264	6.8	40.7	2.3
11-Aug-01	7150	672	9.4	75.9	4.6

The highest daily population estimate (±S.E.) for *Tellina tenuis* at Blackrock, 1,807±118 individuals m^{-2}, was recorded on the first sampling date June-00 (Table 7.4). Estimates for subsequent sampling dates fluctuated between 777 and 1787 individuals m^{-2} for the 27,000m^2 study site, with standard error accounting for between 4.3 and 15.9% of the total population estimates on any given day. The number of individuals and mean shell height for each arbitrary size grouping are presented in Appendix 7.2, these data being utilised as input parameters for each model. The quantity of surface sediment chlorophyll *a* fell to 4.5±0.9 mg m^{-2} during April-01, this value being reached after a steady and continuous decline from the maximum estimate of 29.0±1.6 mg m^{-2} recorded during August-00 (Table 7.4).

Table 7.4. Daily population estimates (±S.E.) for *Tellina tenuis* and total surface sediment chlorophyll *a* (mg m^{-2}), Blackrock.

Date	Population (m^{-2})	±S.E.	% error	Chlorophyll *a* (mg m^{-2})	±S.E.
21-June-00	1807	117.75	6.5	22.06	0.94
22-Jul-00	1554	66.27	4.3	23.73	1.37
20-Aug-00	1114	117.68	10.6	29.02	1.58
23-Sep-00	958	129.52	8.3	22.97	2.47
21-Oct-00	1217	89.39	7.3	20.64	1.22
19-Nov-00	777	83.54	10.8	19.78	1.57
04-Jan-01	1114	193.06	15.9	16.07	0.83
18-Feb-01	1140	134.38	11.8	15.44	1.02
16-Mar-01	984	74.91	7.6	17.28	1.09
14-Apr-01	1295	147.37	12.9	4.52	0.86
27-May-01	1787	86.62	4.8	10.99	0.81
26-Jun-01	1347	123.00	9.1	12.52	1.27

7.3.2 Size-specific grazing relationships

The three methodologies used to calculate a size-specific grazing rate for *Hydrobia* produced slight variations in the total ingestion rate estimates for each sampling date. Use of the size-weight relationship to determine size-specific grazing rates yielded higher estimates than the other 2 methods on all occasions with the exception of the first days sampling when compared with the linear method (Table 7.5). In this instance the ingestion estimate generated by model 2 from the linear method was 5% higher than that for size-weight. The former predicted only slightly higher grazing rates in the 3.5-6mm (SH) size range (Figure 7.3). The majority of the population at Bull Island were within this size range during the first sampling period, with individuals outside of the range being well represented on all other sampling occasions (Appendix 7.1). The size-weight method predicted much higher grazing rates for these individuals.

The methodology based on cumulative growth only resulted in higher overall ingestion estimates for sampling days 3, 4 and 8 when compared with the linear model. This was due to the presence of large number of individuals <3.5mm (SH) corresponding to low numbers of larger individuals at the site on these dates (Appendix 7.1). The cumulative growth method predicted higher grazing at sizes <3.5mm (SH) when compared with the linear method, but the later predicting much higher values at larger sizes. Although the cumulative growth method did not result in higher estimates than the size-weight method at any time, as it only predicted slightly higher grazing rates in the 2-3.5mm (SH) size range (Figure 7.3), it accounted for between 84-98% of the estimate generated using the size-weight method on any given day (Table 7.5).

Although differences in daily population chlorophyll *a* ingestion estimates occurred when using the same model in conjunction with each of the various methodologies used to calculate size-specific grazing rate, these differences were not statistically significant when compared with Chi-square tests (Kruskal-Wallis, $\chi^2=1.59$, df=2, $p \leq 0.01$).

Table 7.5 Percentage difference between daily *Hydrobia* total population chlorophyll *a* ingestion estimates derived using various methodologies for determining size-specific grazing rate. The differences shown apply to the output of model 2.

Date	Method 2 (growth) as % of Method 1 (size-wt)	Method 3 (linear) as % of Method 1 (size-wt)	Method 2 (growth) as % of Method 3 (linear)
22-Jul-00	89	105	84
20-Aug-00	92	95	97
23-Sep-00	98	84	114
21-Oct-00	95	89	106
19-Nov-00	90	96	94
18-Jan-01	90	95	95
06-Mar-01	92	93	99
14-Apr-01	92	86	106
27-May-01	86	88	98
27-Jun-01	84	97	87
14-Jul-01	90	99	89
11-Aug-01	89	92	97

There was a significant difference between the estimates produced when utilising the 2 methodologies for predicting size-specific grazing rate for *Tellina tenuis* (Mann-Whitney, $U=1.0$, $df=1$, $p \leq 0.01$). With the outputs from model 1 as an example, using the size-weight relationship to predict size-specific grazing rate resulted in an overall population chlorophyll *a* clearance estimate of only 42-50% of that predicted by the linear method (Table 7.6). The later method estimated higher ingestion rates for all but the smallest and largest individuals in the population. The greatest differences were predicted in the mid size range, which constituted the majority of the population on all sampling occasions (Appendix 7.2).

Table 7.6. Percentage difference between daily *Tellina* total population chlorophyll *a* ingestion estimates derived for model 1 using each of the methodologies for determining size-specific grazing rate.

Date	Method 1 (size-wt) as % of Method 2 (linear)
21-June-00	45
22-Jul-00	45
20-Aug-00	48
23-Sep-00	45
21-Oct-00	46
19-Nov-00	46
04-Jan-01	49
18-Feb-01	46
16-Mar-01	49
14-Apr-01	48
27-May-01	42
26-Jun-01	50

7.3.3 Hydrobia chlorophyll a grazing estimates

The results presented in the following sections were based on size-specific grazing rates determined by the size-weight relationship of *Hydrobia* only. The estimate produced using this method was not statistically different from those generated using the other methodologies. This method was not considered to have produced more realistic or accurate estimates when compared with the other 2 methods, and was selected for presentation purposes only. A relative estimate of population ingestion can be obtained for the other methods by application of the figures presented in Table 7.5.

Model 1 The effect of density on feeding activity-linear relationship

Maximum estimated population chlorophyll *a* consumption occurred during late September, the period of highest population abundance, reaching 15.9±1.36 mg chlorophyll *a* m^{-2} day^{-1} (Figure 7.11). The lowest level was estimated 2 months earlier on the first sampling date, when total population chlorophyll *a* ingestion of 2.17±0.20 mg chlorophyll *a* m^{-2} day^{-1} accounted for only 14% of that recorded in September. Estimates remained relatively stable during the winter period between November and March. Estimates increased during May to 12.46±0.51 mg chlorophyll *a* m^{-2} day^{-1} with the large additions to the population associated with the spring recruitment, despite the low relatively low individual ingestion rates at small size. Population numbers and, therefore, ingestion estimates fluctuated widely in the following months.

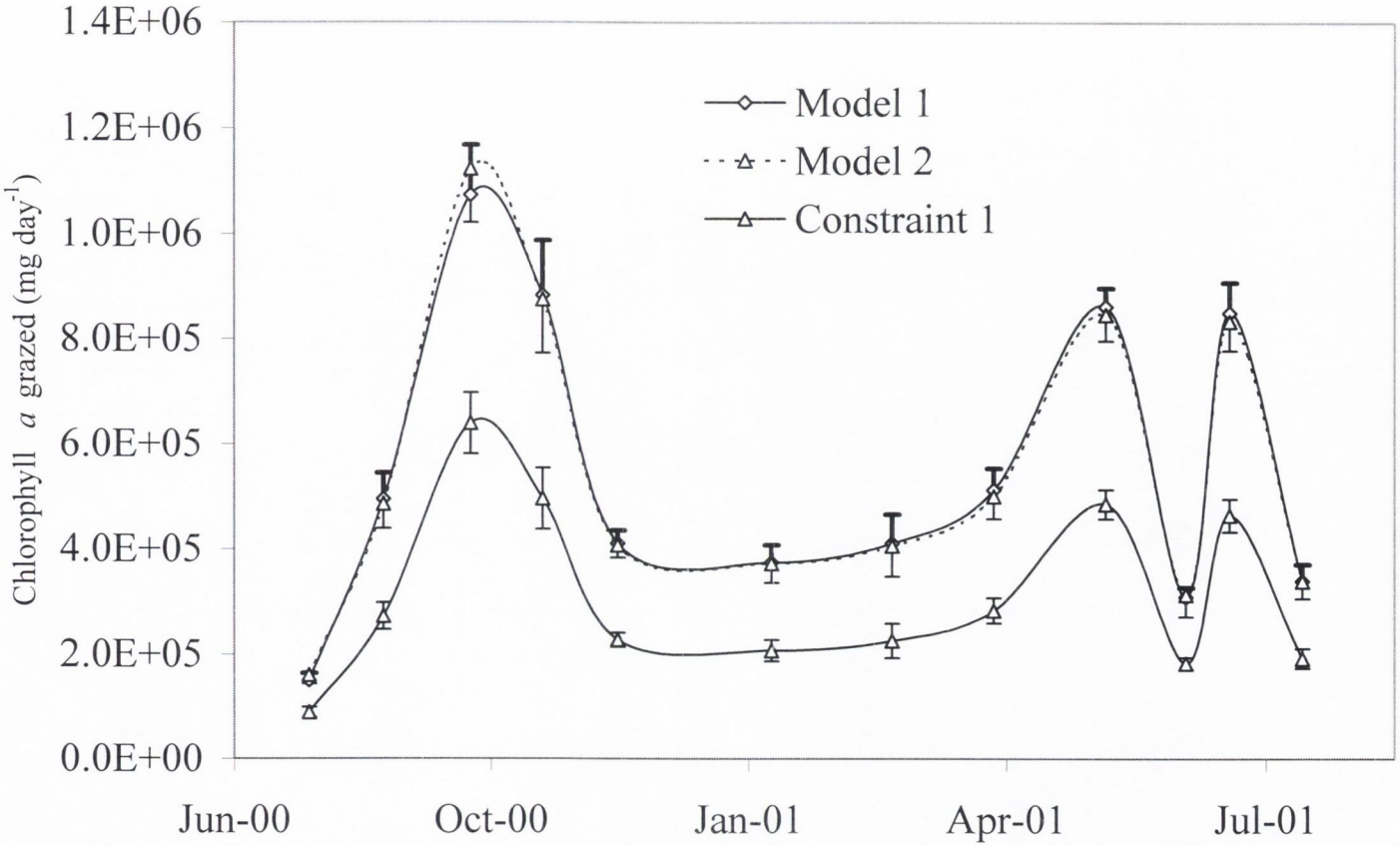


Figure 7.11. Estimates (+S.E.) of chlorophyll *a* ingested by *Hydrobia* at Bull Island each day, July 00-August 01. Only one tail of error shown for models 1 (heavy positive) and 2 (light negative) for clarity.

The total daily grazing estimates for *Hydrobia* were equivalent to between 2-72% of the total surface sediment chlorophyll *a* estimate on any particular sampling day (Figure 7.12). The high of 72% obtained during April-01 was principally due to the occurrence of the low chlorophyll *a* concentration rather than *Hydrobia* numbers, which only approximated the average recorded for all sampling dates at the time. The highest population estimate for *Hydrobia*, which occurred in September, coincided with a high chlorophyll *a* estimate and, therefore, the grazing corresponded to only 19% of the chlorophyll *a* at that time.

Model 2 The effect of density on feeding activity-negative power relationship

The daily total population grazing estimates derived from model 2 were not significantly different from those of model 1 (chi-square test, $p \geq 0.05$). The difference between estimates varied by 7% at most, this occurring between those generated for the first sampling day when density was lowest (Figure 7.11), and was less than 2% for 9 of the 12 sampling occasions. Model 2 only predicted higher grazing rates than model 1 when density was below 7,000indiviuals m^{-2} and above 30,000indiviuals m^{-2}. The former situation occurred only during July-00, June-01 and August-01, the later only at peak abundance during September-00. Due to the similarity in daily estimates derived from models 1 and 2, total grazing estimates as a proportion of total surface sediment chlorophyll *a* were almost identical (Figure 7.12).

As the estimates derived from models 1 and 2 were not significantly different, only the results derived by constraining the later are presented in the following section. The results of model 2 were not considered to be the more appropriate of those produced, particularly considering the lack of experimental data to describe density-dependent effects on feeding rate, and was selected randomly over the linear model for further analysis in order to reduce replication of results.

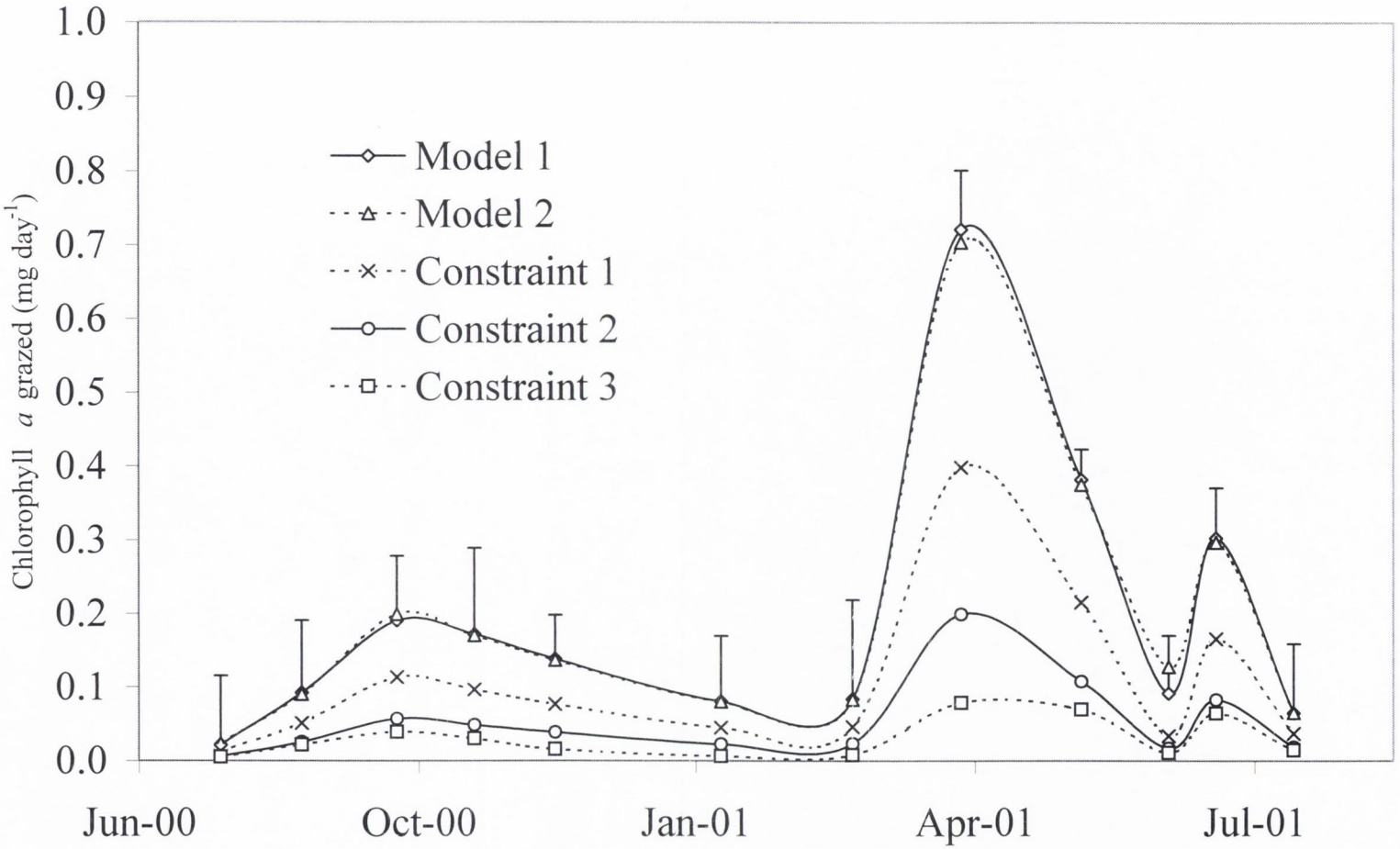


Figure 7.12. Estimates of chlorophyll *a* ingested by *Hydrobia* at Bull Island each day as a proportion of the total quantity of surface sediment chlorophyll *a*, July 00-August 01. Only positive error (S.E.) estimate for model 1 shown for clarity. Error identical but proportional for other estimates.

Constraint 1 - The effect of tidal immersion/emersion on feeding activity

Constraining feeding time to conform to the maximum tidally enforced grazing rate resulted in a statistically significant reduction in total population ingestion estimates. As all daily estimates were reduced by between 41-45%, there was little effect on their magnitude relative to each other (Figure 7.11).

Constraint 2 - The effect of day/night, variation in behaviour and disturbance on feeding activity

The results of this reduction in feeding activity did not impact on the magnitude of daily population estimates relative to one another, and only served to significantly reduce them to levels more likely to occur based on knowledge of the sampling site and species behaviour. The maximum daily ingestion estimate for September was reduced to 4.49±0.42 mg chlorophyll *a* m^{-2} day^{-1} in this step (Figure 7.13).

Constraint 3 - The effect of temperature on feeding activity (Q_{10})

Application of the Q_{10} relationship decreased daily population ingestion estimates from the previous step by between 14-71% (Figure 7.13). The greatest reduction in ingestion rate occurred in Jan 01, when water temperature reached a low of 5.8°C, which resulted in an estimate adjustment from 1.4±1.4 to 0.43±0.04mg chlorophyll *a* m^{-2} day^{-1}. Water temperature in Dublin Bay was only 3°C lower than the experimental temperature of 20°C in August-00, this resulting in the smallest impact on estimated daily population ingestion. When the final adjustments to estimates were made in this model, total daily population ingestion accounted for between 8-24% of the totals predicted in model 2. These final estimates accounted for between 1-8% of the total surface sediment chlorophyll *a* estimates for any given day (Figure 7.12), on 7 of the 12 sampling dates this figure was <2%.

7.3.4 Tellina chlorophyll a clearance estimates

The results presented in the following section were generated using the size-weight relationship to define size-specific ingestion rates for *Tellina*. The daily estimates

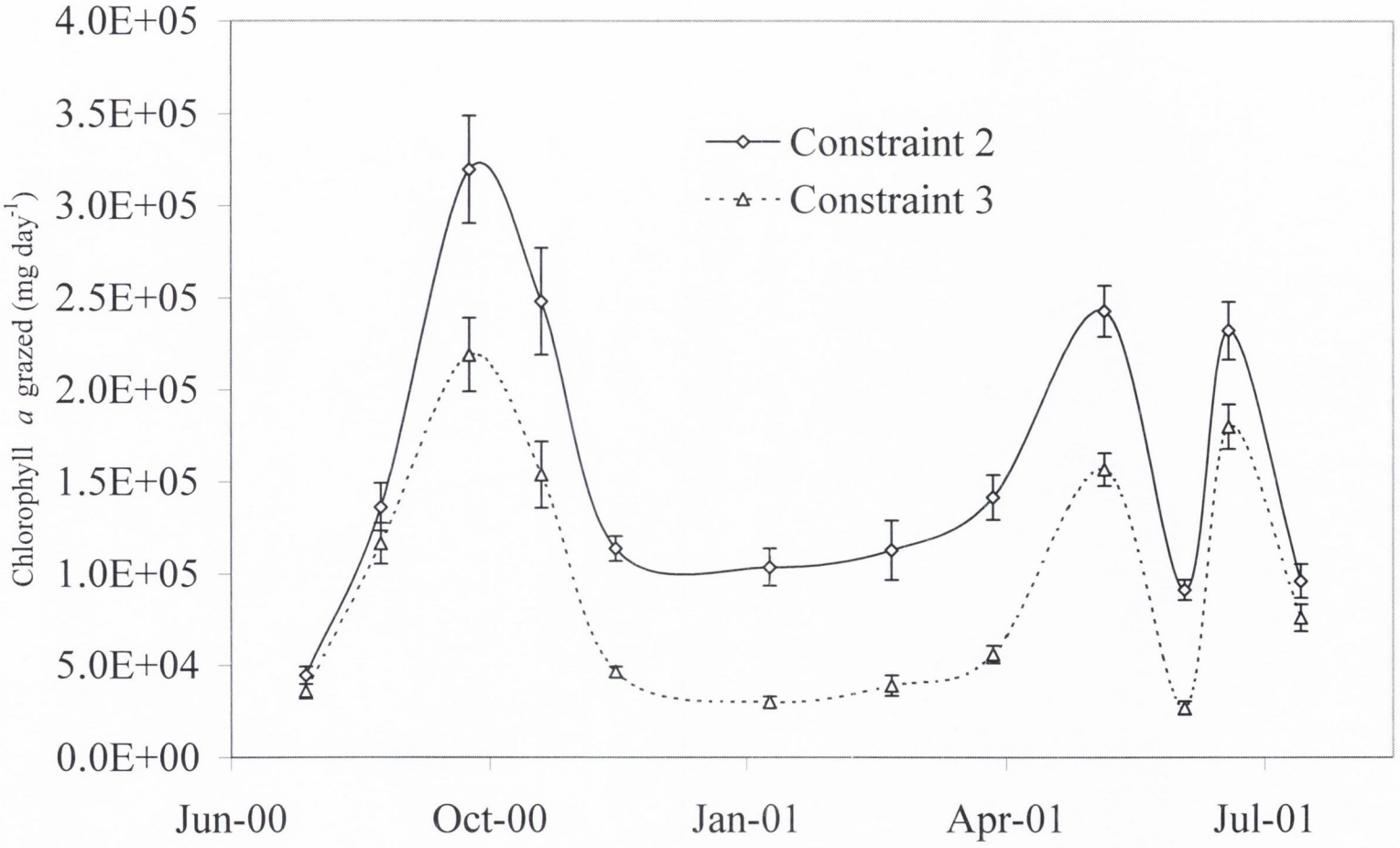


Figure 7.13. Estimates (+S.E.) of chlorophyll *a* ingested by *Hydrobia* at Bull Island each day, July 00-August 01, for constraint 2 and 3 (see methods).

derived from the method that assumed grazing rate to be proportional to shell height (linear) were identical in relative proportion to one another as when the former method was used, and are omitted to reduce replication. It should be appreciated that the estimates derived using the linear method were significantly higher however, and can be obtained by using the factors given in Table 7.6. Results obtained using the size-weight method are presented for ease of comparison to *Hydrobia* results only, and were not considered more reliable than the later.

Model 1-Chlorophyll *a* ingestion estimates

The highest total population chlorophyll *a* clearance estimate of 2.15±0.14 mg chlorophyll *a* m^{-2} day^{-1} was recorded during June-00, coinciding with the highest population estimate recorded during the study period (Figure 7.14). Daily ingestion estimates fluctuated between 1.3-1.8 mg chlorophyll *a* m^{-2} day^{-1} for most other sampling dates, although a low of 0.88±0.09 mg chlorophyll *a* m^{-2} day^{-1} was estimated for November-00. Although the total ingestion rate estimate corresponded to 30% of the total surface sediment chlorophyll *a* during April-01 (Figure 7.14), there was no assumption that the former parameter affected the later in any way as the exact feeding behaviour of this species is yet to be resolved. The total ingestion estimate represented less than 10% of surface sediment chlorophyll *a* on 8 of the 10 sampling occasions.

Constraint 1-The effect of tidal immersion/emersion on feeding activity

The total daily clearance estimates were reduced by between 35-43% by constraining feeding time to the maximum enforced by tidal immersion/emersion, and differed significantly from the predictions of model 1. The greatest reduction occurred for the first and largest estimate for June-00, when high numbers of individuals were located high on the shore, which fell from 2.15±0.14 to 1.22±0.08 mg chlorophyll *a* m^{-2} day^{-1}. Lowest reduction occurred in January and April, when population estimates were low and/or size-frequency distribution skewed toward the smaller size groupings that were located lower on the shore. Only the total daily clearance estimate of April exceeded 10% of total surface sediment chlorophyll *a* (Figure 7.14).

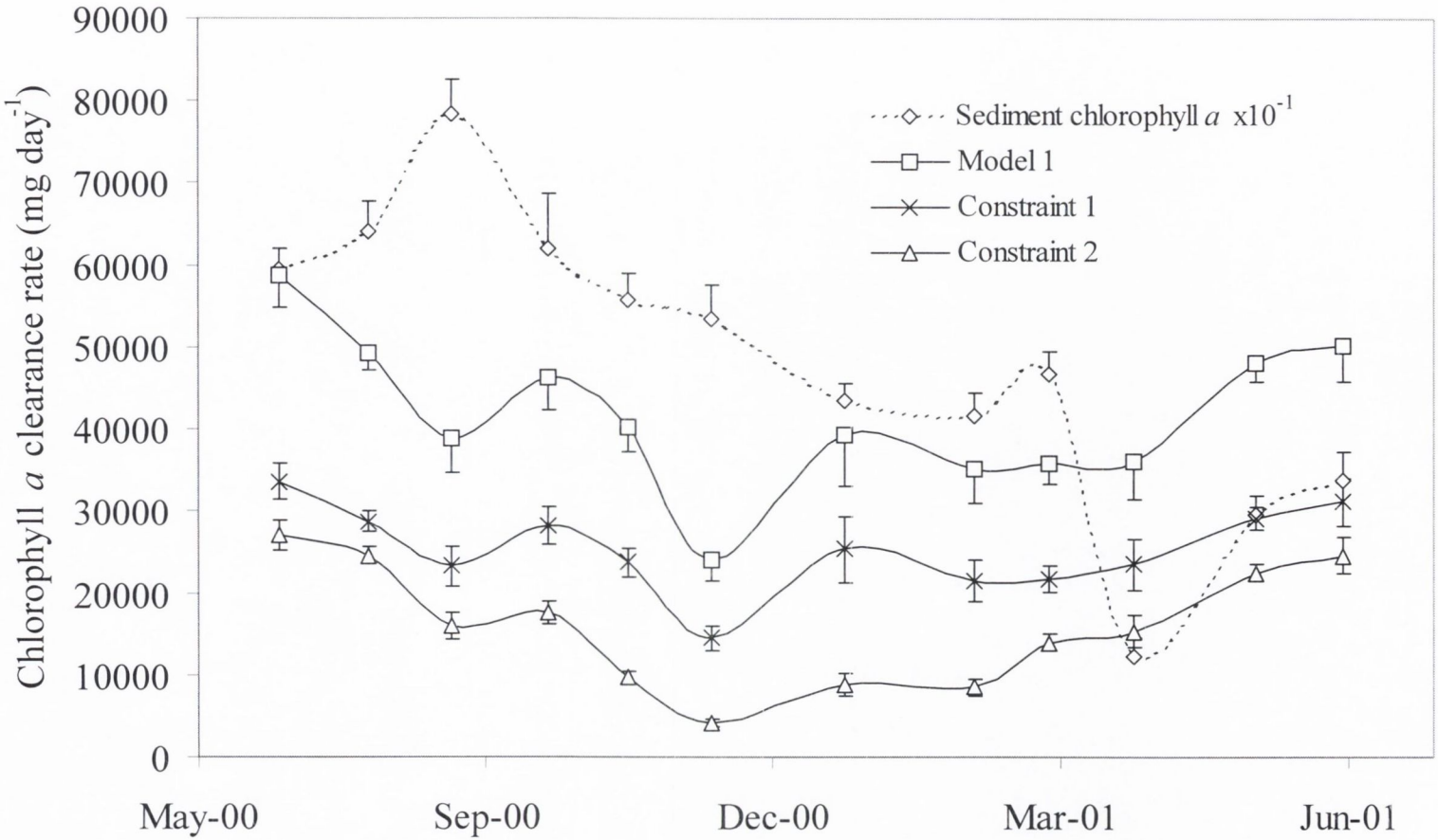


Figure 7.14. Estimates (+S.E.) of chlorophyll *a* ingested by *Tellina* each day at Blackrock and total surface sediment chlorophyll *a*, June 00-June 01.

Constraint 2-The effect of temperature on feeding activity (Q_{10})

The lowest clearance rate estimate predicted after constraint 2 was applied was for November, 0.52±0.06 mg chlorophyll *a* m^{-2} day^{-1}, which fell by 71% to 0.16±0.02 mg chlorophyll *a* m^{-2} day^{-1} when the Q_{10} adjustment was applied. The smallest reduction occurred for June-00, which declined by only 8%. The estimates derived were significantly lower than for models 1 and constraint 2 (Chi-square test, $p \geq 0.05$). Again, only the total daily clearance estimate of April exceeded 10% of the total surface sediment chlorophyll *a* (Figure 7.14), with 8 of the 12 dates <5%. The final estimates produced after application of the Q10 adjustment corresponded to between 18-50% (November and July respectively) of the original clearance predicted from model 1.

When the daily population ingestion estimates for both species had been refined to the Q_{10} stage, the daily *Tellina* estimate represented between 7-76% or 14-158% of the daily *Hydrobia* estimate when either the size-weight or linear relationships were used to calculate size-specific grazing rate for the former species. It should be appreciated that the Blackrock site accounted for only 39% of the area of the Bull Island site however, and these figures were not adjusted for area (see following section). Estimated feeding rates were relatively similar between-species at comparable body sizes, for example grazing rate for a 7mm (SH) individual was estimated to be 70ng chlorophyll *a* hr^{-1} for *Tellina* using the size-weight method to define size-specific grazing rate, and 55-62 ng chlorophyll *a* hr^{-1} for *Hydrobia* depending on density. The majority of individuals within the *Tellina* population had attained a greater size than the approximate maximum of 8mm(SH) for *Hydrobia* however, and these individuals were assumed to achieve much greater proportional feeding rates. The estimated maximum daily ingestion rate (24hr feeding) of a large 7mm (SH) *Hydrobia* corresponded to approximately 30% of that estimated for a 19mm (SH) *Tellina*. Total population chlorophyll *a* ingestion rates observed for *Hydrobia* were significantly increased by their very high abundance however.

7.3.5 Annual population feeding estimates

7.3.5a Hydrobia-Bull Island

Extrapolation of the daily population estimates for *Hydrobia* at the Bull Island site suggested $3.6x10^{11}\pm1.7x10^{11}$ individual *Hydrobia* grazing days occurred at the 69,000m^2 site during the study period. The various methods used to determine total population ingestion estimated that between 3.08±2.78 (model 1) and 0.49±0.44 (constraint 5) g chlorophyll *a* m^{-2} (±95%CI) was ingested at the site during the study period, respectively (Table 7.7). The corresponding organic carbon ingestion estimate (±95%CI) ranged between 8520±7668 and 1360±1224kg C yr^{-1}, or adjusted for the area of the site 124±111 to 20±18g C m^{-2} yr^{-1}, respectively. Due to the multiplicative nature of the estimates, associated error was high. A microphytobenthic primary production estimate (±95%CI) of 30±17g C m^{-2} yr^{-1} or 2087±1173kg C site yr^{-1} was derived from the chlorophyll *a* samples taken at the site during the sampling period. The mean of this estimate represented only 25, 25, 44 and 88% of the mean total organic carbon ingestion estimates for models 1 and 2, and constraints 1-2 respectively. The mean organic carbon ingestion estimate (±95%CI) from constraint 5 of 20±18 g C m^{-2} yr^{-1} accounted for 67% of the annual mean microphytobenthic primary production estimate, although the high degree of error associated with each estimate must be considered. The assumed carbon assimilation rate of 544±489kg C site yr^{-1} would result in the removal of 26% of organic carbon microphytobenthic primary production assuming this was the only food source utilised, with the remaining portion of ingested carbon, 814±734kg (±95%CI), entering the environment as faeces to be broken down into composite nutrients (Table 7.7).

7.3.5b Tellina-Blackock

As daily population ingestion estimates for *Tellina* were significantly different when utilising the 2 methods to calculate size-specific ingestion rates, and no data was available to determine which if either was the more applicable, global results are presented for both in Table 7.8. Estimates of population chlorophyll *a* ingestion (±95%CI) ranged between 15±12 and 5.4±4.4 kg chlorophyll *a* yr^{-1} for the size-weight method and 32±26 and 11±9.1kg chlorophyll *a* yr^{-1} of the linear. Total annual

microphytobenthic primary production (±95%CI) at the Blackrock site was estimated to be 18±5g C m^{-2} yr^{-1} or 486±135kg C site yr^{-1}. Although this was less than the total population organic carbon ingestion estimates from model 1 using the size-weight method and model 1 and constraint 1 utilising the linear, it should be appreciated that the feeding strategies of *Tellina* are poorly understood and that no deficit in dietary organic carbon at the site is suggested. Although only size-weight constraints 1 and 2, and linear constraint 3 predicted annual population organic carbon ingestion to be less than annual microphytobenthos primary production (Table 7.8), other food sources may be readily available to this species. The low assimilation efficiency of the species resulted in all models predicting uptake lower than the microphytobenthos primary production estimate.

Total annual microphytobenthic primary production (±95%CI) at the Blackrock site, 18±5g C m^{-2} yr^{-1}, represented approximately 60% of the estimate derived for the Bull Island site. Due to the smaller area of the Blackrock site, the corresponding total of 486±135kg C site yr^{-1} (±95%CI) was equivalent to only 23% of the mean estimate for Bull Island. A total of $7.6x10^{8}$±$3.1x10^{8}$ individual *Tellina* grazing days were estimated from the extrapolated daily grazing estimates, <1% of that estimated for *Hydrobia* at Bull Island. The greater estimated ingestion rate for individual *Tellina*, due to larger mean body size, resulted in relatively comparable ingestion estimates when both had been constrained to the most severe Q_{10} level. Total population chlorophyll *a* ingestion (±95%CI) estimates of 0.22±0.16 and 0.41±0.34g chlorophyll *a* m^{-2} yr^{-1} for *Tellina* using the size-weight and linear methods, respectively, represented 45 and 84% of the estimate of 0.49±0.44 derived for *Hydrobia*. The lower assumed assimilation efficiency of *Tellina* significantly increased the disparity between estimates for each species however.

Table 7.7. Estimate of mean (±95% C.I.) *Hydrobia* total population ingestion, assimilation and egestion (global estimate and m^{-2}) at Bull Island, July-00-August-01 (385days).

Model	Units	Model 1	Model 2	Constraint 1	Constraint 2	Constraint 3
Chlorophyll *a* ingested	kg $year^{-1}$ *g m^{-2} yr^{-1}*	213±192 *3.1±2.8*	212±191 *3.1±2.8*	118±106 *1.7±1.5*	59±53 *0.85±0.77*	34±31 *0.49±0.44*
Organic carbon ingested	kg $year^{-1}$ *g m^{-2} yr^{-1}*	8520±7668 *124±111*	8480±7632 *122±110*	4720±4248 *68±62*	2360±2124 *34±31*	1360±1224 *20±18*
Assimilation efficiency (%)		40	40	40	40	40
Organic carbon egested as faeces	kg $year^{-1}$ *g m^{-2} yr^{-1}*	5112±4600 *74±67*	5088±4579 *74±66*	2832±2549 *41±37*	1416±1274 *21±19*	814±734 *12±11*
Organic carbon assimilated	kg $year^{-1}$ *g m^{-2} yr^{-1}*	3408±3067 *49±45*	3392±3053 *49±44*	1888±1699 *27±25*	944±850 *14±12*	544±489 *7.9±7.1*

Table 7.8. Estimate of mean (±95% C.I.) *Tellina* total population ingestion, assimilation and egestion (global estimate and m^{-2}) at Blackrock, June-00-01 (370days). The method column relates to the calculation of size-specific ingestion rate.

Model	Units	Size-weight method			Linear method		
		Model 1	Constraint 1	Constraint 2	Model 1	Constraint 1	Constraint 2
Chlorophyll *a* ingested	kg $year^{-1}$ *g m^{-2} yr^{-1}*	15±12 *0.55±0.45*	9.0±7.4 *0.33±0.27*	5.4±4.4 *0.22±0.16*	32±26 *1.2±0.97*	18±15 *0.68±0.56*	11±9.1 *0.41±0.34*
Organic carbon ingested	kg $year^{-1}$ *g m^{-2} yr^{-1}*	589±483 *22±18*	359±295 *13±11*	216±177 *8.0±6.6*	1274±1045 *47±39*	737±604 *27±22*	445±365 *17±14*
Assimilation efficiency (%)		10	10	10	10	10	10
Organic carbon egested as faeces	kg $year^{-1}$ *g m^{-2} yr^{-1}*	530±435 *20±16*	323±265 *12±10*	194±159 *7.2±5.9*	1147±940 *42±35*	663±544 *25±20*	400±328 *15±12*
Organic carbon assimilated	kg $year^{-1}$ *g m^{-2} yr^{-1}*	59±48 *2.2±1.8*	36±29 *1.3±1.1*	22±18 *0.8±0.66*	127±104 *4.7±3.9*	74±60 *2.7±2.2*	45±36 *1.7±1.4*

7.4 Discussion

Estimates of total population chlorophyll *a* ingestion and assimilation would suggest that both *Hydrobia* and *Tellina* play an important role in the transfer of nutrients between primary producers and higher trophic levels at their respective sites. Even though the ingestion rate estimates for individual animals were relatively small, the combined effect of the vast numbers present throughout the year gave rise to large total population ingestion estimates. Although a high degree of error was associated with the estimates generated, they at least represent the approximate range of ingestion values that may occur in the field. The basic inputs to the models, the population and chlorophyll estimates, were robust data measurements of field populations over the course of this study. It is probable that the relative effects and direction of energy flow in the system described by the model are correct. Constraining ingestion rate and duration to be more representative of the conditions that are potentially imposed on individuals in wild populations produced significantly lower estimates than would have been predicted by simple extrapolation of laboratory data. As the design and implementation of multi-factorial laboratory feeding experiments is complex, the current study attempted to resolve this difficulty by the application of field-based data to derive total population ingestion estimates. Although several studies have examined the total population nutrient assimilation potential of particular consumer species (Hughes, 1970b; Sola, 1996; Sauriau & Kang, 2002) or overall secondary production by all primary consumers within a particular habitat (Wolff, 1977; Warwick *et al.*, 1979; Kalejta & Hockey, 1991; Smith & Hollibaugh, 1993; Soetaert & Herman, 1995), no previous studies have attempted to gauge the impact of consumer population ingestion on primary production in this way.

Even if surface sediment chlorophyll *a* concentration was taken to be an indication of the sum total of food available for *Hydrobia* utilisation at the Bull Island, the data presented suggested that this parameter was not limiting population size at this site when environmental constraints on feeding time were imposed. Although total population chlorophyll *a* ingestion represented a significant proportion of that estimated to be available in the surface sediments during the summer period, this is also likely to have coincided with the period when microphytobenthic production rate was highest.

Additionally, periodic tidal delivery and deposition of phytoplankton probably supplemented benthic chlorophyll *a* content during this period. The lowest chlorophyll *a* concentrations were recorded in April which coincided with an influx of juvenile *Hydrobia*, however, similar low chlorophyll levels did not occur in September when a second, larger, entry of juveniles occurred. Stable isotope analysis of *Hydrobia* (Chapter 6) has suggested that this surface deposit feeder consumes at least some phytoplankton during summer months, despite being unable to remove it directly from the water column. Therefore this food source was probably ingested from the sediment surface along with microphytobenthos in as yet undefined proportions. Soetaert & Herman (1995) reported that due to the consistent lower production rates of microphytobenthos throughout the year, compared with the higher but much more seasonal phytoplankton production, the microphytobenthos accounted for 60% of total primary production in an estuary in the Netherlands. In a review of worldwide microphytobenthic production, Underwood & Kromkamp (1999) calculated the microphytobenthos as making a contribution of between 16 and 63% of whole estuary primary production. As peaks in population ingestion estimates were often associated with recruitment pulses of highly abundant newly settled *Hydrobia* in the current study, they rapidly declined due to the subsequent high mortality of this cohort. It seems reasonable to assume, therefore, that primary producers are replenished at a sufficient rate to ensure that food availability does not limit the population size of *Hydrobia* at the Bull Island site when the species was considered in isolation and other primary consumers and their effects on the available primary production not considered.

Whether food availability was limiting for *Tellina tenuis* at the Blackrock site is unclear. Confusion as to the actual feeding strategies of this species remain unresolved, and therefore no direct comparison between estimated food availability and total population ingestion could be made. Although stable isotope analysis (Chapter 6) indicated that the species appeared to consume at least some degree of microphytobenthos throughout the year, the actual feeding behaviour used to utilise this food source was not determined. If *Tellina* does not remove food items from suspension, even if at the sediment-water interface at the Blackrock site, then the laboratory derived ingestion rate of Trevellion

(1971) that was used as the base input in the current study was invalid. If, however, *Tellina* feeds solely on the microphytobenthos, then bottom-up regulation of this population could occur considering the high population ingestion rates derived relative to annual primary production, although this is entirely speculative due to the lack of necessary data. Further stable isotope analysis should be conducted to allow the application of mixing models, which can determine the relative importance of food sources to overall diet. Until the preferred feeding strategy of *Tellina* is determined, or the proportions of time spent deposit and filtering feeding if both strategies are used, it would be unwise to comment further on the nature of primary producer-consumer dynamics for this species at the Blackrock site.

Due to the absence of other similar studies, it is not possible to draw direct comparisons between the population chlorophyll *a* ingestion estimate derived and those for other areas or species. Although some studies have examined the effect of primary consumers on primary production in the laboratory, these results may be considered artificial considering the stringent experimental constraints detailed within each experiment, which do not mirror those that occur in the field (Bianchi *et al.*, 1988; Page *et al.*, 1992; Ingalls *et al.*, 2000; Cartaxana *et al.*, 2003). Estimates of the proportion of surface sediment chlorophyll *a* that was potentially consumed each day from the current study, between 1-10%, did approximate to some laboratory studies. In particular, the mud snail *Ilyanassa* was been shown to remove approximately 10% of surface chlorophyll *a* per day in laboratory conditions (Barnes & Hughes, 1988). Previous field-based studies have used very variable methods to estimate total heterotrophy (all trophic levels) within habitats and related these to some form of primary production estimate. Soetaert & Herman (1995) estimated total heterotrophy for all consumer species to result in the net removal of 380g C m^{-2} yr^{-1} from an estuary in the Netherlands. This was an extremely high value, and many other estuaries have reported lower figures of between 50-200 g C m^{-2} yr^{-1} (Smith & Hollibaugh, 1993). The lower end of this range was not much greater than the figures observed in the current study when all environment constraints on feeding were applied. In contrast to the previous studies, only single species consumption/assimilation

rates were estimated in the current study, and the additional impact of other consumer species was not considered.

The predicted total population chlorophyll *a* ingestion estimates for each species did not appear to be correlated with the quantity of chlorophyll *a* available at the sites. This suggested that at least for the range of primary productivity and total population ingestion estimates derived, primary producers and consumers did not directly influence the abundance of each other in isolation. Although there may have been some bottom-up or top-down regulation at the sites, the relationship was either weak or masked by other controlling factors at the levels observed. The grazing impact of other species, variability between other physical and biological regulatory factors and the interaction between these may have decoupled the relationship between food availability and population size. Although it can be assumed that severe declines in food availability would result in significant decreases in population numbers, significant increases may not necessarily result in an associated expansion of the primary consumers if some other threshold to population size has been reached. For example, intraspecific density-dependent regulation appears to be the most important factor determining the feeding activity (Blanchard *et al.*, 2000) and growth (Chapter 4) of *Hydrobia ulvae*. In the latter case, additional food availability only elicited a positive effect on the growth of small individuals when density was very low. The biological and/or physical parameters that determine population carrying capacity are probably species-specific, and will not in all cases be determined by food availability.

The large degree of error associated with the total population ingestion estimates was a result of the multiplicative nature of the initial population estimates. Due to the uneven distribution of individuals and sizes of individuals at the sites, it is not probable that these estimates could be significantly improved without the investment of extremely large increases in sampling effort. Further refinements to site-specific estimates would more likely be achieved by directing effort toward the improvement of other input parameters. Accurate size and species-specific ingestion rate and feeding duration data, subsequent assimilation efficiency estimates and site-specific primary production estimates in

particular, and the way in which these factors are regulated across the sites over time, would permit significant refinement of global estimates generated for each site. The use of previously published data sources for these parameters in the current study was not considered to be ideal.

The lack of data pertaining to the relationship between size and feeding rate for both species highlighted the importance of reliable input parameters for population modelling, and how estimating these can result in negligible or very significant variation in outputs. Due to the convergence of size-specific grazing rate estimates derived by the application of the different methods at the rarely occurring maximum limit of the size range for *Tellina* (19 mm SH), the greatest disparity between individual feeding rate estimates occurred at the numerically dominant mid-size ranges. This strongly affected the resultant population chlorophyll *a* ingestion estimates. In contrast, the application of the various methods to predict size-specific grazing rates for *Hydrobia* did not affect subsequent ingestion estimates. This was principally due to the convergence of estimates at the well-represented mid-size ranges, with the greatest disparity in size-specific feeding rate estimates occurring at the more poorly represented upper and lower extremes of the overall size range. Therefore, the application of the various methods did not alter individual *Hydrobia* grazing rates sufficiently to affect the total population ingestion estimates, these tending to be more strongly influenced by variations in population number and size-composition. Essentially the difference in the relative contribution of each individual to the overall population ingestion rate for each species lead to greater (*Tellina*) or lesser (*Hydrobia*) sensitivity to variations in particular model input parameters. As the mean size of *Tellina* was greater than that of *Hydrobia*, individuals of the former contributed proportionally more to the overall daily ingestion estimates despite individuals of the same size in each population having similar ingestion rates. This resulted in relatively similar unit area total population ingestion estimates for each species, despite the far higher numerical abundance of *Hydrobia*.

Even at low individual assimilation efficiency, the high total population ingestion achieved was estimated to result in the uptake of a very large quantity of organic carbon

each year. Each species, and individual within the population, will utilise varying proportions of this uptake for physiological activity, maintenance and tissue development. Subsequent egestion, decomposition and digestion will transfer some or all of these nutrients to higher and/or lower trophic levels, depending on the fate of the individual. Although a large proportion of ingested material is apparently egested as faeces, particularly from *Tellina* with its low assimilation efficiency, this faecal matter may, subsequently, represent sites of localised enrichment for detritus feeders and/or bacteria (Forbes & Lopez, 1986). Soetaert & Herman (1995) stated that the detrital portion of the food web was often the most important in estuaries as total heterotrophy often exceeded primary autotrophy. Previous studies recorded a certain proportion of ingested chlorophyll *a* passing through the stomach of herbivorous molluscs totally unaltered (Levinton & Bianchi, 1981; Admiraal, 1984; Hummel, 1985), and presumably capable of re-joining the primary producer standing stock. This would seem feasible when the assimilation efficiency and short gut residence times, 30-40 min for *Hydrobia* (Lopez & Kofoed, 1980), of small primary consumer species are considered. The amphipod *Corophium volutator* has been recorded to feed on material that has passed through the gut of the mudsnail *Hydrobia ulvae* indicating at least some viable food content remains after digestion (Lopez & Levinton, 1978). Although *Hydrobia* were assumed to feed throughout the year, increase in body size was only observed to occur during a highly truncated 2-month period at the Bull Island site (Chapter 4). If biomass also failed to increase during the periods when shell height was stable, then the species would not appear to be as effective at transferring nutrients to higher trophic levels as first thought, but could contribute significantly to the diet of detritus feeders.

Within the constraints of the current study it was not possible to determine the relationship between nutrient availability, standing surface sediment chlorophyll *a* concentration (microphytobenthos) and the regular delivery/removal of microphytoplankton in the water column. It is very difficult to distinguish between the later two groups, and in fact many species regularly occur in and travel between both the water column and sediment media (Baillie & Welsh, 1980; Admiraal, 1984; Heip *et al.*, 1995; Lucas *et al.*, 2001). Regular deposition of microphytoplankton on slack/ebbing

tides may serve to supplement the standing stock of microphytobenthos, or provide a means for the removal of the later if vertical flux occurs. Although a situation could be envisaged where significantly different surface sediment chlorophyll *a* concentrations occurred at successive low tides due to the removal/deposition of microphyto-organisms, this parameter was taken to be a reliable indictor of relative primary production at the sites during the current study. The high variability in sediment chlorophyll *a* concentrations recorded over relatively short sampling distances (Chapter 2), at least at the Bull Island site, may be a reflection of the patchy nature of nutrient supply and/or microphytoplankton deposition, inequalities in sediment composition and associated flora or spatial variation in the grazing pressure of consumers. Other authors have recorded small-scale variability in distribution of microphytobenthos previously (Colijn & de Jonge, 1984; Shaffer & Onuf, 1985; MacIntyre *et al.*, 1996). The use of directional sampling devices (e.g. emergence and deposition traps) to determine substrate-specific nutrient and microphyto-organism flux would reveal much about the productivity dynamics at the sampling sites. These devices can quantify the material passing vertically between the sediment surface and water column.

Density-dependent regulation of *Tellina* population size or ingestion rate has not been described previously due to the apparently sedentary nature of this infaunal species and the relatively low abundance recorded at most sites (McIntyre, 1970; Dekker & Beukema, 1999). For this reason no density-dependent adjustment of feeding rate or duration was made for this species. Density-dependent regulation of *Hydrobia* feeding rate was assumed to be related to the inhibition of feeding activity through direct physical contact between individuals. Additionally, Blanchard *et al.*, (2000) stated that the mucus trails left by the locomotory activity of the species also indirectly influenced feeding potential by inhibiting subsequent grazing of microphytobenthos from the surface of affected sediment particles. This was also determined in land snails (Baur & Baur, 1990). Heavy grazing by *Hydrobia* and subsequent pelletisation of the sediment could also have contributed indirectly to density-dependent regulation of feeding activity. *Hydrobia* do not consume fresh faecal pellets (Lopez-Figueroa & Niell, 1987), and these are relatively slow (4 days) to breakdown in the environment (Forbes & Lopez, 1986).

The high abundance of *Hydrobia* recorded at the study site during certain periods of the year and the species relatively high locomotory and egestion rates would have resulted in much of the area being pelletised and/or covered by mucus trails. It is not clear how long the inhibitory affect of mucus persists on surface sediments, and therefore it is possible that it does not contribute significantly to any inhibitory effects. Although it can be assumed that a quantity of microphyto-organisms will be deposited at the next tidal incursion, at least in the summer months, this may not increase food availability sufficiently to support the entire potential grazing pressure. Additionally, some dietary components utilised by other species such as *Corophium* overlap with those of *Hydrobia* and could strongly influence population regulation due to food limitation (Morrisey, 1988a,b; Hagerthey *et al.*, 2002). Interspecific competition for resources was not considered in the present study, but occurs in intertidal habitats (Connell, 1961; Fenchel & Kofoed, 1976; Kamermans *et al.*, 1992; Grudemo & Bohlin, 2000). *Hydrobia* and *Tellina* represent by far the most dominant macrofaunal primary consumers at the sites studied however, and it was the intention to use these index species as indicators of primary production-consumer dynamics. The future addition of further species to the model is considered desirable.

Not all of the micro-organisms containing the photosynthetic chlorophyll *a* measured during the sampling period may have been available for consumption by *Hydrobia* and *Tellina*. Diatoms dominate the estuarine microalgae with pennate diatoms being the most abundant benthic forms, and centric forms in the plankton (Admiraal, 1984). Grazer species such as *Hydrobia* and *Tellina* often have a specific range of prey types and sizes that they will/are able to ingest (Trevallion, 1971; Lopez & Kofoed, 1980). For example, Fenchel & Kofoed (1976) stated that the smallest of diatom cells escaped grazing by *Hydrobia*. Others are unaltered by ingestion by consumers (Levinton & Bianchi, 1981; Admiraal, 1984). The differential effect of herbivorous deposit feeders on microbial communities are believed to be an important characteristic for reducing interspecific competition. For example, the diet of *Hydrobia ulvae* and the co-occurring amphipod *Corophium volutator* overlap as previously stated. A constant fraction of microalgae remains after *Hydrobia* grazing due to the size-selectivity of this species and absence of

extracellular digestion however, and this can be subsequently consumed by *Corophium* (Morisey, 1988a, b). Additionally, the range of prey items available to an individual within a particular population is known to vary through the lifecycle reducing intraspecific competition for food resources (Hentschel, 1998). Smaller individuals will not be capable of ingesting the range of organisms and sediment particles available to larger conspecifics (Fenchel & Kofoed, 1976). As laboratory based grazing experiments are often conducted on individuals of a fixed size with optimally sized food items in high concentration and stable environments, the feeding rates obtained should normally be considered to represent the maximum possible. It is, however, unlikely that such ingestion rates are attainable by all components of a population under natural field conditions.

Mudflats such as that at the Bull Island site are generally considered to be far more productive than sandy substrates similar to those located at Blackrock. The mean chlorophyll *a* concentration recorded during the sampling period suggested that the Bull Island site was only one third more productive than Blackrock per unit area. This relatively slight difference in the unit area productivity estimates for the two sites may have resulted from the application of the same chlorophyll *a*: primary production conversion relationship to both sites. Varying sediment properties may cause this relationship to vary significantly among sites, and determination and application of site-specific data is desirable before further conclusions are drawn. When comparing the production values to those recorded for similar habitats in other geographic locations, it at first appears that the Bull Island site may be less productive than could be generally expected for intertidal mudflats. On a global scale the majority of primary production estimates for intertidal mudflats were within the range 50 to 200g C m^{-2} y^{-1} (Colijn & de Jonge, 1984). It seems probable that Bull Island was far more productive than was suggested by surface sediment chlorophyll *a* values. Due to the hydrological circulation patterns within the Bay the flooding tide delivers and deposits significant nutrients loads to the site (ERU, 1992). This is in part associated with effluent discharge from the nearby sewage plant, although recent improvements to treatment and discharge protocols have been made. Therefore, it is possible that subsequent strong ebbing tides may result

in a net transport of primary producers away from the lagoon and out into the larger catchment of Dublin Bay. If the surface sediment chlorophyll *a* values recorded at Blackrock were considered indicative of primary production at this site, then it was relatively more productive than would be expected for an open sandflat. Steele & Baird (1968) recorded a production rate between 4-9g C m^{-2} y^{-1} for a sandflat in Scotland. Again the seemingly high productivity at Blackrock may have been associated with the increased levels of anthropogenically-derived nutrients that occur in the Bay, although stable isotope analysis (Chapter 6) did not appear to indicate particularly high levels occurring at the site. Alternatively, the relatively high levels of primary production estimates may have been due to delivery and deposition of significant quantities of microphyto-organisms that were transported from other areas. Although the general circulation patterns are well described for Dublin Bay as a whole, site-specific models are not sufficiently developed to determine or quantify more localised nutrient or productivity flux within the Bay. Further studies should aim to develop such models, and therefore, determine the relative contribution each of the various habitats makes to total productivity.

Due to the complex nature of the marine environment, the total population ingestion estimates derived in the current study were acknowledged to be very broad approximations to those that probably occur in the populations studied. Although in some cases input data were lacking or overly simplistic, the overall results were viewed as a reasonable attempt to address the imbalance between highly constrained and artificial laboratory derived feeding rate estimates and those that occur for whole populations in the field. The application of physical and biological constraints that were based on field data to adjust laboratory feeding rate data to, in turn, estimate total population ingestion was a novel approach to a complex multi-factorial problem. This approach allowed identification of patterns not apparent from traditional statistical methods. It should be appreciated that the accuracy of the model outputs were only as sound as the input parameters used, and the potential for considerable refinement exists. The construction of the model highlighted the existence of knowledge gaps in relation to the species studied, the processes that shape their distribution and abundance and the habitats they

occupy, which in turn gives potential direction for future research. The main knowledge gaps identified were ingestion rates on natural sediment of the studied species especially in relation to size-specific feeding rates. The duration of feeding over a typical 24 hour period and subsequent assimilation efficiencies of different sized animals would also improve model output. Although basic in this early form, it is hoped that the adjustment to existing and additional model components with increasing quality and quantity of input data may progress to the development of a useful multi-factorial model for predicting primary producer-consumer dynamics. A more integrated ecosystem approach will ultimately provide the most reliable estimates of population rate parameters.

Appendix 7.1. Numbers of *Hydrobia* and mean shell height (mm) in each cohort at the Bull Island site (69,000m^2).

Date	Parameter	Cohort 1	Cohort 2	Cohort 3	Cohort 4
22-Jul-00	Number (n)	-	$2.2x10^7$	$1.6x10^8$	$1.5x10^7$
	Shell height(mm)	-	2.71	4.31	5.90
20-Aug-00	Number (n)	$1.0x10^8$	$1.7x10^8$	$4.6x10^8$	$2.1x10^7$
	Shell height(mm)	1.32	2.57	4.22	5.98
23-Sep-00	Number (n)	$7.1x10^8$	$1.1x10^9$	$4.9x10^8$	$7.0x10^7$
	Shell height(mm)	1.49	2.53	4.19	5.66
21-Oct-00	Number (n)	$4.4x10^8$	$4.1x10^8$	$7.3x10^8$	$3.9x10^7$
	Shell height(mm)	1.52	2.63	4.09	5.70
19-Nov-00	Number (n)	$4.4x10^7$	$1.8x10^8$	$3.4x10^8$	$3.6x10^7$
	Shell height(mm)	1.54	2.44	4.32	6.27
18-Jan-01	Number (n)	-	$1.8x10^8$	$3.3x10^8$	$3.4x10^7$
	Shell height(mm)	-	2.01	4.21	6.29
06-Mar-01	Number (n)	-	$3.0x10^8$	$2.7x10^8$	$5.3x10^7$
	Shell height(mm)	-	2.29	4.25	5.90
14-Apr-01	Number (n)	$2.1x10^8$	$2.6x10^8$	$3.6x10^8$	$2.6x10^7$
	Shell height(mm)	1.29	2.40	4.18	6.07
27-May-01	Number (n)	$6.5x10^8$	$5.4x10^8$	$1.0x10^8$	$6.0x10^7$
	Shell height(mm)	2.02	4.17	5.93	7.34
27-Jun-01	Number (n)	$1.6x10^8$	$1.9x10^8$	$4.9x10^7$	-
	Shell height(mm)	2.45	4.56	6.75	-
14-Jul-01	Number (n)	$5.4x10^7$	$2.0x10^8$	$1.0x10^9$	$3.9x10^7$
	Shell height(mm)	1.51	2.87	4.24	6.14
11-Aug-01	Number (n)	$1.3x10^8$	$9.7x10^7$	$2.3x10^8$	$3.6x10^7$
	Shell height(mm)	1.65	2.63	4.39	6.18

Appendix 7.2. Numbers of *Tellina* and mean shell height (mm) in each arbitrary size grouping at the Blackrock site (27,000m^2).

Date	Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
21-June-00	Number (n)	-	$3.9x10^6$	$2.8x10^7$	$1.7x10^7$	$4.9x10^5$
	Shell height(mm)	-	6	10	14	18
22-Jul-00	Number (n)	$8.4x10^5$	$5.0x10^6$	$2.2x10^7$	$1.3x10^7$	$1.3x10^6$
	Shell height(mm)	2	6	10	14	18
20-Aug-00	Number (n)	-	$3.3x10^6$	$1.5x10^7$	$9.9x10^6$	$1.5x10^6$
	Shell height(mm)	-	6	10	14	18
23-Sep-00	Number (n)	$1.3x10^6$	$1.1x10^7$	$1.6x10^7$	$1.1x10^7$	$2.1x10^6$
	Shell height(mm)	2	6	10	14	18
21-Oct-00	Number (n)	-	$4.3x10^6$	$1.6x10^7$	$1.2x10^7$	$6.6x10^5$
	Shell height(mm)	-	6	10	14	18
19-Nov-00	Number (n)	$8.4x10^5$	$4.2x10^6$	$1.0x10^7$	$4.2x10^6$	$1.7x10^6$
	Shell height(mm)	2	6	10	14	18
04-Jan-01	Number (n)	$1.6x10^6$	$9.9x10^6$	$9.2x10^6$	$9.2x10^6$	$3.0x10^6$
	Shell height(mm)	2	6	10	14	18
18-Feb-01	Number (n)	$6.2x10^5$	$7.1x10^6$	$1.1x10^7$	$1.1x10^7$	$6.2x10^5$
	Shell height(mm)	2	6	10	14	18
16-Mar-01	Number (n)	-	$2.1x10^6$	$1.5x10^7$	$7.7x10^6$	$2.1x10^6$
	Shell height(mm)	-	6	10	14	18
14-Apr-01	Number (n)	$1.2x10^6$	$1.0x10^7$	$7.4x10^6$	$9.2x10^6$	$2.5x10^6$
	Shell height(mm)	2	6	10	14	18
27-May-01	Number (n)	-	$1.7x10^7$	$1.5x10^7$	$1.6x10^7$	-
	Shell height(mm)	-	6	10	14	-
26-Jun-01	Number (n)	-	$4.4x10^6$	$1.5x10^7$	$1.6x10^7$	$1.5x10^6$
	Shell height(mm)	-	6	10	14	18

General Discussion

The aim of this study was to assess the impact of two primary consumer species on the distribution and biomass of primary producers in the respective intertidal habitats where they occur. Chlorophyll a and phaeopigment concentration were used as indicators of the primary production present. The results provide the first detailed account of the way in which the population dynamics of the primary consumer species, and the availability of food resources, vary both spatially and temporally at the study sites. Although it was not possible to determine all regulating factors, initial indications would suggest that a complex suite of interacting variables shape population size and distribution at any one time. As primary consumer species represent a vital pathway for the constant recycling of energy and nutrients to higher and lower trophic levels, they are often considered keystone species. Knowledge of the medium- to long-term changes in their population dynamics may therefore be considered a good indication of ecosystem change and health.

Although it is difficult to compartmentalise the various food sources occurring in marine sediments, the measurement of sediment chlorophyll *a* was taken to be a good indictor of relative primary productivity, and therefore a reliable index of food availability (for review MacIntyre *et al.*, 1996). Results suggested that with reasonable constraints on consumer feeding activity, food availability in isolation is unlikely to limit the population size and growth of *Hydrobia* at the Bull Island site for most if not all of the year. If the microphytobenthos represents the main dietary component for *Tellina*, as was suggested by isotope analysis, a similar situation could occur at the Blackrock site. The feeding strategy of *Tellina* under varying environmental conditions remains unresolved (Trevallion, 1971), and no such conclusions can be drawn until this is formally described. Although it was not possible to determine whether 'top-down' or 'bottom-up' regulation of population biomass of either the primary producers or consumers occurred at the study sites, results suggested that other regulatory factors, such as population size for *Hydrobia*, may determine the threshold carrying capacities and potential primary and secondary productivity at the sites (Blanchard *et al.*, 2000). The factors regulating population size are variable, and probably elicit differential effects both spatially and temporally

(Robinson & Tully, 2000b, c). Population regulation is particularly difficult to define when high spatial heterogeneity occurs (Hassell, 1987). The lack of a direct significant relationship between primary production and consumer abundance in this study could indicate that monitoring the population of these species in isolation would, not itself, be sufficient to determine the influence of ecosystem changes in the short- to medium-term. Further time-series datasets of the population parameters of *Hydrobia* and *Tellina* at the intertidal mud and sandflat sites in which they occur, and the way in which variation in the physical and biological environment shape their population sizes and distributions would be necessary before establishing them as reliable indicator species for Dublin Bay.

The variation in the spatial distribution of different sized individuals at both sites in the current study suggested that regulatory factors elicit different influences on various components of the consumer species populations. Laboratory and field data suggested that food availability influenced the distribution of *Hydrobia* at least to some extent. Similar to other studies (Haubois *et al.*, 2004), adults of the species were more abundant in areas with high chlorophyll *a* concentration at Bull Island, and also displayed a preference for food rich sediments in the laboratory. The increased availability of food higher up the shore would seem even more important in determining the distribution of adults when the higher physical stresses associated with inhabiting this zone are considered (Boaden & Seed, 1993). The differences in size-specific distribution patterns at both sites could, also, have been due to variation in the food sources utilised, species interactions or the distribution of resources other than food (Sandulli & Pinckney, 1999; Aljetlawi *et al.*, 2000; Rossi, 2002). Ontogenetic changes in diet of species have been recorded in deposit feeders previously with juveniles generally requiring a high quality diet of diatoms and other microphytobenthic species and adults utilising a less specific range of lower quality food sources (Henstchel, 1998; Kang *et al.*, 1999; Bouillon *et al.*, 2002). Population density and, presumably, interaction among species results in a reduction in effective feeding time (Blanchard *et al.*, 2000), and, in this study, growth. This may have resulted in the different size-specific distribution patterns observed in the wild if a differential effect was elicited on individuals of varying size. Further multi-factorial experiments such as those that were conducted in the present study would assist

in determining the size-specific parameters that regulate both population distribution and size.

Feeding experiments to determine ingestion rates were not successful for either species examined in the current study. The accuracy and applicability of any laboratory-based feeding experiments has been questioned (Jorgensen, 1996; Rueslink, 2000; Riisgard, 2001). Numerous methodological problems exist due to the requirement to constrain many key variables at perceived optimal levels for a particular species so that the effect of the single variable under examination can be determined. Laboratory experiments are generally conducted over short duration and the results obtained for a small number of individuals extrapolated for a population in the wild. As many species feed on food items of a particular size and quality, the appropriateness of the food culture used to determine feeding rate has often been questioned (Bayne *et al.*, 1989; Petersen, 2004; Riisgard, 2004). These problems are exacerbated when the exact feeding strategy of the species is not known (Lopez & Levinton, 1987). The confusion surrounding the exact feeding strategy of *Tellina* (Trevallion, 1971) hampered the determination of a feeding rate for this species, despite a number of methods used for detection of either filter or deposit feeding. The controversy surrounding the reliability and precision of laboratory feeding rate experiment in relation to those that occur in the field has at least stimulated significant debate as to the need for standardised, universally acceptable methodologies (Jorgensen, 1996; Bayne, 2004; Petersen, 2004; Riisgard, 2004). The feeding strategies of species are highly variable, and 'best possible' methodologies for one species may not be appropriate even for others that appear to have similar habits and habitats. The most suitable method for determining the feeding rate for an individual will, therefore, require a considerable amount of 'trial and error'. The estimates obtained may still not be representative of the feeding rate and duration achieved in the wild (Ruesink, 2000), and the constraints on models as applied in this study should be considered a useful step in adjusting laboratory derived data. Although the estimates of total population grazing were rudimentary due to the lack of robustness of input parameters, they were at least considered to be a more realistic indication of potential impact when compared with the simple extrapolation of laboratory data. They suggested that considerable quantities of

autotrophic biomass are transferred into secondary level production by the two consumer species. The shortcomings of the model require significant improvements in input parameters. Accurate size-specific grazing rate, knowledge of the food sources utilised by each species and assimilation efficiency in particular would refine model output. Site-specific primary production estimates would also prove extremely useful in defining the extent of subsequent grazing activity (Colijn & de Jonge, 1984; Serôdio & Catarino, 2000). Although both consumer species studied were the dominant primary consumers at the respective study sites, habitat-specific estimates of total primary producer-consumer flux should be developed to incorporate other species of meio- and macrofaunal grazers.

Pre- and post-settlement processes together shape population size-frequency and distribution (Gaines & Roughgarden, 1985; Gaines *et al.*, 1985; Roughgarden *et al.*, 1988; Robinson & Tully, 2000a, b, c). Variations in recruitment success are known to be particularly important in defining future adult population size for relatively sedentary and intertidal species (Connell, 1985). Although there was some indication of recruitment variability in the current study, information on reproductive development, planktonic dispersal and early benthic life history of the two species studied is lacking (Fish & Fish, 1974; Barnes, 1990; Cardoso *et al.*, 2002). Recruitment failure is common for many marine invertebrate species, although the causative factors are unclear (Dekker & Beukema, 1999). Density-independent regulation of future population size associated with the planktonic phase, such as unfavourable dispersal and poor larval supply, influence settlement and subsequent recruitment to the benthos (Rumrill, 1990; Roughgarden *et al.*, 1988). Once settlement occurs, the influence of density-dependent regulatory factors increases, often resulting in very high early benthic phase mortality and patchiness (Gosselin & Qian, 1997). This can be sufficiently strong to decouple the relationship between larval supply and subsequent population numbers. Further studies should aim to quantify inter-annual variability in recruitment success, and determine whether this has a significant effect on the populations studied. Studies incorporating abundance of *Hydrobia* and *Tellina* in the larval phase at the study sites, relating inter-annual changes in density to physical factors such as water temperature, and investigating subsequent cohort strength would help establish the extent of density-independent factors

prior to settlement. In actuality, population size and distribution is most likely shaped by a combination of density-dependent and independent factors acting on the population both before and after settlement. Further studies should attempt to elucidate the factors regulating the populations studied. This would include the quantification of the relative influence of variations in recruitment and post-settlement effects. The factors affecting population size could then be monitored along with population parameters to identify both natural and anthropogenically derived changes in the ecosystem. The effect on population density of extreme weather events such as storms or unusually cold weather were not examined in the current study and such investigations could explain some of the variation encountered. Monitoring the numbers of recruits of *Hydrobia* in during April and September each year to determine the medium- to long-term recruitment success would be a useful monitoring tool should major changes in the Dublin Bay ecosystem occur. Sampling on one occasion only per month would not be roecommended, however. *Tellina* is more sensitive to adverse environmental conditions than *Hydrobia* and as such would be a useful indicator of the impact of unexpected pollution events. Recoverability of *Tellina* to short-term unfavourable conditions is high, however, due to the time length for juveniles to mature (approximately 2 years) a population that was significantly diminished would likely need up to 5 years to be completely recovered.

Stable isotope analysis indicated that *Hydrobia* and *Tellina* utilised microphytobenthic and phytoplanktonic food sources. This was not surprising due to the regular deposition and re-suspension of these components with physical wave and tide action (Baillie & Welsh, 1980; Varela & Penas, 1985; Shaffer & Sullivan, 1988; de Jonge & van Beusekom, 1992; Brotas & Catarino, 1995; Conde *et al.*, 1999; Lucas *et al.*, 2000). As *Hydrobia* is a surface deposit feeder any phytoplankton contributing to its diet results from the ingestion of organisms deposited onto the mudflat surface. The feeding method of *Tellina* has still not been determined conclusively and the suggestion that it utilises both phytoplankton and phytobenthos from the isotopic ratios recorded in the current study cannot be without further investigations to determine feeding strategy. Direct observation of feeding behaviour is desirable to validate the isotope data. Stable isotope analysis is a useful tool in determining food sources of species such as deposit feeders,

which due to their habit of ingesting large quantities of material but only utilising some are difficult to define (Hummel, 1985; Herman *et al.*, 2000). The effect of natural seasonal (Machas *et al.*, 2003) and stochastic variations, and anthropogenic effects can also be examined using this technique. The impact that anthropogenic influences such as high nutrient loads from wastewater treatment plants have on the food web can be determined using isotope analysis (Owens, 1987; Riera *et al.*, 2000) and, when used with consideration of spatial scale, give an important insight into the area affected by such inputs. Despite only primary treated sewage being discharged into Dublin Bay at the time of this study no impact of this increased nutrient load on primary consumers was apparent. Microphytobenthos can exert an important influence on the nutrient balance and may significantly contribute to de-eutrophication, (Meyercordt & Meyer-Reil, 1999) which may have reduced any obvious effects at the study sites. Using the data presented in the current study as a baseline, further seasonal sampling and analysis could be useful for monitoring the impact (positive/negative) of the progressive improvement to sewage treatment and wastewater management that is ongoing in the Dublin Bay catchment area. In the current study financial constraints prevented more widespread use of the technique however. Further studies should aim to catalogue the isotopic signatures of all potential food sources within Dublin Bay. This data could then be used in mixing models to predict the flow of energy and nutrients within the surrounding system. The use of such techniques in determining food webs has been useful in recent years (Riera *et al.*, 1998; Kang *et al.*, 1999).

Primary production of the two intertidal sites examined in the current study was expected to be high due to the abundance of consumer species at the sites. High nutrient loads can occur during certain periods due to outflow from the wastewater treatment plant at Ringsend, which can often result in the presence of nuisance macroalgae at certain times of the year (Jeffrey *et al.*, 1995). Comparison of sediment chlorophyll *a* concentrations, which were used as an index of microphytobenthic biomass and probably incorporated some relative indication of phytoplankton productivity due to deposition, to previously published values from other similar habitats suggested that primary productivity was relatively low at the study sites (MacIntyre *et al.*, 1996; Underwood & Kromkamp,

1999). Although high values were recorded occasionally during the study period, values remained low relative to other geographic locations. A possible reason to account for the apparent deficit of chlorophyll in the Dublin Bay system may be the regular and comprehensive tidal flushing of the area (ERU, 1992). This could result in the exportation of biomass, nutrients and detritus to inshore areas outside of the bay. This may in turn suggest that the bay represents an important source area that increases near-shore productivity. At the present time, this is purely speculative, and a more detailed account of the localised hydrodynamics of the bay and surrounding area is warranted. This data could be used in conjunction with biological sampling data to predict energy and nutrient flow within the area.

The importance of considering spatial and temporal variability in environmental parameters over appropriate scales is essential for understanding of population and ecosystem dynamics (Hughes, 1999). Inappropriate sampling design may result in failure to identify significant patterns and variations in population dynamics. The high variability in the spatial distribution of the primary producers and consumers in the current study highlighted the dynamic and transient nature of marine intertidal environments. Patchiness in the rocky intertidal zone has been well documented (Connell, 1985; Boaden & Seed, 1993), and often considered to be far in excess of that occurring in apparently less dynamic soft substrates. The results of the current study have shown that despite the relatively uniform appearance of the habitats within the study sites, significant variation in population parameters can occur over relatively short durations and distances. Quantification of sediment chlorophyll *a* concentration or consumer species population size-frequency distribution and/or abundance in one particular area was not always representative of those in others. This was also true of temporal sampling, when the population parameters recorded during one period did not reflect those in others, both intra- and inter-annually. For example, settlement of the spring cohort of *Hydrobia* occurred significantly earlier in 2000 when compared to 2001. To have sampled at one period during the settlement season, as occurred in April 2000, may have missed the peak in settlement and recruitment, leading to errors in inter-annual comparisons of these variables. Robinson & Tully (2000a, c) stated that significant intra-

annual variability in the spatial and temporal distribution of settlement and recruitment rendered inter-annual comparisons invalid if sampling frequency was low, particularly when the very high and rapid mortality rate of early benthic phase individuals was considered. In these cases, population parameters recorded at periods when the population is more stable (for example during winter) may be just as useful for inter-annual comparison and identification of population change. In the current study these relatively stable population densities occurred for *Hydrobia* in winter (January and February) and also in the summer (June and July). *Tellina* did not show major fluctuations over the temporal study but stable population density would be most likey to occur over the winter period. As *Tellina* spawning occurs annually from March but mainly in May and June, and the planktonic larval phase duration is approximately 1 month, sampling during the summer months could contain newly settled recruits if sieve size was small enough.

Data on the dynamics of single dominant keystone species provide a basis for the development of models that can be applied to the management of entire ecosystems. The ecosystem approach to marine environmental monitoring, assessment and management has gained much momentum in recent years, but the successful application of such methods is still rarely achieved due to the complex nature of the environment. Single species models are useful 'building blocks' for the development of ecosystem models when they can be expanded to incorporate data relating to habitat and species interaction. The open and dynamic nature of most marine populations necessitates the collection of robust biological and physical data over relatively long periods to fully understand and appreciate the influence of the factors regulating population size and distribution. The results presented in this thesis represent a starting point for the establishment and rational development of medium- to long-term monitoring of the target populations at the two study sites. Although significant knowledge gaps still exist, the results highlight where additional research efforts may have the greatest potential benefit in developing marine biological monitoring programmes for Dublin Bay.

Specific knowledge gaps that would benefit from further study are site-specific primary production rates to determine if the Dublin Bay ecosytem is more productive than appeared from the current study. Size and species specific ingestion rates determined on natural sediment, the duration of feeding over a typical 24 hour period and subsequent assimilation efficiencies would allow more accurate energy flow determinations. The change in feeding behaviour as individuals age would also be useful. Although density-dependent regulation of feeding has been investigated for *Hydrobia*, this is not the case for *Tellina*. Other features worth investigation focus on the feeding strategy of *Tellina* and determining if suspension or deposit feeding is the customary feeding mode and the triggers that determine the switch from one mode to another. The use of stable isotope analysis techniques could aid in the determination of the extent of feeding on phytoplankton versus microphytoplankton. Experiments using this technique could be used to answer many of the knowledge gaps highlighted in this study.

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