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MARL LAKE (HABITAT 3140) SURVEY AND ASSESSMENT METHODS MANUAL

Cilian Roden, Paul Murphy, James Ryan & Philip Doddy

An Roinn Tithíochta,
Rialtais Áitiúil agus Oidhreachta
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Cilian Roden, Paul Murphy[1], James Ryan & Philip Doddy[2]

[1]Eireco, [2]Woodrow Sustainable Solutions

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Executive Summary

This *Irish Wildlife Manuals* volume describes methods for field survey and conservation condition assessment of the Habitats Directive Annex I habitat 'Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.' (habitat code 3140) in Irish marl lakes. The vegetation of marl lakes is unusual in that it is dominated by cyanobacterial (cyanophyte/microbial) crusts in the shallows (down to a maximum of two metres depth), followed by three or more charophyte zones to depths of eight to twelve metres. Marl lakes in *Good* conservation condition have very low values for both total phosphorus and water colour. Two field survey methods are recommended: snorkel or scuba survey of charophytes and other macrophytes; and shoreline survey of the cyanobacterial crust. Macrophyte samples are 2 m x 2 m relevés, along transects from the shoreline to below the limits of vegetation growth. Cyanobacterial crust is sampled in 30 cm–50 cm water depth at shoreline stations with limestone substrate (bedrock or boulders). Eleven parameters, or indicators, based on these macrophyte and cyanobacterial crust data, are used to assess the conservation condition of a marl lake. The key indicators are

- The number of vegetation zones, and the presence of deeper water charophyte zones

- The euphotic depth (maximum depth of vegetation colonisation) and

- The percentage cover of cyanobacterial crust.

Further work is recommended to develop indicators appropriate to coastal charophyte-dominated lakes and to recognise degraded marl lakes where vascular plants have replaced the characteristic alga-dominated vegetation.

Marl lakes have a rich and distinctive flora dominated by charophyte and other algae and Irish marl lakes are home to charophyte species that are rare or absent from neighbouring countries. Shallow, well-mixed marl lakes are more common and larger in Ireland than most other parts of Europe, thus Ireland has significant responsibility for the protection of this habitat. However, there has been a steady erosion of habitat quality in Ireland with reductions in area and depth of colonisation of submerged vegetation (euphotic depth), loss of populations of charophyte species, increasing water colour, chlorophyll and nutrient levels, and introductions of invasive species.

Acknowledgements

1   Introduction

1.1   Purpose of this *Irish Wildlife Manuals*

This *Irish Wildlife Manuals* volume details field survey methods for monitoring the ecological or conservation condition of marl lakes based on underwater examination of lake vegetation, with careful sampling of charophyte vegetation (Chapter 2) and analysis of the cyanobacterial crust (Chapter 3). It also proposes methods for assessing conservation condition as *Good*, *Poor* or *Bad* based on these vegetation data and using on a range of defined parameters and targets (Chapter 4). These methods are used by NPWS to inform reports to the EU, under Article 17 of the Habitats Directive, on the national conservation status of the habitat (see NPWS, 2013a, b, 2019a, b), as well as in preparing site-specific conservation objectives for the habitat in Special Areas of Conservation (SAC) (see, for example, NPWS, 2019c). The methods also have application in survey conducted for ecological assessments, particularly Appropriate Assessment under Article 6(3) of the Habitats Directive, and for identifying and planning necessary conservation measures.

Two separate *Irish Wildlife Manuals* detail the application of the methods between 2011 and 2018 to Irish marl lakes, both within and outwith SACs (Roden & Murphy, 2013; Roden *et al*., 2020). Roden *et al*. (2020) provide further information on the distribution, natural variation and condition of marl lakes in Ireland (see also NPWS, 2019b).

1.2   Background

Hard-water, or marl, lakes (EU Habitats Directive code 3140: Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.) are characteristic of the Carboniferous limestone of the Irish midlands, but also occur along the coast and on other basic rock types. Research over many decades has shown that they have a rich and distinctive flora and vegetation dominated by charophyte algae (King & Caffrey, 1998; Reynolds, 1998). More recently, research has demonstrated that much of their shallow-water substratum is covered by characteristic cyanobacterial (cyanophyte) crusts (Doddy, 2019; Doddy *et al*., 2019a, b; Kennedy *et al*., 2012). Because they occur in lowland limestone areas, marl lakes are often adjacent to intensively farmed land and large towns and are consequently at risk from eutrophication due to agricultural run-off and sewage (Pentecost, 2009).

Marl lakes, especially shallow, well-mixed lakes, are uncommon in a European context (Wiik *et al*., 2014). These lakes are more common and larger in Ireland than in some neighbouring European countries, and this abundance of marl lakes is reflected in the presence of species ranging from charophytes (*Chara tomentosa*), White-clawed Crayfish *Austropotamobius pallipes*, insects (the aquatic coleopteran species *Ochthebius nilssoni*) (Nelson *et al*., 2019; O'Callaghan *et al*., 2009) and other organisms, which are rare or absent from neighbouring countries. They are also the location of some of the country's finest Brown Trout *Salmo trutta* fisheries (*e.g*. Lough Mask and Lough Corrib) (Reynolds, 1998). Thus, Ireland has a European responsibility to protect this habitat. However, there has been a steady erosion of habitat quality, including increasing chlorophyll levels, loss of populations of species such as charophytes and Arctic Char *Salvelinus alpinus* and a possible decrease in water clarity with the consequence that the depth of the euphotic zone or the area covered by benthic vegetation is decreasing (NPWS, 2008, 2013a, b, 2019a, b; Stewart & Church, 1992). In addition, introduced species, (including Curly Waterweed *Lagarosiphon major*, and Zebra Mussel *Dreissena polymorpha*) are severely damaging certain lakes such as Lough Corrib.

1.3   Defining and identifying marl lakes

Most naturalists in Ireland will conflate the terms charophyte lake and marl lake, and the Habitats Directive habitat Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. (3140) is defined largely in terms of *Chara* vegetation, clear water and high pH. In general terms, a marl lake is a lake in which $CaCO_3$ consists of more than 50% of the sediment (see Pentecost, 2009); however it does not follow that such lakes will always have a charophyte and cyanobacterial crust vegetation.

Free *et al.* (2005), amongst others, noted the importance of alkalinity in defining different types of macrophyte vegetation in lakes, and suggested a lower alkalinity boundary of 100 mg/l for Irish marl lakes. The alkalinity of charophyte-dominated lakes examined by C. Roden and P. Murphy since 2011 has ranged from 92–230 mg/l, (median 159 mg/l) (Roden *et al.*, 2020). Extensive charophyte beds however are found in lakes with alkalinity in the range 40–100 mg/l; for example, extensive beds of *Chara curta* are found in Kindrum Lough and Port Lough in Co. Donegal with alkalinities of 73 mg/l and 35 mg/l respectively. These intermediate alkalinity lakes differ from typical marl lakes in many other aspects of their vegetation such as the presence of *Isoetes* species (Roden *et al.*, in prep.), and are classified as a separate Habitats Directive Annex I habitat (EU Habitats Directive code 3130: Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or *Isoeto-Nanojuncetea*) (see NPWS, 2019b; O Connor, 2015). Provisionally, we suggest the lower alkalinity boundary for Irish marl lakes lies between 80 mg/l and100 mg/l, but stress that further analysis of lake vegetation is necessary to firmly establish this boundary. The majority of marl lakes have much higher alkalinity values, exceeding 200 mg/l in some cases.

The vegetation of Irish marl lakes is unusual in that much of the phytobenthos, down to a maximum depth of about two metres, consists of microbial crusts (Figure 1), followed by three to four separate charophyte species zones to a depth of eight to 12 m (Figures 2 to 4). Typically angiosperms have low abundance or are even absent. These characteristics make many standard methods of lake assessment based on macrophyte vegetation inappropriate for monitoring Irish marl lakes.

Figure 1   Cyanobacterial or cyanophyte crust zone (also referred to as microbial crust and termed 'krustenstein' by Roden & Murphy (2013)). All photographs, unless otherwise stated, Cilian Roden.

While Irish marl lakes have been the subject of much research, accurate maps of sub-littoral vegetation based on snorkelling/scuba observation are not common (King & Caffrey, 1998). Heuff and Ryan did study a wide variety of lakes by snorkelling (Heuff, 1984), while John *et al*. (1982) examined a number of hard-water Co. Westmeath lakes by a combination of methods (grapnel-sampling, glass-bottomed bucket and diving). King and others (King & Champ, 2000; Krause & King, 1994) studied sub-littoral vegetation of Lough Corrib and Lough Carra using grapnel samples. Roden (1999, 2000, 2001) and Bruinsma *et al*. (2009) used snorkel/scuba to map vegetation in marl lakes both on Carboniferous limestone and calcareous machair (Roden & Murphy, 2013; Roden *et al*., 2020.).

Figure 2   *Chara curta* zone.

Figure 3   *Chara rudis* zone.

Figure 4 *Chara virgata* zone.

Marl lakes are threatened by nutrient-pollution (Roden & Murphy, 2013) and it is recognised that they need to be assigned specifically-tailored ecological standards, due to their particular vulnerability, and that appropriate environmental quality standards (EQSs) should be developed for them (Free *et al*., 2016).

Under the Habitats Directive, the conservation status or condition of a habitat is largely defined by the structure and function of its biological communities and typical species. In this volume, 'conservation condition' or 'condition', is used for individual lakes in preference to 'conservation status' or 'status'. There is little difficulty in recognising marl lakes in *Good* conservation condition, such as those in the Burren. These have the well-defined, shallow cyanobacterial crust zone and three-to-four charophyte zones detailed above. A more difficult problem is recognising degraded marl lakes, *i.e.* lakes which once may have supported charophyte vegetation but no longer do so. For example, Summerhill Lough in Co. Fermanagh and Co. Monaghan is a designated SAC for the marl lake habitat (3140), yet when examined in 2012, it contained a vegetation of *Nuphar lutea* and *Elodea canadensis* (Roden *et al*., 2020). Brigid's Lough in east Co. Clare, lying on limestone bedrock and with an alkalinity of 212 mEq/L, contains a similar vegetation. Wiik *et al*. (2015) using palaeolimnological techniques showed that some lakes in England used to support *Chara* vegetation, but this is now absent from the sites. This issue is important for determining the natural habitat type of lakes with no previous botanical data and in the context of setting objectives to restore damaged lakes in SACs. Historical data on species composition and/or palaeolimnological evidence may be required to underpin objectives to restore the marl lake habitat (3140) in lakes where cyanobacterial crusts and charophyte vegetation are significantly reduced or absent, *i.e.* lakes in *Bad* conservation condition. As it is apparent that marl lakes have very low values both for total phosphorus and water colour, a restoration objective will require demanding and restrictive controls in the lake catchment.

2   Sampling charophytes and other macrophyte vegetation

This chapter explains how to prepare for and conduct field survey of macrophytes (charophytes, some other larger algae and vascular plants) in marl lakes using snorkel or scuba techniques, usually from boats. Macrophytes are sampled in 2 m x 2 m relevés along transects perpendicular to the shore. Relevés are sampled, by snorkelling or scuba, from the shoreline to below the limits of vegetation growth and their placement is determined by defined criteria. Each species within a relevé is recorded and its abundance estimated. The separate methods necessary to survey the cyanobacterial (cyanophyte) crust, are described in Chapter 3.

2.1   Planning field work

Before field survey can commence a map and, if possible, a bathymetric chart of the chosen lake must be obtained. Ordnance Survey of Ireland Discovery Series (1:50,000) Maps are ideal for planning access points, estimating lake area, selecting transect locations and noting inflowing and outflowing streams. The GEOHIVE.IE website (http://map.geohive.ie/mapviewer.html) is an excellent source of mapping and aerial photography. The Environmental Protection Agency (EPA) hold a substantial amount of lake bathymetry data that are available upon request.

2.1.1   Access

Ideally, marl lake macrophyte survey uses a boat (see Section 2.2.2), however not all marl lakes are easily accessible. The larger lakes are mostly in public ownership and public slipways can be used to launch boats. Smaller lakes may be accessible by private roads or tracks, but permission to use these should be sought. In cases where it is not possible to launch a boat, a light-weight Canadian canoe can be brought to the shore by portage (see Section 2.2.3). A minority of important marl lakes, such as Cooloorta/Travaun Lough in the Burren, Co. Clare can only be accessed on foot over rough ground. In these small lakes, boat cover must be dispensed with and the recorder must remain on the shore.

2.1.2   Selection of sampling transects

A minimum of two transects should be sampled in each lake, one along a sheltered shore (normally north- or east-facing) and one along an exposed shore (normally west- or south-facing). Where bathymetric charts are available, these should be used in selecting transect positions. Experience suggests that steeply sloping transects have poorer development of vegetation zones than more gently sloping transects, therefore transects which slope at an angle of 30° or less are preferable.

As euphotic depth, or the maximum depth of macrophyte colonisation, is an important metric, it is necessary that each sampling transect extend from the lake edge, perpendicular to the shore, to below the limits of vegetation growth.

Inflowing and outflowing streams should be avoided when selecting sampling transects. In the case of larger lakes, each basin should be treated as a subunit and at least one transect taken in each basin. While irregular shorelines may create almost-isolated bays, these are of lower importance unless they are deep enough to exceed the euphotic depth. In that case, they should also be sampled. As euphotic depth seems not to vary within a single basin, it is not necessary to sample more than two to four transects per basin. However, if the purpose of the survey is to map vegetation, more transects will be required.

An experienced team can typically survey three to four transects per day. This figure can be used to estimate the number of days required to survey a lake.

2.1.3   Field data-sheets

A printed field data-sheet or recording-form should always be used (see sample provided in Figure 5). A separate data-sheet is used for each transect. A sufficient number of data-sheets should be brought on each survey to allow for additional transects. Data-sheets should ideally be printed on card or waterproof paper, rather than standard paper, as water damage is a frequent problem.

Figure 5 Field data-sheet with data entered for a transect on Annaghmore Lough in Co. Roscommon. Note original field data in pencil; followed by annotations in black ink added later by the diver/sampler. Sampling date was 31/08/2012 not 1/09/2012. This error was later corrected during data entry.

2.2 Equipment

2.2.1 Vehicle

A suitable, road-worthy and properly maintained vehicle is required, one capable of towing boat and trailer and launch and recovery on gravel or sandy shores (Figure 6). A four-wheel drive vehicle, while not essential, allows for a greater range of launch and recovery sites to be used.

2.2.2 Boat

A shallow draft boat or RIB with optimal length 4 m or 4.5 m provides a safe working platform allowing sufficient work space for crew and equipment (see Figure 6). The boat should be capable of launch and recovery by a two person crew and should have sufficient stability and appropriate design to allow for a snorkeller/diver to re-enter the boat in open water. The engine should be four stroke to avoid oily discharge to water in exhaust. The trailer for transport of boat should have a hand winch for recovery.

Figure 6   Appropriate boat, vehicle and boat-trailer for marl lake survey.

2.2.3 Canoe

A Canadian canoe (see Figure 7) allows for access to shallow lakes or where launch of a larger boat is impossible, but can only be safely operated in conditions of Beaufort Force 3 or less.

2.2.4 Boat equipment

A full set of safety equipment should be taken on every voyage no matter how short. This includes

1. Oars

2. Hand held VHF radio

3. Flares

4. Spare fuel

5. Anchor (which should be ready/accessible at all times in case of emergency)

6. Tools for minor repairs

7. First-aid kit in a waterproof container

A *Diver down flag* should be flown when the snorkeller is in the water. A nominated on-shore person must be informed of planned activities, sampling and launch sites and estimated time of return to port.

Figure 7   Canadian-style canoe for use in lakes with no suitable launching site for larger boats.

2.3.5   PPE Equipment

Each crew member should have the following personal protective equipment

1.   Life jacket

2.   Sufficient warm and waterproof clothing

3.   Warm hat

4.   Sun cream

5.   Sunglasses

6.   Drinks

7.   Food

8.   Mobile phone and numbers of emergency services

2.2.6   General survey equipment:

The following equipment is required for each survey

1.   Hand-held Global Positioning System (GPS) and spare batteries

2.   O.S. maps of appropriate scale, bathymetric maps (if available) and aerial photographs of lake. These should be laminated or in a waterproof case. Alternatively, a tablet capable of displaying digital maps and photographs can be used in a waterproof case

3.   Hard-copy field data-sheets in a weather-writer (or similar) with clutch pencil (and spares)

4. Plastic bags for plant samples (zip-lock preferable) and indelible markers (including back-ups/ spares) for labelling

5. Secchi disc

6. Hand lens (x10).

2.2.7   Snorkelling gear

The following snorkelling equipment is required

1. Wet suit (suitable thickness to allow for sustained periods of immersion without getting cold in water temperatures which may be in low double figures)

2. A diving hood and booties of similar, suitable thickness

3. A weight belt with sufficient weight to enable the snorkeller to remain submerged in shallow depths (1–2 m)

4. Fins, mask and snorkel

5. Diver's depth gauge accurate to 1 m

6. Underwater camera with hand-loop attachment

7. Mesh sample collection bag on hand loop attachment.
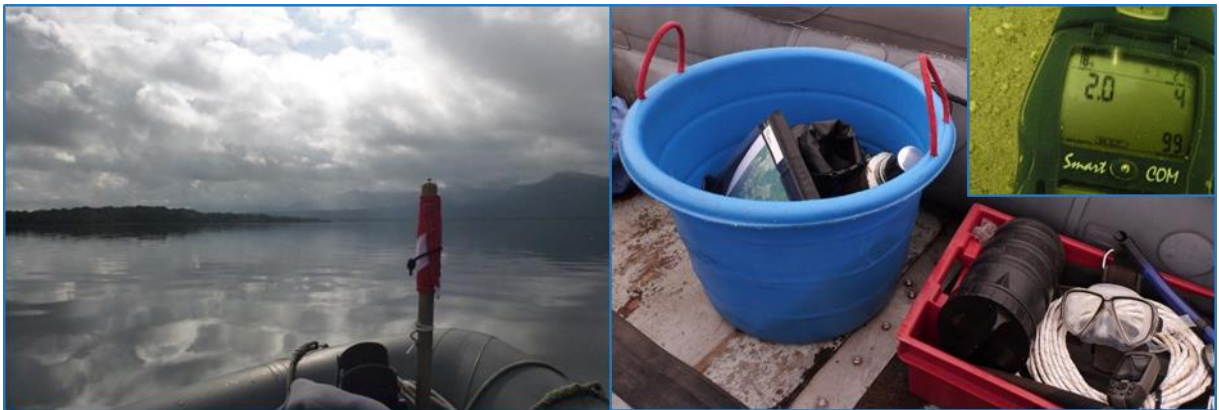
Figure 8   Marl lake survey equipment. Note (right-hand photograph) that equipment on board is stored in large, water-proof containers. The following are visible: weather proofed document holder, GPS receiver, Secchi disc (rope only), face mask and water-proof container (for keys, mobile phones *etc.*), depth gauge (top right).

2.3   Biosecurity

2.3.1   Biosecurity risks

Before sampling commences all aspects of biosecurity must be considered and a plan for managing biosecurity risk devised and executed as described below. The biosecurity risks that must be considered include the following

• The risk of transfer of Crayfish Plague and other diseases, given that marl can lakes have populations of the protected White-clawed Crayfish, *Austropotamobius pallipes*

• The risk of accidental transfer of other aquatic invasive alien species (including, *inter alia*, *Dreissena polymorpha*, *Hemimysis anomala*, *Elodea nuttallii*, *Elodea canadensis*, *Lemna minuta*, *Hottonia palustris* and *Angullicola crassus*)

- The risk of accidental transfer of terrestrial invasive plant species including *Fallopia japonica*, *Heracleum mantegazzianum* and *Impatiens glandulifera*

The biosecurity measures must cover all equipment being used on any particular survey including

- Scuba equipment such as tanks, regulators, buoyancy control devices (BCDs), harness, safety lines and any other equipment used

- Any vessel used for survey including engine, trailer, ratchet straps, *etc*.

2.3.2   Biosecurity protocol

The following operational protocol is recommended

Pre-survey

Prior to going on-site, all equipment is subject to disinfection using prescribed measures. Guidelines on biosecurity, including a protocol for field survey (IFI, 2010) and disinfection guidelines for boats, boating equipment, scuba diving equipment and paddle sports are available from https://www.fisheriesireland.ie/Biosecurity/biosecurity.html. See also below.

Post-survey

Step 1 (Drying and Cleaning)

All field equipment will be visually inspected before leaving the site and all visible material including mussels or other invasive species removed and left at the source water body. Particular attention will be given to places where organisms or plant fragments could be accidentally trapped, such as the treads of boots and waders, corners of weights, hinges of benthic grabs, the cooling systems of outboard engines, *etc*.

Step 2 (Disinfection)

All field equipment will be cleaned by soaking, spraying, dipping in, or scrubbing with a suitable disinfectant solution. A 1% solution of Virkon ®Aquatic, with a contact time of at least 15 minutes, is recommended owing to the risk of encounter with crayfish plague.

For items of equipment which are unsuited to this method, the equipment will be washed with water (>60°C where possible) and allowed to dry completely before next use. However freezing (for at least 24 hours) of some items may also be carried out. Boats and trailers will be power-hosed prior to re-use in a different system.

2.3.3   Reporting on invasive alien species

In the event that an invasive alien species, evidence or indication of disease on target species or other biosecurity hazard is recorded during the survey, the details will be reported to

- The National Biodiversity Data Centre (via the online invasives recording form, the Biodiversity Data Capture phone app or by e-mail to invasives@biodiversityireland.ie; see http://www.bio diversityireland.ie/projects/invasive-species/submit-sightings/ for further information)

- IFI (via e-mail to info@fisheriesireland.ie, the IFI Invasive Species App or LoCall 1890-347424

- Relevant NPWS staff.

The information provided should include species sighted, dates, geo-location and photographs.

2.4   Field survey

This section describes the procedure for sampling lake macrophytes in marl lakes. Two survey techniques, snorkelling and scuba, are outlined, either of which may be used for marl lake survey. Each technique has advantages and problems, but in our opinion, the balance of advantage lies with snorkel survey, owing to reasons of cost, logistics and convenience, and we have used the method with success in surveying many Irish lakes (see Roden *et al.*, in prep).

2.4.1   Snorkel survey

A minimum of two personnel is required for snorkel survey. Person One (the recorder and Coxswain/boat handler) is responsible for boat handling, recording all data, operating the GPS receiver and navigation. Person Two (the sampler or diver) is a snorkeller and is responsible for data collection, photography and sample gathering (see Figure 9). Depending on available skills, these roles can be reversed during a survey.

The main disadvantage of snorkelling is the short period of time available for observation on a single breath-hold. In most Irish marl lakes however, only a small number of species grow below 6 m, so prolonged scrutiny is not necessary for accurate recording of vegetation. In addition, underwater photography allows permanent records of bottom vegetation to be made and re-examined later. For shallow lake survey the advantages of snorkelling are many. Provided the recorder/boat handler is also a trained snorkel-diver, it is not necessary to have two divers in the water at the same time for safety reasons. The snorkeller's equipment is minimal and easily transportable, even on foot, and compressor access is not necessary.

Figure 9   Snorkel survey of marl lake macrophytes. Note charophyte sward with stems of *Phragmites australis*.

2.4.2   Scuba survey

While scuba-diving is generally not necessary to assess marl lake vegetation, it may be used in situations where the euphotic depth is exceptionally great. In the event that scuba equipment is required, a three

person team is essential. Either two divers operate in-water together (see Figure 10), or a second operative must be in a position to act as standby diver ready to enter the water to assist the primary diver. The Coxswain/recorder, in this instance, will operate as dive supervisor. All members of the dive-team should hold suitable diving qualifications, and have sufficient experience to undertake their respective roles.

Figure 10 Scuba survey of marl lake macrophytes.

The fundamental advantage of scuba is the diver can remain below surface for long periods of time and, consequently, make very detailed observations. In deep water survey, scuba-diving is essential, however, in lakes with a maximum euphotic depth of 12 m the advantage of remaining submerged for long periods has to be weighed against the many inconveniences of scuba. These include the additional equipment requirements for scuba, such as access to a compressor. As a typical survey day can involve up to six hours in the water, several air cylinders may be necessary and would require refilling for the next day's survey. This can prove problematic when surveying multiple lakes in remote locations. For health and safety, a minimum dive team of two suitably qualified and experienced divers is required for all survey work. This increases considerably the amount of equipment that must be carried to more remote lakes, which may not accessible by road.

2.4.3   Position location

The exact position of the initial shallow-water relevé is determined by GPS. As GPS does not function underwater, for subsequent relevés it is necessary for the diver to surface and indicate position to the recorder. Obviously this method introduces some error in position determination. If a weighted buoyed rope was used and the diver took up any slack on the rope, more accurate positioning would be possible. However, such a method would be time-consuming and it is not clear that the more accurate horizontal-positioning would yield more useful data for the purposes of macrophyte assessment. Vertical data from the depth gauge are of greater ecological value than horizontal position. Provided accurate depth data are collected, the estimated horizontal positions are acceptable.

2.4.4   Sampling and recording data

The position of a transect at the lakeshore is determined using GPS. The eastings and northings should be recorded on the field data-sheet immediately, as well as the numbered waypoint, so that the data can be checked later. This lakeshore location then represents the first relevé of the transect. Each relevé should describe a homogenous area with a depth difference less than 20 cm. Areas of temporarily flooded marginal vegetation should be excluded. Typical marginal species that should be excluded include *Ranunculus flammula*, *Mentha aquatica*, *Carex* spp. and *Schoenus nigricans*, however emergents such as *Phragmites australis* or *Schoenoplectus lacustris* are included within the survey transect. The sampler examines each relevé, a 2 m x 2 m area, for all macroscopic plant species and for cyanobacterial crust. Each species observed is written down by the recorder. Estimates of each species' cover abundance are made using a modified Braun Blanquet scale (see Table 1) and values recorded. Cyanobacterial crust is treated as a single taxon in relevés.

Table 1   Modified Braun-Blanquet cover scale

Braun-Blanquent scale	Braun-Blanquent % cover	Modified marl lake scale	Modified marl lake % cover
+	<5%; few individuals	0.1	<1%
1	<5%; numerous individuals	1	1–5%
2	5–25%	2	5–25%
3	25–50%	3	25–50%
4	50–75%	4	50–75%
5	75–100%	5	>75%

Samples of all problematic species are collected and placed in labelled plastic bags by the recorder. Labels must include date, transect and relevé numbers, GPS waypoint and depth. It should be noted that a licence is required from the Minister for Housing, Local Government and Heritage to collect specimens of plant species protected under the Wildlife Acts' Flora (Protection) Order (S.I. No. 356 of 2015, at time of writing[1]). These include various vascular plants, mosses, liverworts, lichens and stoneworts. Further information on legislation and licensing is available from www.npws.ie.

Substrate type is noted and total vegetation cover recorded as a percentage of total relevé area. Sample depth is recorded, by visual estimate in water less than one metre depth (1 m) and by depth gauge thereafter. A photo or photos are taken and photograph serial number(s) recorded. If possible, the diver should briefly examine surrounding vegetation at the same depth to ensure that the relevé taken is typical or representative of the location.

The diver then swims away from the shore and selects the next position for sampling. Relevé position selection is based on the first to occur of one of three possible events

1.   A change in benthic vegetation

2.   A depth increase of 1 m, or

3.   A horizontal distance of 20 m.

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[1] It is an offence to cut, pick, uproot or otherwise take, injure, damage, or destroy, purchase, sell or be in possession of any plant, whether whole or part, of a species mentioned in the Order, or wilfully to alter, damage, destroy or interfere with the habitat of such a species.

The diver measures relevé depth using the depth gauge and then surfaces, keeping position vertically above the sample area, until the recorder can log the position using GPS. The procedure outlined above is then repeated resulting in a list of species present, their cover values, total vegetation cover, substrate type, exact position and depth, as well as photograph numbers, if taken.

The euphotic depth is determined as the depth at which photosynthetic macrophytes are no longer present. The diver should attempt to cross the vegetation—no vegetation boundary at several points to establish if this depth varies greatly. In most cases, the boundary tends to be a sharp transition at a constant depth and is easily recorded. As with relevés, the diver should ascend vertically and hold position until the recorder notes his or her position.

Sufficient relevés are recorded to ensure that all vegetation types have been sampled. An approximate guide is that the number of relevés sampled should equal the euphotic depth in metres.

The field data-sheets are filled in by the Coxswain/recorder in the boat/canoe (see Figure 11), who shadows the diver's movement in the water. The recorder should also make sketch maps and notes describing any points of interest visible from the boat (such as floating plants, reedbeds, environmental disturbance on shore, inflows). For lakes that are not accessible by road, where boat cover cannot be provided for the diver, the recorder must stand on the shore and the diver call out transect data.

Figure 11 Recording marl lake macrophyte data in the field.

2.4.5 Automation

There is an obvious opportunity to record directly onto a tablet or laptop computer and amalgamate vegetation and positional data into a combined database in the field. Difficulties to be overcome include truly weatherproof equipment, as work in small boats in a wet climate always means equipment is exposed to water. It would also be necessary to modify data after taxonomic work in the laboratory and ensure adequate quality assurance.

2.4.6 Additional data

The additional data to be recorded at each lake includes

1. Observations on water level, including any incidents of exposure of cyanobacterial crust or charophyte zones

2. Underwater springs – an interesting feature of some marl lakes. These appear to be favoured by bryophytes and vascular plants, rather than charophytes and cyanobacterial crust. As they are little studied, they should be sampled where possible, but not used in lake assessment. Springs have been recorded in Lough Bane, Lough Carra and Lough Owel (Roden, 2010; Roden & Murphy, 2013; Roden *et al.*, 2020)

3. The position of any large banks of submerged vascular vegetation (*e.g. Myriophyllum verticillatum*) should be recorded using GPS, as these are indicative of eutrophication

4. Reedbeds – while these may be sampled as part of normal transects, the distribution and extent of reedbeds is often indicative of lake nutrient status. Mapping reedbed margins, particularly the lake-ward boundary, using GPS yields a useful baseline for repeat surveys in future years. Species, density, height or other measurements of reedbed structure, may also provide useful indicators of eutrophication

5. Any other signs of eutrophication, such as excessive growth of Blanket Weed (*Cladophora* sp.) or algal fouling on macrophytes should be noted

6. Under water clarity and the extent of visibility should be assessed by the diver on each transect. Visibility less than 1.5 m suggests high turbidity and possible environmental problems

7. Secchi disc depth on at least one sample per basin/lake. The depth recorded is the mean of the disc disappearance depth and disc reappearance depths. In most cases, Secchi disc depth does not vary significantly over distances of less than 1 km, but depth will vary over time depending on algal blooms and inflow of coloured water. Consequently, euphotic depth is a more useful measure of light penetration as it averages over time. Nevertheless, the ease of sampling and immediately available information on colour makes Secchi readings useful

8. If the lake is not monitored by the EPA/Local Authority, water samples should be taken and sent immediately to a certified laboratory for total phosphorus, chlorophyll *a* and water colour analyses

9. Surveyors should be alert for the presence of invasive species such as *Elodea nuttallii* or *Lagarosiphon major*. If possible, the population size of invasive species should be estimated. Where possible, additional transects should be snorkelled to determine the extent of the population

10. Any visible pressure and threats should be recorded. These may include: evidence of pollution from waste-water treatment plants or other point sources; agricultural nutrient sources, such as slurry-spreading or chemical fertiliser use; signs of land disturbance leading to excess sediment entering the lake; turf-cutting; afforestation or clear-felling, particularly on peat soils; and excessive abstraction (see also Section 4.5).

2.4.7   Photography

Underwater digital cameras, operating to 15 m depth are now more affordable and are an essential tool in lake survey, as they can provide a permanent visual record of plant communities at specific locations. The core problem with lake photography is low light levels and particle-rich water. The former requires either flash or wide aperture lens, but the latter results in backscatter if flash is used. Cameras with faster lens speed (lower f-stop) will produce better work, but even a minimum f-stop of 3.3 will yield worthwhile images. High ISO settings will also counter the effect of dim light. High ISO or low f-stop both allow faster shutter speed. This is essential when snorkelling, as remaining still underwater is an art that is learnt slowly.

Initial settings should be a minimum shutter speed of 1/100th of a second, and allow the camera exposure meter to set the f-stop. ISO of up to 800 will produce acceptable images on modern cameras, and even higher ISO settings are worth using in water close to the euphotic depth limit.

As noted above, photographs provide a permanent record of the not-easily accessed underwater environment. As digital images are virtually cost free, and given the challenge of taking well-focused images underwater, a large number should be taken. Unwanted images should be discarded later.

Most digital cameras also have a video function and a useful practice is to take a video recording of a transect from shallow water to the maximum euphotic depth. Such a record is a useful *aide-memoire*

when analysing results later. The commonest problem in making such videos, is swimming too fast to capture clear images.

Because there is a certain similarity in the vegetation of most marl lakes, and given the energy use associated with such storage, it is arguable whether every photo taken needs to be retained. Those that are retained should be filed in a database with location details and a description of species present.

2.4.8 Follow up on shore

All recording cards should be photographed, either during sampling or immediately after landing. Along with collected samples, they are the only record of the work and must be duplicated as soon as possible. GPS data should be uploaded to a laptop or other computer, equally promptly, along with all photos.

2.5 Storage of field data

Within three days of sampling, the following data-sets should be available

1. Recording cards (one per transect), showing lake name, surveyor names, sampling date, euphotic depth, Secchi depth, number of relevés sampled, corrected species list with cover estimates, total cover, substrate, depth and serial numbers of photos taken

2. Uploaded spreadsheet with GPS data including date, time, northing, easting and waypoint number

3. Uploaded digital photos

4. Plant specimens pressed or preserved.

Where possible these data should be duplicated and stored in separate locations.

2.6 Identification of plant specimens

Marl lake vegetation is dominated by cyanobacterial crust and charophytes. It is not practical to identify species in the crust on a routine basis and its quality is assessed in other ways (see Chapter 3), but it is essential that charophytes and other macrophytes are identified to species level. The majority of species, including charophytes, can be identified in the field and surveyors must have this competence to be effective. Some taxa require examination under the microscope to confirm identification and samples of these must be collected in the field (see list below). While it is outside the scope of this manual to provide a guide to species identification, a list of relevant literature is provided in Chapter 7.

Plant identification can be postponed for up to three days, provided the plastic specimen bags are stored in cool dark conditions. All additional taxonomic data are noted on the original recording cards, specifying the relevé in which the specimen was gathered. Any undetermined or questionable material should be pressed, or for bryophytes dried in paper envelopes, labelled and submitted to a specialist for identification.

Charophytes can be pressed or preserved in alcohol. They have a deserved reputation for being fragile and brittle when pressed, and an alternative is to preserve in liquid-usually 70% ethanol. The latter is more convenient in the short-run, but requires much storage space. A more serious problem is that alcohol can evaporate unless sealed glass jars are used; standard plastic sample containers do not last in the long-term.

When pressing charophytes, it is useful to place a sheet of fine, non-absorbent gauze between specimen and drying paper to prevent the specimen gluing to the paper. Old plankton netting is ideal.

The following equipment is required in the laboratory

1. Stereoscopic microscope with x 20–60 magnification

2. Dissecting needles, scalpel, forceps, white trays

3. Herbarium paper

4. Plant press.

The taxa that require examination under the microscope to confirm identification include

- Deep-water charophytes; in the majority of cases, *Chara virgata* is the dominant form at depth, but the related *C. globularis* may occur. In addition, deep water forms of *C. contraria* or even *C. denudata* occur. Therefore several samples of deep water taxa should be collected and their identity confirmed in the laboratory

- *Chara rudis*. This taxon is universal in Irish marl lakes but the related *C. hispida* can occur rarely; any unusually spiny *C. rudis* should be collected and examined under the microscope

- Small or dwarf forms of shallow water charophytes are frequent, these are often *C. virgata* var. *annulata*, but occasional specimens should be collected to check if *C. aspera* or *C. contraria* is present

- Narrow leaved *Potamogeton* species should always be collected and checked to see if *P. berchtholdii*, *P. pusillus*, *P. friesii*, *P. filiformis* or *P. pectinatus* are present. Any unusual pondweeds (*Potamogeton*) could be hybrids and should be collected

- Aquatic mosses are uncommon in marl lakes with the exception of *Fontinalis antipyretica* which is relatively easy to recognise. All other species should be collected and identified

- Filamentous algae can be easily identified to order or even genus using a compound microscope. The genus *Cladophora* is worth noting, as it may indicate eutrophication if abundant.

2.7 Data management and analyses

This section provides suggested approaches, based on the experience of the authors. The data entry step can be postponed until after the field season, if necessary. Data in the recording cards should first be transferred to an excel spreadsheet. An example is shown in Figure 12. Column A contains an alphabetical list of all plant species likely to occur in Irish marl lakes (part of this list only is shown in Figure 12). Row one is the waypoint number of each relevé, row two is relevé depth, row three is relevé total cover as a percentage, row four is substrate, row five is transect euphotic depth, row six is transect number, row seven is lake name or an abbreviation of same.

Data from the original recording cards are now entered into the database, resulting in a matrix containing all recorded environmental and vegetation data. Data from all lakes sampled should be included in this matrix, in order to allow subsequent data analysis.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	WP	508	509	510	511	512	513	514	515	516	517	518	519
2	depth	0.3	0.4	0.6	0.9	1.2	1.2	1.2	1.6	2.6	3.5	4.5	6.1
3	cover	40	15	80	80	80	80	100	100	100	100	100	100
4	substrate	marl stones	marl stones	gravel	marl on peat	soft marl	soft marl	soft marl	soft marl	soft marl	soft marl	soft marl	marl
5	euphotic depth	6	6	6	6	6	6	6	6	6	6	6	6
6	Transect	1	1	1	1	1	1	1	1	1	1	1	1
7	relevé	1	2	3	4	5	6	7	8	9	10	11	12
8	Secchi	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
9	Alisma plantago-aquatica												
10	Baldellia ranunculoides												
11	Bryophytes												
12	Chara virgata											5	5
13	Callitriche hermaphroditica												
14	Ceratophyllum demersum												
15	Chara annulata												
16	Chara aspera	0.1	0.1									2	2
17	Chara contraria												
18	Chara denudata												
19	Chara curta					1	4						
20	Chara globularis												
21	Chara hispida												
22	Chara polyacantha					4	2						
23	Chara rudis							4	4	5	5		
24	Chara tomentosa												
25	Chara vulgaris												
26	Cladium mariscus												
27	Cladophora sp.												
28	Eleocharis palustris												
29	Elodea canadensis					0.1			4		1		
30	Equisetum fluviatile												
31	Fontinalis antipyretica												
32	Hippuris vulgaris								4				
33	Juncus articulatus												
34	Juncus bulbosus												
35	Cyanophyte crust	3	2	5	5	5							
36	Lemna minor												
37	Lemna trisulcata												
38	Lichen												
39	Littorella uniflora												
40	Mentha aquatica												
41	Myriophyllum alterniflorum												
42	Myriophyllum spicatum								4				
43	Myriophyllum verticillatum												
44	Nitella flexilis												
45	Nitella tenuissima												
46	Nuphar lutea								1	1			

Figure 12 Field survey data transferred to excel spreadsheet. 'WP' is way point number, a number automatically assigned by GPS device. Relevé depth ('depth'), euphotic depth and Secchi depth are all given in metres. Cover abundance of individual species is in keeping with Table 1.

A second spreadsheet containing GPS or positional data is now opened (Figure 13). The first database is transposed so row one (waypoint number) is now column A. Both spreadsheets are sorted on ascending waypoint number and the data from the GPS sheet inserted into the vegetation sheet, making sure waypoint numbers match. Some additional waypoints such as Secchi disc positions, may need to be deleted from the combined spreadsheet, as there will not be a corresponding waypoint in the vegetation data.

The database now contains all survey data gathered during the field survey and is ready for analysis (Figure 14). Copies should be made and stored securely.

Such data are frequently interpreted using multivariate statistics both to distinguish separate vegetation units and identify relationships between species distribution and environmental factors. For the purpose of lake assessment this is not required. Each transect is analysed separately and, as explained in Chapter 4, environmental condition is based on the least favourable metric from all transects made in each lake.

	A	B	C	D	E	F	G	H
1	waypoint no.	Comment	Date Time	Zone	Easting	Northing	Altitude	Lake
2	508	31-AUG-12 4:41:39PM	08/31/2012 15:41:39.000	IM	89499	83787	51.3	Annaghmore
3	509	31-AUG-12 4:47:17PM	08/31/2012 15:47:17.000	IM	89504	83774	50.4	Annaghmore
4	510	31-AUG-12 4:50:13PM	08/31/2012 15:50:13.000	IM	89509	83757	51.3	Annaghmore
5	511	31-AUG-12 4:51:47PM	08/31/2012 15:51:47.000	IM	89511	83744	50.8	Annaghmore
6	512	31-AUG-12 4:59:35PM	08/31/2012 15:59:35.000	IM	89524	83695	52	Annaghmore
7	513	31-AUG-12 5:01:45PM	08/31/2012 16:01:45.000	IM	89531	83685	52.8	Annaghmore
8	514	31-AUG-12 5:03:53PM	08/31/2012 16:03:53.000	IM	89537	83679	51.6	Annaghmore
9	515	31-AUG-12 5:08:29PM	08/31/2012 16:08:29.000	IM	89543	83671	51.6	Annaghmore
10	516	31-AUG-12 5:10:32PM	08/31/2012 16:10:32.000	IM	89543	83666	52.3	Annaghmore
11	517	31-AUG-12 5:12:20PM	08/31/2012 16:12:20.000	IM	89545	83656	52.8	Annaghmore
12	518	31-AUG-12 5:13:59PM	08/31/2012 16:13:59.000	IM	89544	83652	53.2	Annaghmore
13	519	31-AUG-12 5:16:14PM	08/31/2012 16:16:14.000	IM	89559	83644	52.3	Annaghmore

Figure 13 A sample of the data downloaded from GPS device.

waypoint no.	Comment	Date Time	Zone	Easting	Northing	Altitude	Lake	relevé	WP	euphotic depth	Secchi	depth	substrate	cover	Alisma plantago-aquatica	Baldellia ranunculoides	Bryophytes	Chara virgata	Callitriche hermaphroditica	Ceratophyllum demersum	Chara annulata	Chara aspera	Chara contraria
508	31-AUG-12 4:41:39PM	08/31/2012 15:41:39.000	IM	89499	83787	51.3	Annaghmore	1	508	6	3.5	0.3	marl stones	40								0.1	
509	31-AUG-12 4:47:17PM	08/31/2012 15:47:17.000	IM	89504	83774	50.4	Annaghmore	2	509	6	3.5	0.4	marl stones	15								0.1	
510	31-AUG-12 4:50:13PM	08/31/2012 15:50:13.000	IM	89509	83757	51.3	Annaghmore	3	510	6	3.5	0.6	gravel	80									
511	31-AUG-12 4:51:47PM	08/31/2012 15:51:47.000	IM	89511	83744	50.8	Annaghmore	4	511	6	3.5	0.9	marl on peat	80									
512	31-AUG-12 4:59:35PM	08/31/2012 15:59:35.000	IM	89524	83695	52	Annaghmore	5	512	6	3.5	1.2	soft marl	80									
513	31-AUG-12 5:01:45PM	08/31/2012 16:01:45.000	IM	89531	83685	52.8	Annaghmore	6	513	6	3.5	1.2	soft marl	80									
514	31-AUG-12 5:03:53PM	08/31/2012 16:03:53.000	IM	89537	83679	51.6	Annaghmore	7	514	6	3.5	1.2	soft marl	100									
515	31-AUG-12 5:08:29PM	08/31/2012 16:08:29.000	IM	89543	83671	51.6	Annaghmore	8	515	6	3.5	1.6	soft marl	100									
516	31-AUG-12 5:10:32PM	08/31/2012 16:10:32.000	IM	89543	83666	52.3	Annaghmore	9	516	6	3.5	2.6	soft marl	100									
517	31-AUG-12 5:12:20PM	08/31/2012 16:12:20.000	IM	89545	83656	52.8	Annaghmore	10	517	6	3.5	3.5	soft marl	100									
518	31-AUG-12 5:13:59PM	08/31/2012 16:13:59.000	IM	89544	83652	53.2	Annaghmore	11	518	6	3.5	4.5	soft marl	100						5		2	
519	31-AUG-12 5:16:14PM	08/31/2012 16:16:14.000	IM	89559	83644	52.3	Annaghmore	12	519	6	3.5	6.1	marl	100						5		2	

Figure 14 A sample of the combined, final database.

Figure 15 Field discussions among snorkel surveyors.

3   Sampling and analysing the cyanobacterial crust

This chapter explains how to survey the cyanobacterial (cyanophyte) crust of marl lakes. The method involves estimating crust cover in the field, and taking samples for measurement of chlorophyll *a* concentration and enumeration of chlorophyte cells under microscope.

3.1   Site selection

Before sampling, a decision must be made on the number of sampling stations to be used in each lake and their locations selected. In multi-basin lakes, such as Lough Carra, one station per basin should be sampled to get a representative sample of the lake. In single-basin lakes, such as Lough Rea, a single station may well be sufficient, unless certain areas of the lake are known to be more nutrient-polluted than others. It is recommended that fixed stations be established for repeated sampling. The first round of samples can be used to determine baseline values for crust metrics at that station, to which future measurements can be compared.

All crust-sampling can be done without a boat, provided that access to the shore is available. Easily accessed stations are preferable, to allow sampling when necessary. However, areas in frequent use should be avoided, such as areas beside boat slips, because disturbance and abrasion can be expected to affect crust cover. Stations must have an available limestone substrate, suitable for crust growth. Therefore, areas with a soft sediment-covered shore, or dense reed-beds, are not suitable. Bedrock or submerged boulders are suitable as a substrate, but small stones and cobbles are not; this is because these small stones can be shifted by waves, preventing normal crust-growth. Areas beside a drainage outlet (such as a ditch or stream) are not suitable for general lake-monitoring, as they may not give results which are representative of the lake overall. However, crust metric results from such areas may well give a useful indication of nutrient-pollution in water entering the lake, so these areas may be considered for secondary sampling. In order to measure changes in lakes over time, it is recommended to establish fixed stations for repeated sampling, so that medium-term to long-term trends in crust characteristics can be recognised.

3.2   Fieldwork equipment

The following equipment is required for fieldwork

- Chest waders
- Life jacket
- Mobile phone and numbers of emergency services
- Aquascope (bathyscope)
- GPS device (for determining location)
- Graduated cane in metric/centimetres (for measuring depth)
- Random number tables
- Plastic sample containers with lids, at least 12 cm x 10 cm x 8 cm in size
- A stainless steel spatula, with blade at least 8 cm x 10 cm in area (for lifting crust samples)
- A knife (for cutting around the edge of crust samples)
- Prepared sampling sheets, a clipboard and pencils
- Disinfectant (*e.g.* Virkon ®Aquatic) for cleaning equipment between sites
- An opaque box or other container, with lid, for transporting sample containers.

3.3   Fieldwork 1: preparation

Sampling should be done during average or low water level, normally between spring and autumn, as water levels in many lakes rise in winter during which time crust zones may not be accessible. The period from May to September has been found to be most suitable in this regard. Significant seasonal changes in crust metrics are thought unlikely to occur during this period, based on current information. Nonetheless, where a particular station is being repeatedly sampled in successive years, it would be ideal, though not essential, to sample during the same month. All sampling and measures of crust cover are done in the 30 cm–50 cm depth zone. On gently-sloping lakeshores, this can consist of quite a broad band, whereas on steeper shores it will be a narrower area.

Equipment used in sampling (waders, aquascope *etc.*) should be disinfected before approaching the lake, and once sampling is complete, to prevent the spread of invasive species (see also Section 2.3). For safety reasons, two people are required for sampling: for convenience, they are called the 'sampler' and the 'assistant'. The assistant should remain on dry land at all times, and should have on his/her person a mobile phone and numbers of the emergency services in case assistance is required. The sampler should wear chest-waders and a life-jacket. The sampler must know at what depth (s)he is sampling; therefore, waders which have the appropriate depths (30 cm and 50 cm) marked on them can be useful, or a cane/staff marked with these measurements can be used. As lakeshore rocks and crusts can be very slippery, a hiking/walking-pole or cane is also useful for stability. Sample containers should be labelled in advance for convenience.

3.4   Fieldwork 2: estimating crust cover

Crust cover is calculated as the percentage of sample points where cyanobacterial crust is present along a length of shoreline. A 25 m length of shoreline should be measured out, and the halfway point recorded by GPS. All sampling and spot-checks should be done within this length of shore, in the 30 cm–50 cm depth zone. Crust cover is measured by carrying out 25 spot checks. The assistant calls out pairs of numbers, taken from random number tables, to the sampler. The sampler then takes a number of strides (corresponding to the first number) parallel to the shore, and then a number of strides (corresponding to the second number) perpendicular to the shore. If the upper or lower depth limit, or the end of the 25 m sampling area, is reached, the sampler simply turns around and continues in the opposite direction for the remaining number of strides. Having reached a particular point, the sampler then lowers the aquascope into the water, and checks for the presence of crust in the circle formed by the base of the aquascope. If any crust is present, this is a positive record. For each point, the sampler calls out the result (positive or negative) to the assistant, who notes it down on the sampling sheet. Aquascopes of the same basal diameter should be used in all situations where results will be compared; a base diameter of 30 cm, generally available, is recommended for standard use.

3.5   Fieldwork 3: crust sampling & transportation

Five samples of 10 cm x 8 cm area should be taken at each station. This is accomplished by cutting around a piece of crust of this size (or larger; precise measurements are not essential at this point) using a knife, then sliding a stainless steel spatula underneath the base of this piece of crust and transferring it to a sample container (Figure 16). In this manner, the full depth of crust is sampled, irrespective of its thickness. Crusts generally range from about 0.5 cm to about 5 cm in thickness, but may sometimes exceed this range.

In situations where much of the sampling area (*e.g.* >50%) is covered by crust, points for sample collection can also be randomised. Starting at one end of the 25 m sampling area, the sampler is again given random numbers from the assistant and proceeds to take that number of strides parallel to the shore, staying at a depth of 50 cm. If crust is present at the determined spot, a sample is taken there. If

not, the procedure is repeated until crust is found. Five samples are taken in this manner. If the sampler reaches the end of the 25 m sampling area, (s)he simply turns around and continues back in the opposite direction.

Figure 16 Cyanobacterial crust sampling.

However, in situations where crust is patchy and hard to find, this method is not practical and the sampler will need to seek out crust by sight (using an aquascope if necessary). Five samples should be taken, at 50 cm depth if possible or at least in the 30 cm–50 cm depth zone.

Collected samples should be packed in an opaque container (to exclude light) with a tight lid and kept in a cool, dark space in so far as possible (not in direct sunlight). They should be returned to the laboratory or storage facility on the same day. When sample containers are opened, any liquid should be drained off. At this point, a 1 cm x 8 cm (approximately) strip of crust should be cut off the main sample for microscopy, wrapped in aluminium foil, placed in a plastic box or bag, and frozen at -20°C until microscopy is to be carried out. The remainder of the sample, to be used for pigment analysis, should be placed in a suitable lidded storage container, and also frozen at -20°C until pigment extractions are to be done.

3.6 Microscopy

Samples should be removed from the freezer shortly before microscopy. It has been found, in some cases, that the preparation of slides is easier if done before the sample is fully de-frosted. This is because it is often easier to cut a partly-frozen sample accurately than one which is fully de-frosted. Three slides are prepared from each sample, using material from three different points of the 1 cm x 8 cm subsample. In each case, a 2 mm wide section of crust is cut and laid flat in a petri dish. The orange lines in Figure 17 show how the section should then be cut in order to obtain the material for making the slide. A blade is used to reduce the section to a width of 5 mm. The top 1 mm is then cut off to exclude plankton or debris which may have settled on the surface of the crust. A 1 mm slice (shaded) is then removed and placed on the glass slide; this will be a piece of crust of 5 mm x 2 mm x 1 mm in size. One drop of water is added, and the sample homogenised by grinding with a steel-tipped probe, on the centre of the slide,

for one minute. A 22 mm x 22 mm cover slip is then placed on top and gently pressed until the homogenised material spreads out to the edges of the cover slip.
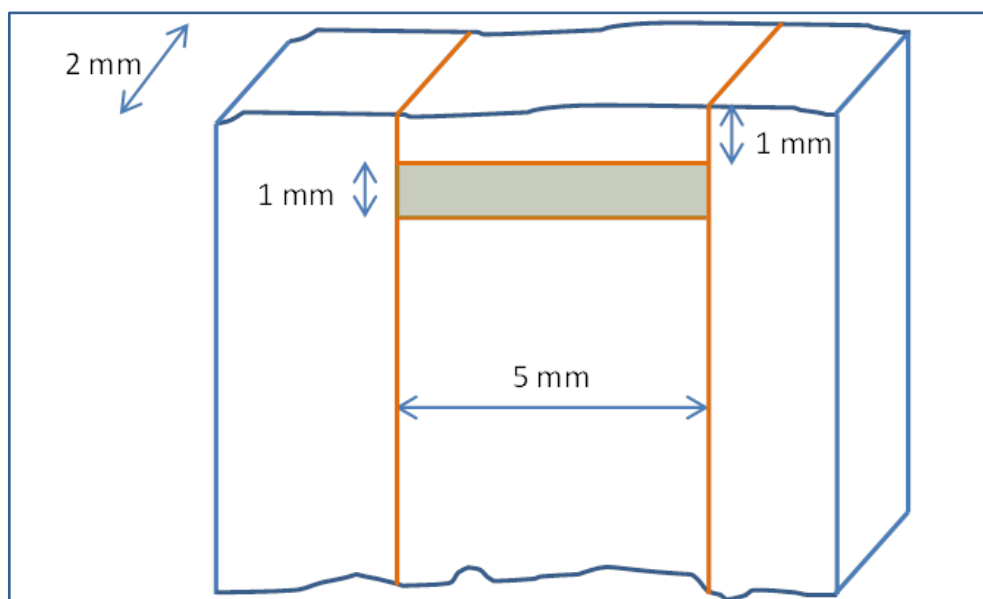

Figure 17 Sections of crust are cut as shown by the orange lines to obtain material for making each slide. A 5 mm x 2 mm x 1 mm piece of crust (shaded) is used for each slide.

Twenty random fields of view at ×400 magnification are then examined using light microscopy, and the presence or absence of chlorophyte cells (as defined by John *et al.*, 2011) noted in each case. Each count result, N (out of a total of 20), can then be converted to frequency percentage (N x 5), and the mean obtained for all counts from a particular lake station. Since 20 observations are made on each slide, three slides are prepared from each sample, and five samples are taken at each station, the estimate of chlorophyte abundance for each station is based on 300 observations.

3.7 Pigment analysis

Chlorophyll *a* is measured per unit volume of crust. When crusts are more than a centimetre in thickness, the upper one centimetre (1 cm) is used for pigment analysis, any crust below this being removed and discarded. Again, this may be easier with some crusts if done before the samples are fully de-frosted, as they are firmer for cutting at this stage. While a simple 1 cm³ cube of crust from each sample could be used, the following method is recommended in order to make volume measurements more accurate and measured values potentially more representative

- Measure out approximately 3 cm³ of crust and grind with a pestle and mortar for one minute. This results in a thick, homogenised paste

- A plastic cube, open at the bottom and top, with internal volume of precisely 1 cm³, is useful for measuring volume; such cubes can be made by cutting sections of plastic cuvettes to the right size. Place the plastic cube on a petri dish and fill with the paste using a small spatula, pressing down firmly so that no air spaces remain. Skim off any excess material from the top of the cube (*e.g.* using a glass microscope slide)

- Transfer this material to a glass test-tube. Graduated test tubes or cylinders are convenient for this purpose. Then add a 90% acetone solution, to a total volume of 10 ml. Using a glass rod, grind the sample in the test tube for three minutes. Cover the top of the test tube using Parafilm or a stopper. Refrigerate in darkness at 4°C overnight

- Proceed with spectrophotometry as described in Parsons *et al.* (1984). For this procedure, the extract from each sample is pipetted into a glass cuvette of 1 cm light path. Having set up and calibrated the machine to measure absorbance, according to the manufacturer's instructions, the extract is scanned at a wavelength of 665 nm and 750 nm. The sample is then removed from the spectrophotometer, and acidified using HCl according to Parsons *et al.* (1984) (in a fume cupboard). The lid is placed on the cuvette, and the sample then re-scanned at the same wavelengths as before. The wavelength at 750 nm is subtracted from that at 665 nm for both 'before' and 'after' readings, and chlorophyll *a* calculated according to Parsons *et al*. (1984).

Each of the five samples per station should be processed as above, and a mean figure calculated for chlorophyll *a*.
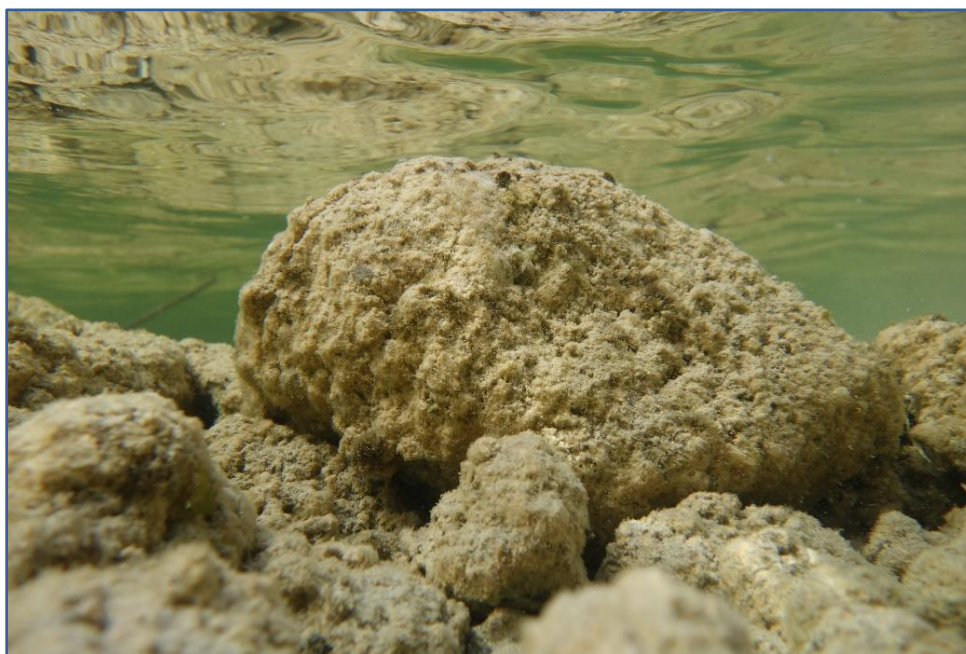

Figure 18 Cyanobacterial crust on cobble at Lough Bunny, Co. Clare. Photograph Philip Doddy.

Figure 19 Sampling cyanobacterial crust at Lough Carra, Co. Mayo. Photograph Caitríona Douglas.

4   Assessing the conservation condition of marl lakes

4.1   Defining reference conditions for Irish marl lakes

Most lowland habitat in Western Europe is extensively modified by human activities. Therefore, it is difficult to define reference conditions for lowland lakes. Data presented in Roden *et al*. (2020) suggest that in certain Burren lakes, total phosphorus and lake colour are very low (<0.01 mg/l and <10 Hazen colour units, respectively). For example, in Cooloorta Lough in the Burren, total phosphorus is 0.003 mg/l and colour is 9.7 units, while in Lough Rea, total phosphorus is 0.007 mg/l and colour is 5.7 units. Such lakes are characterised as having an extensive cyanobacterial crust zone, three or four charophyte zones and low total cover for vascular plant species. These lakes are taken as being close to reference condition. Such lakes appear to resemble the inferred former vegetation of now eutrophied lakes studied by Wiik *et al*. (2014, 2015).

At present many marl lakes show high colour but low total phosphorus (*e.g*. Walshpool), consequently they have a euphotic depth less than 6 m. It might be argued that colour was never low at such lakes, but here we assume that high colour (>20 Hazen units) reflect catchment disturbance such as drainage of bogland.

A small number of coastal lakes in calcareous sandy basins have both clear water and extensive charophyte vegetation (Aillebrack Lough and Fahy Lough in Connemara, Co. Galway; Magheradrumman Lough, Fanad, Co. Donegal). Few are deep enough to support either deep water charophytes or a deep-water/aphotic plant zone (maximum depths recorded in Roden (1999) are between 2 m and 8 m). Furthermore they tend to support angiosperms (*Potamogeton perfoliatus*, *P. pectinatus*) at depth, and have characteristic angiosperm species including *Ranunculus* species and *Myriophyllum spicatum* not found in reference grade Burren marl lakes. It seems probable that such lakes are naturally more nutrient rich than inland marl lakes.

There are also little known lakes on metamorphic rock with marble bands which support charophyte vegetation but these have not been examined as yet, *e.g*. Lough Talt, Co. Sligo. At present we have studied too few lakes of this type to propose reference conditions.

4.2   Parameters for assessing marl lake conservation condition

Based on the analyses of Roden *et al*. (2020), 11 conservation condition assessment parameters are proposed as indicators of the structure and functions of marl lakes on Carboniferous limestone (Table 2). These parameters are an expanded version of those proposed by Roden & Murphy (2013). For each parameter, targets are defined for *Favourable* (*Good*) conservation condition. Boundaries are also provided to distinguish *Poor* and *Bad* condition. Values are calculated for each parameter on each transect. Sections 4.2.1 to 4.2.9 describe how each is derived. Section 4.3 explains how these data are combined to give an overall assessment of the conservation condition of a lake.

The changes to parameters and targets since the 2011 survey (Roden & Murphy, 2013) are based on increased field knowledge of Irish marl lakes and include

- The cyanobacterial crust zone is included in assessing the number of vegetation zones and the lowest zone of *Chara denudata*/*Nitella flexilis* is not made an obligatory character of *Good* conservation condition, as it is not universal in marl lakes in *Good* condition

- The target for euphotic depth is decreased from 8 m to 7 m

- The cyanobacterial crust metrics are expanded based on the work of Dr Doddy

- Angiosperm abundance is now replaced by % cover of charophytes and cyanobacterial crust (C&K score), which is inverse to angiosperm cover

- Secchi depth is not used as it is not strongly correlated to euphotic depth.

Table 2 Conservation condition assessment parameters and corresponding targets for *Favourable* or *Good* condition

Parameter	Favourable or Good	Unfavourable-Inadequate or Poor	Unfavourable-Bad or Bad
Area	Stable or increasing	Decrease <10%	Decrease ≥10%
Number of vegetation zones	4 or more*	2–3*	1 or none†
Euphotic depth m	>7	4.5–7	<4.5
crust cover %	>70	70–20	<20
Crust chlorophyll *a* $\mu g/cm^3$	<45	>45	undefined
Crust chlorophytes % frequency	<45	>45	undefined
C&K score	>0.6	0.3–0.6	<0.3
Lake level	at or above cyanobacterial crust	>50% of cyanobacterial crust exposed	*Chara curta* visible above water
Total phosphorus (TP) mg/l	≤0.01	0.011–0.02	>0.02
Colour Hazen units	<15	15–30	>30
Index(TP × Colour)	<0.1	>0.1<0.5	>0.5

* Lough Rea is an exception, as the *Chara rudis* zone is (apparently naturally) absent, hence the target for *Good* is 3 or more vegetation zones, whilst 2 would indicate *Poor* condition. See also Section 4.2.2.

† And where charophytes only occur in shallow water (<2 m)

4.2.1   Area

Lake habitat area could be set as the surface area of the lake (calculated at a scale of 1:5,000); in this case significant changes in habitat area would only occur as a consequence of substantial drainage work. In many lakes, the macrophyte zone does not cover the entire lake bottom. An estimate of macrophyte area could be calculated as lake circumference multiplied by the average width of the euphotic zone. The latter can be calculated as the distance from the shore to the base of the euphotic zone, using transect waypoints as markers. If only two transects are sampled this is obviously an approximation, but resampling transects will highlight changes in area of macrophyte habitat. If bathymetric charts are available, the area could be calculated directly as the entire lake bottom shallower than the euphotic depth. We propose that *Good* is equal to no change or increase, *Unfavourable-Inadequate* (*Poor*) is less than 10% decrease in area and *Unfavourable-Bad* is a decrease in area of greater than or equal to 10% between sampling rounds.

4.2.2   Charophyte species and vegetation zones

Marl lakes are dominated by charophytes and their conservation value increases with increasing charophyte diversity. Loss of species between surveys is a sign of habitat damage. Species present in all marl lakes in *Good* condition include *Chara curta, Chara rudis, Chara virgata*; other species are widespread including *Chara aculeolata, Chara aspera, Chara contraria, Chara virgata* var. *annulata*, while some are very local, such as *Chara hispida* (apparently a species of ponds rather than marl lakes), *Chara denudata* and *Chara tomentosa* — the last two are Red Data Book species with fewer than 15 known Irish localities.

Nitella tenuissima has been recorded from two marl lakes in small quantity but like *C. hispida* may not be typical.

For previously surveyed lakes, there should be no loss of charophytes in subsequent surveys and the status of any red data book species should be documented regularly. Any loss of species should result in an *Unfavourable-Inadequate* (*Poor*) rating.

In general, lakes in reference condition will have the following zones between water surface and euphotic depth

- A cyanobacterial crust zone with occasional colonies of *Chara virgata* var *annulata*

- A *Chara curta* zone

- A *Chara rudis* zone

- A *Chara virgata* zone and

- In some lakes, a *Chara denudata* or *Nitella flexilis* zone.

For a zone to be present, cover values of 3 (*i.e.* more than 25% cover) or more should be recorded in relevés on the transect. Transects with four or more zones are rated as *Good*; if no zone is found deeper than the *Chara rudis* zone, the transect is rated as *Unfavourable-Inadequate* (*Poor*); if charophytes are absent or only occur in shallow water (<2 m), it is rated *Unfavourable-Bad*. As charophytes decline, angiosperms especially *Potamogeton* species and *Elodea* tend to increase, and overall euphotic depth will decrease usually to less than 4.5 m. *Lemna trisulca* and *Ceratophyllum demersum* are also negative indicators.

One exception to this scheme includes the very clear water of Lough Rea where the *Chara rudis* zone is all but absent and instead *Chara contraria* is abundant to the euphotic limit.

4.2.3   Euphotic depth

One value for euphotic depth (maximum depth of vegetation colonisation) is recorded per transect. For each lake, or basin, the maximum euphotic depth measured is used for assessment, as smaller values may reflect change in slope, the presence of rock or other natural factors. In lakes with several basins, the least favourable basin value is used for assessment. Euphotic depth of more than seven metres is *Good*; 4.5–7 m is *Unfavourable-Inadequate* (*Poor*); *Unfavourable-Bad* is less than 4.5 m.

4.2.4   Crust metrics

Doddy *et al.* (2019a) found that marl lakes of good ecological quality have a high degree of crust cover in the defined sampling zone, particularly on bedrock or large stones, although other submerged surfaces can also be encrusted. For lakes to be considered *Good*, they should have a minimum of 70% cover as measured by the method detailed in Chapter 3. Lakes which have cover of between 20% and 70% are assessed as *Unfavourable-Inadequate* (*Poor*), as it is very likely that these lakes have suffered some degradation from their natural state as a result of nutrient-pollution. Lakes where cover is less than 20% are seen as *Unfavourable-Bad*; in severe cases, no crust at all may be recorded.

Figures for chlorophyte abundance and chlorophyll *a* concentration tend to occur roughly in proportion to each other: a lake with high chlorophyte abundance will normally have high crust chlorophyll concentrations too. In lakes which have no surviving crust cover at all, these measures cannot, of course, be applied. However, in lakes which are in decline from their natural state, these metrics have been found to be useful and reliable indicators (Doddy *et al.*, 2019a, b). Lakes in *Good* condition will have chlorophyte percentage frequency of no more than 45% (often much less). Values above this are a sign that degradation is occurring. Considering crust chlorophyll *a* concentration, lakes in *Good* condition generally range from about 20 µg/cm³ to about 40 µg/cm³; mean figures of 45 µg/cm³ or higher should be seen as indicative of degradation.

While chlorophytes and chlorophyll *a* are both good indicators, chlorophytes are particularly highly correlated with phosphorus levels in lakes (Doddy *et al.*, 2019a). Therefore, while chlorophyll measurements may be easier and faster, a good argument can be made for enumerating chlorophytes as well. Figures for these two indicators can be expected to rise roughly in unison until breakdown and fragmentation of crust occurs. By this stage, crusts will often be noticeably greenish in colour and may have a partial cover of aquatic bryophytes.

4.2.5   Charophyte and cyanobacterial crust score (C&K)

A very noticeable feature of marl lakes in *Good* condition is the small contribution to vegetation cover made by vascular plants and bryophytes. Thus, the proportion of cover represented by charophytes, cyanobacterial crust and vascular plants are indicators of marl lake condition. Bryophytes typically constitute a very small part of total cover. Proportion of cover is calculated as follows

1.   The species list is divided into four groups, cyanobacterial crust, charophytes, bryophytes and vascular plants

2.   For each relevé, total cover of each group is summed

3.   For each transect, average cover of each group is calculated as the sum of cover of all relevés/ number of relevés

4.   A combined charophyte and cyanobacterial crust (referred to as '**K**rustenstein' by Roden & Murphy (2013)) score (C&K score) is calculated by dividing the sum of the cover of both groups by total cover.

This method is dependent on the adequate sampling of each transect as detailed in Sections 2.1.2, 2.4.4 and 3.4) and assumes an even distribution of relevés amongst the vegetation zones present. Oversampling a zone could bias the results. Nevertheless, data based on more than 30 lakes indicate that lakes in *Good* condition have C&K cover proportions of greater than 0.6 (Roden *et al.*, 2020). *Unfavourable-Inadequate* or *Poor* is set at 0.3–0.6 for the C&K score. *Unfavourable-Bad* is set at less than 0.3 for the C&K score. A vascular plant score could be derived, but is simply the inverse of the combined charophyte and crust (C&K) score and, therefore, not an independent measure of lake quality. It is accepted that this index may need to be further tested and refined.

4.2.6   Hydrological regime

In undisturbed lakes, fluctuations in lake level follow predictable seasonal trends, and the data necessary to demonstrate significant hydrological change can only be obtained by an automatic water level recorder. However certain relationships exist between vegetation zones and seasonal water levels. These can be measured in the field and if they break down, the lake must be deemed to be in unfavourable conservation condition

•   In summer, water level should never be lower than the top of the *Chara curta* zone. The lake should be rated *Bad* if the *Chara curta* zone is exposed.

•   In summer, the norm is that more than 90% of the crust zone is covered. If more than half the cyanobacterial crust zone is exposed, it is a matter for significant concern and the lake should be rated *Poor*.

•   In winter, all zones should be submerged and it is usual for the surrounding lake side vegetation to be inundated to a depth of perhaps 50 cm.

Excessively high winter levels are not known to damage lake condition, but very high summer levels are unusual and if persistent, indicate a change in lake structure and function.

4.2.7   Total phosphorus

These data may be available from the EPA or, alternatively, must be collected by the researcher. Single samples may be unreliable indicators and a sampling programme is necessary to determine meaningful values. Roden *et al*. (2020) analysed available data relating macrophyte composition to lake total phosphorus (TP) and water colour, and their findings are the basis for the boundary levels. While some lakes may have higher average total phosphorus concentrations, the majority of lakes in *Good* condition have total phosphorus below 0.01 mg/l (*e.g.* Cooloorta or Lough Rea, see also Section 4.1 above). Lakes where total phosphorus is >0.02 mg/l are invariably in *Poor* or *Bad* condition. *Good* condition is set at less than or equal to 0.01 mg/l, *Poor* 0.011–0.02 mg/l and *Bad* >0.02 mg/l total phosphorus.

4.2.8   Colour

As with total phosphorus, data on colour must be obtained from the EPA or measured by the investigator. Unsurprisingly, there is a correlation with euphotic depth, and nearly all lakes in *Good* condition have low colour (<15 Hazen units, *e.g.* Lough Bane 4.3 units). Here we set *Good* condition at <15 Hazen units, *Poor* at >15<30 Hazen units and *Bad* at > 30 Hazen colour units.

4.2.9   Index

An empirical index defined as the product of total phosphorus in mg/l and water colour in Hazen units (TP × Colour) has significant correlation with other indicators of lake quality such as euphotic depth or charophyte cover (Roden *et al*., 2020). Here we set *Good* conservation condition as <0.1, *Unfavourable-Inadequate* (*Poor*) as 0.1–0.5 and *Unfavourable-Bad* at >0.5.

4.3   Overall assessment of the conservation condition of an Irish marl lake

As multiple samples are taken and a variety of metrics are used to assess ecological condition of marl lakes, it is probable that different or possibly contradictory results may be obtained. Here we discuss how to obtain an overall lake assessment and resolve any contradictory indications.

4.3.1   Assessing the conservation condition of individual basins

In our experience, little variation is found among transects taken in the same lake basin. In very sheltered areas, vascular plants may be more abundant but charophyte diversity at depth will not be affected. For each basin, all transects are evaluated separately and the best (most favourable) transect used. For lakes with a simple structure comprising a single basin, likewise the best transect is used in the assessment. This choice is based on field observation that, within a single basin, most transects are similar and determined by similar water transparency. Slight variations reflect local factors. Large differences imply some local but strong influence, such as sewage discharge or stream inflow.

4.3.2   Combining conservation condition assessments for two or more basins

In complex lakes, such as Lough Carra, different basins can have very different scores. This situation indicates growing ecological change and deterioration in conservation condition. Therefore, the conservation condition of the lake as a whole is determined by the worst basin rating.

4.3.3   Combining the results of multiple condition assessment parameters

While we quantify boundaries for most of the indicators used, it should be remembered that these occupy gradients rather than abrupt state changes, therefore account may need to be taken of some

margin of error around the proposed boundaries in certain cases. Generally, the different indicators used co-vary, but an anomalous value will require expert judgement to interpret.

We have lower confidence in some indicators than others, for example lakes with similar total phosphorus values can have different overall conservation assessments. At Lough Owel, for example, total phosphorus was 0.012 mg/l in 2011 and 0.010 mg/l in 2018 and the lake was in *Good* conservation condition on both occasions (Roden & Murphy, 2013; Roden *et al.*, 2020). By contrast, Lough Arrow had similar average total phosphorus of 0.010-0.012 mg/l, but was in *Poor* conservation condition in 2012 and 2019, owing to low euphotic depth, decline in charophyte species richness and abundance and the expansion of angiosperms, including alien invasive species (Roden & Murphy, 2020; Roden *et al.*, 2020; Bradley *et al.*, 2015). We suggest the following order of priority for the proposed indicators

1. **Vegetation zones** — all lakes which lack zones deeper than *Chara rudis* should be deemed at least *Unfavourable-Inadequate* (*Poor*) irrespective of other measurements. As each zone contains a different species, a lake in *Good* condition should contain, as a minimum, the following species; cyanobacterial crust, *Chara curta*, *Chara rudis*, *Chara virgata*

2. **Euphotic depth** — all lakes with euphotic depth less than 6 m should be rated at least *Poor*, irrespective of other measurements

3. **Cyanobacterial crust** — the complete absence of crust on exposed limestone rock will always indicate *Bad* conservation condition. Chlorophyll *a* content and proportion of chlorophyte algae in crust have a similar weighting to C&K score (below)

4. **C&K score** — the combined charophyte and cyanobacterial crust cover score (C&K) is probably less reliable than the previous indicators and should not override euphotic depth or number of vegetation zones. Nevertheless, a C&K score <0.6 probably indicates some nutrient enrichment and suggests at least *Poor* condition.

The remaining indicators are assumed to influence ecosystem structure and function but the exact manner is not determined. Therefore, conservation assessments based on these indicators should not override those listed above, which directly measure structure and function. Unfavourable values may however indicate existing pressures or future threats to the ecosystem. On an empirical basis it appears that the combined index of total phosphorus × water colour closely tracks conservation condition as determined by other indicators, and values greater than 0.5 probably indicate lakes in *Poor* or *Bad* condition. Three examples (Loughs Arrow, Bane and Owel) are provided in Table 3.

Table 3   Worked examples of overall conservation condition assessments for three marl lakes. Data for Lough Arrow courtesy of the CANN project (Roden & Murphy, 2020). Data for Loughs Bane and Owel from Roden *et al.* (2020). Cyanobacterial crust data from Doddy *et al.* (2019a).

Parameter	Arrow (2019)		Bane (2018)		Owel (2018)	
Number of vegetation zones	3	Poor	5	Good	6	Good
Euphotic depth m	4.5	Poor	9	Good	8.0	Good
crust cover %	0	Bad	92	Good	50	Poor
Crust chlorophyll *a* μg/cm^3	-		40 (±1)	Good	46 (±1)	Poor
Crust chlorophytes % frequency	-		14.7 (±1.3)	Good	37.7 (±11)	Poor
C&K score	0.59	Poor	0.642	Good	1.0	Good
Lake level	above crust	Good	above crust	Good	Below	Poor
Total phosphorus (TP) mg/l	0.012	Poor	0.006	Good	0.010	Good
Colour Hazen units	21	Poor	4.23	Good	6.85	Good
Index(TP × Colour)	0.25	Poor	0.046	Good	0.0685	Good
Overall assessment		Poor		Good		Good

All indicators for Lough Bane demonstrate *Good* conservation condition and the lake is rated *Good*. Average concentrations of total phosphorus have been above the 0.1 mg/l target in the past and crust chlorophyll *a* is close to the *Good*/*Poor* boundary (45 µg/cm³), possibly indicating early signs of nutrient enrichment. In Cullaunyheeda, all indicators are *Poor* or *Bad*, but both euphotic depth and vegetation zones are *Poor* meaning some charophyte vegetation is still present. In the most degraded lakes, no charophyte vegetation can be found and these are rated *Bad*. On this basis, Cullaunyheeda is rated *Poor*.

4.4   Other indicators of marl lake condition

Other attributes used in NPWS site-specific conservation objectives (see O Connor, 2015 and NPWS, 2018, 2019c) may be useful indicators, but cannot currently be used in assessing lake conservation condition. This may be because of challenges with measurements/quantification or low statistical certainty. In consequence, they should be measured and examined, where possible, but should not over-ride the assessment based on the metrics discussed above. These are discussed individually below.

4.4.1   Substrate

In general, marl lakes are dominated by limestone bedrock, calcareous silt and sand and loose stones. The appearance of large expanses of raw peat would indicate excessive sediment input. Sediment also accumulates phosphorus and can release phosphorus into the water column. Future work should attempt to clarify acceptable sediment phosphorus concentrations.

4.4.2   Attached algae

Occasional blooms of filamentous algae occur in marl lakes. These do not always imply excess nutrients; especially if species of the orders Zygnematales or Oedogoniales are responsible. Drifting masses of *Cladophora* species however may indicate a decline in water quality.

4.4.3   Water Framework Directive biological quality elements or metrics

The EPA have extensive data for many Irish lakes. These data are used to determine lake status for the Water Framework Directive. Macrophyte data collected by the EPA are not directly comparable to the conservation condition data described here owing to very different methods used. However chemical and physical data on lake water are very useful in understanding marl lake ecology. The EPA data-series are generally the most complete available for Irish marl lakes, but many smaller lakes have not been sampled.

Water colour and total phosphorus help determine vegetation structure. The first by directly controlling light penetration and, hence, euphotic depth, the second by indirectly controlling light penetration through promoting phytoplankton growth leading to shading. Total phosphorus also determines species composition by influencing the relative growth rates of charophytes and angiosperms, as it appears angiosperms outcompete charophytes when more nutrients are available.

When EPA data on total phosphorus and water colour are available, they should be used as described above to assess conservation condition.

4.4.4   Secchi disc transparency

Where a series of measurements is available, this metric indicates water clarity. However single measurements are less useful than a time series and often show little correspondence to the euphotic depth. We prefer to measure euphotic depth directly by observation, but a decreasing trend in Secchi depth would indicate declining water quality.

4.5 Pressures and threats

In general, human impacts on marl lakes are indirect, in the sense that the pressures occur in the lake catchment rather than in the lake itself. Consequently, it is often not possible to detect pressures and threats by direct observation, rather analysis of aerial imagery or data on inflowing streams are necessary. Such analysis is outside the scope of this manual and here we only offer a short summary of likely pressures and threats, which should be noted during field survey.

4.5.1 Increases in nutrient loading

A general rise in nutrient (nitrogen and phosphorus) loading is known to have occurred in many Irish lakes. This increase reflects increased use of fertilisers on farms and in forests, as well as discharges from sewage works and an increase in septic tanks around many lakes. The impact of this pressure is often summarised as an increase in phosphorus (measured as total phosphorus), an element which frequently limits plant and algal growth and is known to correlate with a decline in water quality and lake conservation condition. Data on phosphorus inputs may be obtained from studies by the EPA or others (*e.g.* Teagasc, Local Authorities (environment sections, LAWPRO) or academic/research institutes). Increases in farming, forestry or housing activities detected from aerial imagery might be related to changes in lake total phosphorus. In general, it is not possible for field observation to directly monitor and detect changes in nutrient loading, even though it is probably the greatest threat to Irish marl lakes.

4.5.2 Increases in lake colour and sediment load

Again, it is not easy to detect these factors by direct observation. Aerial imagery will show whether cutover bog-land drains into the lake. In addition, as many marl lakes are fed from ground water, drains and other artificial channels may indicate anthropogenic sources of sediment. Where possible, such features should be noted and inspected during field survey. An advantage of boat survey is that discharge points around the lake shore can be easily checked for excessive plant or algal growth. Sediment accumulation, darker water or increases in vascular plants at the point a drain or channel enters the lake all indicate nutrient and sediment pressures on the lake. It should be noted that increases in nutrient loading may vary independently from increases in lake colour and sediment load.

4.5.3 Excessive water abstraction

Marl lakes are often the source of clear, low-nutrient water, and consequently are used by public and private water schemes. In the past, excessive abstraction was known to have reduced water levels to the *Chara rudis* zone at one site. Such rates of abstraction are extremely damaging and should be reported if observed. All abstraction schemes should incorporate continual monitoring of lake levels.

4.5.4 Invasive species

Some invasive species have had a large impact on the ecology of marl lakes. These species include *Lagarosiphon major* in Lough Corrib, the Zebra Mussel *Dreissena polymorpha* in several marl lakes and *Elodea nuttallii* in Lough Arrow. It is noticeable that these species all occur in lakes rated as *Unfavourable-Inadequate* (*Poor*). It is possible that invasive species impact on lake structure and function only when nutrients are elevated. Nevertheless, the presence of introduced species is a potential threat to marl lakes and should be noted.

4.5.5 Other pressures

Other pollutants, such as herbicide and pesticide usage close to the lake may have damaging impacts. Lake infilling, or similar severe morphological change, inevitably damages littoral communities.

5   Recommendations on monitoring and other future work

5.1   Monitoring programme

As marl lakes are an unusual and vulnerable habitat, an extensive monitoring programme should be undertaken to ensure the habitat is not further degraded or lost entirely, as has happened in other countries. Less than 10% of the known area of marl lakes is estimated to be in *Good* conservation condition (NPWS, 2019b) and the greater part of the remaining is rated as *Bad*. The first priority is to ensure no further losses of *Good* habitat. Ideally, all known lakes in *Good* conservation condition should be included in any monitoring plan or programme. Lakes in *Poor* conservation condition (but not *Bad*) should also be monitored, ideally as part of a restoration programme. Lakes in *Bad* conservation condition – the majority of marl lakes – will benefit less from monitoring, as their restoration will prove very difficult.

1. Examples of lakes in *Good* conservation condition which should be monitored are Loughs Bane, Rea, Owel, Annaghmore, Spring, White Lough and all the lakes in the Burren, as well as coastal lakes such as Fahy Lough or Cross Lough.

2. Lakes in *Poor* conservation condition that should be monitored include Loughs Corrib, Carra, Mask, Urlaur, Erritt, Ennel, Lene, Finn Lough and other lakes in east Co. Clare.

3. Many former marl lakes are now lacking most signs charophyte vegetation, such as Lough Derg and Lough Ree on the Shannon, or Lough Bridget. These must be rated as in *Bad* conservation condition.

All monitoring programmes are constrained by expense, here we propose a possible monitoring programme

1. All known *Good* lakes should be sampled at least once every ten years. In addition, exploratory work should aim to establish if further marl lakes in *Good* conservation condition exist, which is still probable. Smaller water bodies in the midlands of Ireland, should be prioritised for exploratory survey

2. Some lakes in *Poor* conservation condition should be monitored, especially those in SACs and those where there is some possibility of restoration, as is required by the Water Framework Directive. Frequency of monitoring should be linked to any attempts at lake restoration, with frequent monitoring if a restoration programme is agreed or under way

3. Monitoring of lakes in *Bad* conservation condition is less worthwhile, but those in SACs ought to be monitored occasionally

4. Monitoring of lakes using the cyanobacterial crust method is a novel but useful approach; its greatest advantage being that it is rapid, requires few personnel and little equipment. Crust samples could be easily gathered from many if not all lakes in *Good* conservation condition. A monitoring programme using crust alone might allow effective and cheap monitoring of all *Good* lakes much more frequently than either snorkelling or current EPA protocols. With a longer time series it may be demonstrated that small changes in crust chlorophyll or chlorophyte count reflect subtle changes in lake structure and function owing to eutrophication.

5.2   Further work

The proposed scheme is based on extensive fieldwork on more than 30 marl lakes in Ireland, as well as reports of similar lakes elsewhere. Some improvement in technique is always possible especially with

further developments in electronic recording. Nevertheless, the data obtained on macrophyte vegetation and cyanobacterial crust composition are reasonably comprehensive for marl lakes on Carboniferous limestone. The obvious difficulty with the vegetation survey method is the need for snorkelling or scuba diving, which are expensive and require special skills. To what extent cyanobacterial crust monitoring is sufficient for determining marl lake ecological condition remains to be tested in future sampling programmes, but the method offers obvious savings in time and sampling effort.

Some differentiation between crust monitoring and submerged macrophyte monitoring is possible. In general terms, crust will respond directly to increased nutrient inputs rather than to decreases in light caused by excess plankton or water colour. Submerged macrophytes will respond to light limitation. Additional data on both sets of indicators may allow a distinction between the impact of excess colour resulting from bog destruction or drainage and the impact of excess phosphorus.

Marl lakes on Carboniferous limestone are common in central and western Ireland but, to date, we have not encountered evidence that more than one type of marl lake exists other than as a result of human interference in nutrient cycles and drainage. In future, further research on kettle hole type lakes in glacial drift may suggest different natural vegetation types. Only further palaeoecological research can establish this point, which is of more than theoretical importance if the objective of restoring lakes to their 'natural' state is to be achieved. We do know, however, that other charophyte dominated lakes exist especially along the coast on machair but also in areas of metamorphic limestone or marble, and these are very rare. Such lakes have not, in general, been sampled in EPA monitoring programmes because of their small size and inaccessibility. Preliminary work by Roden (1999) suggests machair loughs are naturally more nutrient rich, shallower and have some distinctive species but further survey is necessary to define reference conditions. Those outlined above are probably unsuitable without modification.

Figure 20 It can be useful to have both a boat and canoe during survey – the canoe is useful for reaching isolated basins separated by very shallow water.

6   References

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Figure 21 Cilian Roden with specimen of cyanobacterial crust. Photograph Philip Doddy.

7   Identification guides

Here we list some reference books for charophyte identification. Standard floras are adequate for vascular plants with the exception of pondweeds and *Callitriche* species. For charophytes, the best single text is Bryant *et al*. (2011). Moore (1986) is very clear but the nomenclature used is no longer current. Krause (1997), although in German, is very comprehensive and refers to Irish material. Stewart & Church (1992) is an excellent guide, even though described as a Red Data Book. Urbaniak & Gabka (2014) has beautiful colour photos and includes most Irish species.

Preston (1996) is indispensable as a guide to Pondweeds (*Potamogeton* species), which are very common in marl lakes. Lansdown (2008) will be referred to less often as *Callitriche* species are not frequent in marl lakes.

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