Natural England Commissioned Report NECR230

Designing a methodology for surveying fish populations in freshwater lakes

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Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

This report was commissioned to review available fish survey methods and the limitations and constraints presented by the lakes within the SSSI series, to design a standardised, practical, and representative method for fish survey which could be reasonably applied throughout the SSSI lakes to achieve comparable results.

The report then goes on to design a study which applies this fish survey design to a selection of SSSI lakes to ascertain whether fish are likely to be contributing to their unfavourable condition.

This report has been used to inform a follow up study which seeks to apply the fish survey techniques to a range of SSSI lakes to ascertain information about their fish populations and their likely impact on SSSI condition.

The report also includes a comprehensive review of fish survey methods and concludes with a recommended standardised fish survey method which can be applied consistently across different SSSIs to provide comparable quantitative results. It is expected that this methodology can be applied in subsequent fish surveys commissioned on SSSI lakes, to provide more robust and repeatable results.

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Mirror carp captured by point-abundance sampling by electric fishing © Martin Perrow

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1 INTRODUCTION & AIM

Natural England (hereafter NE) are responsible for advising on the management of around 300 lakes and ponds notified as SSSIs for the importance of their aquatic habitat or for water dependent species such as amphibians or assemblages of invertebrates or birds, in a regional and national context.

The suite of SSSI water bodies incorporates a considerable variety of types in terms of hydrology, size, depth, origin, ecology and trophic status, as well as use and ownership. Preliminary work on the ecological status of SSSI units by Skeate & Perrow (2007) for NE suggested that some 83% of lakes were unlikely to meet their conservation targets, which was in close agreement with the earlier work by Carvalho & Moss (1995) who found that 84% of the 102 SSSI lakes they examined to be adversely affected by eutrophication. Common Standards Monitoring (CSM) condition assessment has subsequently confirmed that 98 of the 119 lakes assessed (82%) were in unfavourable condition (Goldsmith 2012, Burgess *et al*. 2014). Moreover, in the first tranche of lakes assessed, over half of the few (14) in favourable condition were deemed to be at significant risk of deterioration (Goldsmith 2012).

A range of symptoms of habitat degradation were reported, with the most frequent being:

- Eutrophication and general water quality problems.
- Species-poor aquatic macrophyte assemblages.
- Evidence of recent loss of indicative plant species.
- High incidence of non-native, invasive plant species.

Other than the presence of non-native invasive plant species, the underlying cause of unfavourable condition could typically not be established. For this to be achieved, further investigation by specific NE staff with responsibility for that particular geographic area is undertaken. However, as identified by Skeate & Perrow (2007) determining the likely root of the problem at a particular lake, typically in the absence of specific data, requires considerable expertise and experience (i.e. expert judgement), often beyond the broader-based skill-set of NE conservation officers. Specific training should be considered to fill this knowledge gap.

After compiling all available information, Carvalho & Moss (1995) were however able to determine that of the 96 sites they investigated (revised from the initial 102), 79 (84%)

showed signs of eutrophication. Of these, the cause in 15 and possibly up to 21 (27%) was attributed to the effects of fish, especially non-native Common Carp (hereafter Carp) *Cyprinus carpio* introduced for the purposes of recreational angling.

The effects of fish are generally thought to be more intense in shallow rather than deep lakes as fish typically reach higher numerical or biomass density per lake volume in shallow lakes where resources are concentrated in the photic zone and fish may easily exploit both benthic and pelagic resources without the need for vertical migration, thereby also promoting benthic-pelagic coupling (Jeppesen *et al*. 1997). In many standing water bodies, and particularly those found in relatively shallow lakes typical of the SSSIs considered above, fish are known to promote the effects of eutrophication via both top-down and bottom-up processes within the trophic chain. For example, zooplanktivorous fish such as Common Roach (hereafter Roach) *Rutilus rutilus* may reduce or even eliminate large cladoceran (Daphnid) zooplankton through size-selective predation, which in turn reduces the grazing pressure upon phytoplankton with consequences for algal abundance, biomass and community structure in a top-down trophic cascade (Carpenter & Kitchell 1993, Townsend *et al*. 1986, Phillips *et al*. 1996, Moss *et al*. 1996, Perrow *et al*. 1999a).

In contrast, through turnover of bottom sediments as they forage, large benthivorous fish such as Carp or Common Bream (hereafter Bream) *Abramis brama* may exert a more complex mixture of top-down and bottom-up processes through re-suspension of fine lake sediments to reduce water clarity (Breukelaar *et al*. 1994), release of nutrients available for algal uptake (Tatrai *et al*. 1990, Cline *et al*. 1994) and direct uprooting of submerged plants (Ten Winkel & Meulemans 1984, Zambrano *et al*. 1999).

Herbivorous species such as Common Rudd (hereafter Rudd) *Scardinius erythropthalmus* may structure macrophyte communities through selective grazing of one species over another with consequences for palatable, vulnerable species (Lake *et al*. 2002). All fish may also process nutrients through their bodies and transfer them in a form available for algal uptake and growth through egestion into the water column (Tatrai & Istvanovics 1986).

Such is the relative strength of fish-induced processes that different fish assemblages tend to be associated with different water quality and habitat variables. Using correspondence analysis for 28 UK shallow lakes, Zambrano *et al*. (2006) demonstrated a clear separation between different functional groups of fish. Dominance of zooplanktivorous fish was associated with chlorophyll *a* concentration whereas benthivorous fish were linked to nitrogen concentration. Only piscivorous species were associated with both the cover and species richness of submerged macrophytes.

Simple knowledge of water quality and habitat parameters may thus provide some insight into the fish community present. However, rigorous estimates of fish density and biomass will be required to determine if there is a problem. For zooplanktivorous fish in shallow lakes, Perrow *et al*. (1999) provide some evidence of a threshold density of 0.2 ind. m^{-2} of underyearling Roach, above which an effect upon zooplankton and thus algal populations is likely in structureless environments. The existence of refuges for zooplankton amongst stands of submerged macrophytes may reduce although not entirely eliminate predation and large bodied-cladoceran grazers may persist at fish densities to ~1 ind. m^{-2} in more structured environments.

In relation to fish biomass, which may be primarily linked to larger, typically benthivorous species, NE and EA have provided joint guidance that limits the projected estimates for biomass of total fish production in lake SACs[1] (Special Areas of Conservation) to 200 kg ha^{-1}, with a presumption against stocking of Carp and Bream in particular. The threshold appears to be based on the suggestion of Smith (2001) that a submerged macrophyte community can no longer be sustained in shallow lakes at a fish biomass of 150-250 kg ha^{-1}, with submerged macrophytes absent from lakes with a biomass of >300 kg ha^{-1}. The simple analysis underpinning this statement was from a limited sample of 11 mainly UK, but also Dutch lakes, with six lakes represented more than once to provide a total sample size of n=19. Further work would ideally be undertaken to refine confidence in the use of a threshold value of fish biomass as a basis for management.

The pervasive effect of fish upon lake structure and function means that biomanipulation of fish stocks, that is the removal of zooplanktivorous/benthivorous fish or occasionally the introduction of piscivorous species to control the other groups, is seen to be a valuable and cost-effective tool for shallow lake restoration (Perrow *et al*. 1997, Jeppesen & Sammalkorpi 2002, Skov *et al*. 2002, Søndergaard *et al*. 2007, 2008) in both cold temperate and more recently, warm tropical climes (Jeppesen *et al.* 2012a). In warm lakes, benthivory and the

[1] These guidelines are now being incorporated into the EA's operational instruction for the new Live Fish Movement Scheme covering both coarse and game fish in relation to SSSIs as well as SACs (G. Madgwick pers comm).

recycling of phosphorus may be more important than the relative strength of zooplanktivory in ultimately determining algal standing crop (Jeppesen *et al.* 2012a). In cold temperate lakes, the opposite may be true, although in fact the relative strength of one over the other as a structuring force in a given situation is poorly understood. It is therefore unsurprising that biomanipulation tends to aim to tackle both zooplanktivorous and benthivorous species whilst promoting the relative contribution of piscivores.

Moreover, it should also be noted that the strength of different trophic interactions and thus the effectiveness of any management involving fish varies markedly according to lake depth. Researchers (e.g. Moss *et al.* 1997) have found that a maximum depth of approximately 3 m effectively divides lakes into shallow and deep subsets, with observable and significant consequences for their functioning in the present context. Most significantly, the entire water column of shallow lakes may be occupied by submerged macrophytes, which may help buffer the effects of eutrophication through a variety of mechanisms such as out-competing algae for nutrients, suppressing the growth of algae through the production of alleopathic substances, offering refuges for grazing zooplankton and preventing the resuspension of bottom sediments by wind, waves and fish (see Jeppesen *et al*. 1998 for a discussion of all factors). For a given nutrient concentration, shallow lakes may exist as clear, macrophyte-dominated or turbid, phytoplankton-dominated alternative stable states with fish as the catalyst driving one to the other. Deeper lakes on the other hand usually stratify through the growing season with nutrients effectively locked below a thermocline and unavailable for algal uptake. In deep lakes, any submerged macrophytes are limited to the edges of the lake where they can only have a more limited structuring role in lake dynamics.

As also noted above, the effects of fish are generally thought to be more intense in shallow rather than deep lakes (Jeppesen *et al*. 1997). The fish communities of shallow and deep lakes also tend to be different, although this is also linked to nutrient status, with salmonids and such as Arctic Charr *Salvelinus alpinus* and percids such as European Perch (hereafter Perch) *Perca fluviatilis* dominating deep lakes whereas cyprinids such as Carp, Bream and Roach become prevalent in shallow lakes.

At this stage, around 130 SSSI lakes of varying size and depth are known to support a fishery or to have been stocked with fish at some time, with the majority (77%) being subject to recent applications to the Environment Agency (hereafter EA) to stock more individuals of the same or different species to those already present. Approximately 90 of the 130 SSSI fisheries are assessed as being in unfavourable condition, with the presence of fish and/or fishery management identified as a potential contributor to poor condition in more than half of them (~50), including some that are not actively stocked (G. Madgwick *pers comm*). However, NE holds very little data to support this perception. Without detailed information on the fish assemblage and especially density and biomass of the species populations alongside additional information upon water quality parameters and ecological status, as well as other potential pressures and issues, there is no possibility of effective management.

Accordingly, it is the primary aim of this project to identify the means of effectively sampling the fish community of a variable set of SSSI lakes in order to provide an accurate description of the fish present and meaningful measures of fish density and biomass. This information can then be used to inform site-based management in order to secure favourable ecological condition. What follows is a brief review of available fish sampling techniques in both shallow and deeper lake systems, coupled with a discussion of their relative strengths and weaknesses. A number of recommendations are then made on the use of particular techniques, or a suite of techniques in different circumstances, including within shallow or deep and small or large lakes.

2 REVIEW OF SAMPLING METHODS

2.1 Underlying principles

Fishery scientists employ a wide range of methods to sample fish, with many of these originally based on artisanal methods to capture fish as a source of food. Technological advancement means that the range of means of sampling fish without capture (e.g. hydroacoustics, underwater cameras) has increased considerably from the basis of a few limited situations where fish can be counted by naked eye (e.g. in clear shallow waters). Methods involving capture typically fall into two categories: *active* methods where the observer uses and moves specific gear to actively capture fish and *passive* methods, where the movement of the fish brings it into contact with the gear and it effectively captures itself. Active methods may be best employed when fish are less active and able to avoid capture, such as during cooler seasons in temperate climes, whilst passive methods work best in warmer seasons when fish, as poikilotherms, are more active.

This distinction means that passive methods tend to be qualitative (i.e. where fish capture is not linked to a unit of measurement) whereas active methods may be both qualitative and quantitative, with the latter most usefully expressed as fish density per unit area (e.g. individuals m^{-2} or ha^{-1} or as biomass in g m^{-2} or kg ha^{-1}). Density measures are typically given expressed relative to surface area rather than volume and are thus independent of the depth of the water body. Quantitative measures allow direct comparison between water bodies of different types and sizes.

In fact, despite significant recent developments in a range of sampling techniques there is still no method that is truly quantitative for all functional groups and species, as different methods are selective and have inherent biases. For example, the size of fish affects their susceptibility to capture, as do differences in behaviour, both between species (e.g. slow or fast moving species, shoaling or solitary, cryptic and refuging) and within species with differential activity according to diel and seasonal patterns. It may also be surprisingly difficult to define the area that is actually being sampled by gear such as seine nets as the shape of the set net influences the area sampled considerably, which influences the resultant density estimates.

Moreover, different methods lend themselves to different habitats. For example, nets may work more efficiently in the absence of plants or detritus, which may otherwise clog the meshes or prevent effective deployment in the first place. Underwater obstructions (tree roots or branches or artificial structures) similarly mean that some types of net cannot be used at all due to snagging. As a result of a range of biases, a number of authors have indicated that no one method can be applied in all situations, and monitoring programmes incorporating several methods are most likely to be successful (e.g. Kubečka *et al*. 2009, Winfield *et al*. 2009, Emmrich *et al*. 2012).

Typically, different methods are performed in different habitats (e.g. Jaarsma 2007) and the respective samples weighted according to the area sampled to produce a combined overall estimate. In lakes, this division may simply be between the littoral and limnetic (open water) zones or could be further divided if there are a variety of distinctly different habitats, such as areas of submerged vegetation. The littoral zone is of particular functional importance providing spawning, fry, refuge and ambush habitat as well as specific food resources for different species. It is especially important in small lakes where its size is relatively large compared to the open water. But even in large lakes with a low littoral:limnetic area ratio, its size may belie its importance as refuge habitat during the day for species/age groups that then migrate into the limnetic zone at night to feed (e.g. Winfield 2004). Such diel migration may be as important as the vertical migration that is often described for deep lakes (Mehner 2012).

Even where a method is thought to be suitable, the manner in which it is performed has considerable bearing on its efficiency. Subtleties of how a net is pulled or a trawl is towed may produce different catches. A method such as electric fishing, which requires active sighting and collection of fish is particularly susceptible to operator bias. This is because effective capture of fish demands the development of a series of search images both for different species that respond differently to the gear (for example, some cryptic species may remain so even when stunned) as well as different sizes of individuals (large fish are always easier to see than small ones but may be much more difficult to actually capture). In this case, the range of estimates produced by different teams of operators may be considerable if based on a single catch or run, rather than a series to produce a projected estimate. Similarly, highly technical techniques such as hydroacoustics present the operator with a vast range of operational settings that must be optimised and/or standardised to facilitate robust absolute or relative comparisons to be made across lakes and surveys (Hateley *et al.* 2013).

In lakes, the choice of methods is heavily dependent on the depth of the lake and to a lesser extent its size. A method such as electric fishing can only be used to sample the entire water column in a shallow lake although it could be used to sample the littoral margin or the upper surface layers of a deep lake perhaps especially for a particular species or pelagic fry. Similarly, it may not be practically possible to sample the entire water column in a very deep lake with a standard seine net. Nevertheless, a purse seine could be used, whereby the net only reaches part of the way down the water column, with the bottom of the net drawn together by a string to make a bag that is then hauled. The other issue with large lakes is one of sampling a sufficient area with each sample in order to provide reasonable confidence that the fish assemblage is sampled effectively. The opposite may be true on a small pond, where the sampling method used may have to be scaled down to be able to generate multiple samples. As with all sampling, it is desirable to have a number of samples to provide some measure of variance around a mean or median value, even if this is lost when integrating multiple sample methods (see above). Opting to survey when fish are likely to be more evenly distributed, bearing in mind that some species virtually always occur in patchily distributed shoals, may produce more confidence in the estimates. Alternatively, sampling in the winter months when fish may be heavily aggregated in a few locations in a lake may be problematic in that many samples may contain zero values.

It is suggested that a fish sampling strategy for SSSI lakes designed to help define the likely role of fish in the trophic interactions within the water body, must aim to achieve the following:

- Generate quantitative estimates, or at least estimates, of the three main functional groups: zooplanktivores, benthivores and piscivores.
- Sample all main habitat zones within the lake, specifically the littoral as well as the limnetic zone.
- Maintain the potential to produce at least broadly comparable measures (i.e. numerical and biomass density of the different species present) across deep and shallow and large and small lakes.
- Be broadly repeatable should conditions change within the lake, and be replicable by different teams of operators
- Be generally cost-efficient bearing in mind the maximum likely budget of £45,000 per year over two years for the main project in which it is desirable to sample as many lakes as possible.

In the following section we briefly discuss the relative advantages and disadvantages of the main methods of sampling lake fish communities. The limited time available to this project means that this process is not definitive and we draw heavily on our personal experiences and expertise generated by over 60 years combined experience of sampling fish in a wide variety of lakes in a number of countries in Europe and elsewhere as well as the UK. We appreciate that much EA fish sampling is based on guidance produced by the European Inland Fisheries and Aquaculture Advisory Commission (EIFAC) in the form of European Standards (CEN) such as European Standard EN14962:2006 on general sampling approaches and other more specific standards. However, such guidance is relatively high level with little detail and so as a starting point we draw upon the much more detailed review of fisheries census methods by Perrow *et al*. (1996a) updated by Coté & Perrow (2006) in the authoritative and influential *Ecological Census Techniques* in two editions by Cambridge University Press. On the basis of being an updated version, we will refer to Coté & Perrow (2006), unless there is specific material only represented in Perrow *et al*. (1996a).

2.2 Sampling methods

Coté & Perrow (2006) provide a summary of the use of all methods thought to be suitable to census fish in a wide range of habitats from freshwater to marine systems including coral reefs. The methods generally suitable for use in freshwater and brackish lakes are shown in

Table 1 as adapted from Coté & Perrow (2006) using a classification of the method as being *usually applicable*, *often applicable* and *sometimes applicable*.

In addition, we have added the potential for the method to provide quantitative estimates of numerical and biomass density, accepting that truly quantitative estimates of all functional groups is difficult to obtain as a result of gear selectivity and bias (see 2.1 above). Some methods are often viewed as semi-quantitative in that some level of estimate may be provided for some groups, perhaps in the form of a catch per unit effort (CPUE), especially if the method is applied in a particular way. We have included whether the method is generally quantitative, often semi-quantitative or qualitative. Linked to this, electric fishing has been separated into two rather different techniques, although the basis of fish being attracted to an anode and temporarily immobilised (stunned) is the same. The reason for separation is that point-abundance sampling (PASE) by electric fishing always attempts to quantify the area fished at multiple pre-determined points. Standard electric fishing on the other hand involves exploration of habitats in a much more unrestricted manner, although of course this may be applied within a defined area, such as within a stop-net. In fact, point-abundance sampling could also be undertaken using a small net that is thrown (cast-net) or lifted (scoop-net) or comes to the surface on its own (buoyant net), mostly adapted from local fishing gears. There is considerable scope for such methods to be used, particularly for specific groups of fish, especially small ones, but as this has been rarely been achieved in the UK, these are excluded from the selection of methods considered here.

The division of lakes into large, small, shallow, deep, open and vegetated is mostly arbitrary but is designed to cover the suite of SSSI lakes and to help refine the choice of methods for a particular lake. The various divisions should also be used in combination with each other when applied to a particular case i.e. large and deep. In this circumstance, the limitation of the method may be because of depth not lake size, but if there is a blank cell under either heading then it should be assumed the method is not applicable. For example, electric fishing is not generally suitable in deep lakes and thus is automatically excluded in both large and small deep lakes. However, electric fishing may be undertaken in the shallow margins of deep lakes, which in most, although not all cases, are vegetated. Vegetated may therefore be applied to submerged or emergent or even overhanging vegetation.

Table 1 may thus also reveal the suitability of the method when specifically applied in a particular circumstance. This is important when considering integration of different methods applied separately to the limnetic and littoral zones, or even to different habitats within these, especially in very large systems. Qualifications surrounding the applicability of the method in different circumstances and the quality of the output along the spectrum from qualitative to quantitative are provided within each section for each method.

Table 1. Applicability and quality of the estimate supplied by the different fish sampling methods that may be used in lakes of different characteristics. Applicability is represented as follows: !! = usually applicable, ! = often applicable, ? = sometimes applicable. The quality of the estimate is represented as follows: """ = generally quantitative, "" = often semi quantitative and " = qualitative.

Method	Lake characteristics					
	Large	Small (<2 ha)	Shallow (<3 m)	Deep (>3 m)	Open	Vegetated
Visual observation	? "	!! ""	!! ""		!! ""	? "
Underwater cameras	! "	!! ""	!! ""	! ""	!! ""	? "
Electric fishing	!! "	!! ""	!! "		! "	!! ""
PASE	!! """	!! """	!! """		! ""	!! """
Seine netting	!! """	!! """	!! """	? """	!! """	? ""
Trawling	!! ""	? ""	! ""	! ""	!! ""	? ""
Hook and line	!! "	!! "	!! "	!! "	!! "	! "
Gill netting	!! ""	!! ""	!! ""	!! ""	!! ""	! ""
Traps (fykes)	!! "	!! "	!! "	!! "	!! "	!! "
Hydroacoustics	!! """	? ""	?	!! """	!! """	
ARIS	! "	!! ""	!! ""	! ""	!! ""	? "
Proxy measures	? "	? "	? ""	? "	? "	? "

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Qualifications surrounding the applicability of the method in different circumstances and the quality of the output along the spectrum from qualitative to quantitative are provided within each section for each method.

2.2.1 Visual observations

In some circumstances, such as small, shallow, clear systems sampled in bright conditions, bankside observations may be used to provide a rough estimate of population size of especially large, readily observable species, such as Carp. In a small (3.95 ha) linear lake we sampled recently, an estate worker had estimated ~300 carp to be present from observations especially during the spawning season. From the known average individual weight from anglers' catches, an approximate biomass for the lake (500 kg ha^{-1}) could be derived. This was between the two estimates supplied from seine nets and PASE, but closer to that delivered by seines (83 kg ha^{-1}), which were otherwise thought to have underestimated the biomass of carp as a result of the presence of large quantities of submerged vegetation which affected the performance of the net in relation to fish near or on the lake bed. Potential issues of using PASE to estimate biomass of rare, large fish are discussed in 2.2.4 below.

It is possible that similarly useful observations could be made from a boat in good conditions, with even large, shallow clear water bodies sampled along a series of transects with a defined visibility distance from the boat akin to that used for surveys of marine mammals (Hammond *et al*. 2013) or seabirds at sea (Camphuysen *et al*. 2004).

Visual distance sampling has recently been applied underwater, and in the study of Pink *et al*. (2007) the use of this technique provided similar estimates to mark-recapture of individuals. The underwater census of fish along both fixed and roving transects using snorkelling or SCUBA that is particularly well developed in coral reef habitats may also be applied in clear lakes (see Brosse *et al*. 2001), in much the same way as has recently gained popularity for surveying submerged vegetation (e.g. Capers 2000). In fixed transects, the numbers and sizes of fish of each species occurring within a given distance of the predetermined transect line are recorded. This may only be around 1-2 m even in good visibility in many temperature lakes and obviously detection visibility becomes so small as to be useless in turbid lakes. It is thought that Secchi depth in the summer months in most SSSI lakes is <1 m and very rarely > 3 m (G. Madgwick *pers comm*). Roving transects are more qualitative as a result of being timed swims that begin at a random location, in which species but not numbers of individuals are recorded in different time intervals. This is a less useful technique in that species diversity is the main deliverable.

Point-counts provide semi-quantitative estimates in a similar way to PASE (see 2.2.4 below) by taking samples at a series of points at which the visible distance around the observer can be defined (Brosse *et al*. 2001). The numbers and sizes of fish swimming through the observable cylinder from lake-bed to surface are then recorded, typically over set time intervals. Estimates are semi-quantitative if there is no adjustment for encounter rate and swimming speed of the different species and sizes.

Overall, visual observations may provide useful supplementary information, particularly for large species that may be difficult to sample quantitatively by other means. It is generally not recommended that specific effort is made to undertake specific visual observations, but rather that any data gathered by lake managers or users should be used. Equally, planned surveys of aquatic vegetation could readily incorporate useful data of fish sightings.

2.2.2 Underwater cameras

Underwater cameras may be applied in a similar way to underwater visual observations (see 2.2.1 above) to produce semi-quantitative estimates of fish density with the camera operated by the observer or perhaps by a remotely operated vehicle (ROV). Underwater cameras have been previously used to assess various types of fish habitat both in the form of a camera mounted on a hydroacoustic transducer (Winfield *et al*. 2007) and on an ROV (Miller *et al*. 2015). A moving image in the form of digital video lends itself well to a transect sample design as is often undertaken in surveys of seabirds, although equally a series of stills may perform a similar function and where these are separated will allow greater replication (Buckland *et al*. 2012). Moreover, stills as periodic 'snapshots' (see Camphuysen *et al*. 2004 for the principles) may be used to eliminate the bias of different swimming speeds of fish relative to each other and the observer, and ultimately allow a more precise estimate of density.

Alternatively, cameras may be used in a more qualitative manner. For example, CEFAS have used underwater video to assess the efficacy of predator refuges in the form of underwater cages in coarse fisheries subject to the attentions of Great Cormorant *Phalacrocorax carbo* (CEFAS 2002, Natural England 2011). A similar system could potentially be used to confirm the presence of large fish. A still camera could be deployed in a similar way, perhaps with time lapse to take pictures at set intervals over a relatively long time period or in the form of a ‘camera trap’, with activation of the camera in the presence of a moving subject.

Overall, underwater cameras, like direct visual methods, have not been widely used. They do however, have reasonable potential, perhaps especially if used in a qualitative manner or as a relative measure to confirm the presence and relative abundance of fish that are difficult to survey by other means such as large benthivores such as Carp or piscivores such as Pike. A standard protocol would require considerable development and it may be better to use a system on an individual site basis. A key advantage of the use of underwater cameras where deployed and left *in situ* would be relatively small effort and cost if the equipment is already available.

2.2.3 Electric fishing

Electric fishing (or electrofishing) involves passing an electric current through water between an anode(s) and cathode. Different currents may be used, with DC being the more popular as it induces attraction (galvanotaxis) to the anode, where fish are temporarily immobilised and may be captured with hand nets. The higher power requirement for DC is overcome by the use of pulsed DC. Most systems operate at 50-100 Hz, although higher frequency output (600 Hz) is especially useful in waters of low conductivity (<50 microsiemens). Electric fishing may also be used in brackish as well as freshwater conditions by increasing current supply to the electrodes or reducing the size of electrodes, albeit at a cost of reduced stunning efficiency. Moreover, using high frequency square-wave pulsed DC with a short duty cycle Lamarque (1990a) was able to fish in water at conductivities to 40,000 $\mu S\ cm^{-1}$ using generators as small as 3 kVA.

It is the depth to which the anode can be inserted into the water and the depth at which fish can be retrieved with nets that determines the depth to which the method can be used. In practical terms this is around 2-2.5 m and preferably less. Electric fishing is therefore not suitable for sampling the entire fish community in deep open waters, although it could be employed to capture surface-dwelling species and individuals, especially at night should vertical migration be demonstrated by the species concerned. The experience of the one of the authors (IJW) with floating gill nets suggests that many species, apart from obligate benhivores such as Ruffe *Gymnocephalus cernuus*, may undertake vertical migration.

In shallow waters, electric fishing may be conducted along pre-determined transects in a semi-quantitative manner with the sampling area determined from the length of transect and the effective width (stunning radius – see PASE in 2.2.4 below) of the anodes. This width may be increased considerably by the use of multiple anodes along a boom, with several operators collecting stunned fish. The 'boom-boat' used is typically powered and set to cruise at a constant speed. Such systems have been developed particularly for use in large river systems and canals (e.g. Cowx *et al*. 1988). Efficiency in open water may however be relatively low should fish actively avoid the vessel, apart from small fish that have a slower swimming speed than the cruise speed of the vessel. However, there may be more confidence in more structured habitats where fish are not displaced and as a result of the galvanotaxis of electric fishing gear, in that fish are 'drawn' from cover (dense reed beds, tree root systems and macrophyte beds).

Electric fishing may thus be used to sample habitats that cannot be sampled by other active methods (see 1.1 above). In lakes, electric fishing is therefore seen as particularly useful in sampling the littoral zone (shoreline) and is incorporated into European Standards (CEN) in order to implement the European Union's Water Framework Directive (EU-WFD - 2000/60/EC) (2000) that includes fish as a biological quality element for assessing the status of inland waters. The European standard for fishing with electricity (EN14011) requires a minimum fished length of shoreline of >50 m (although Jaarsma 2007 states a minimum of 100 m). In Austria, more precise guidelines require 'small' lakes of <1 km^2)[2] to be sampled at >4 sites, with larger lakes sampled at one additional site per km^2 of surface area. Thus, in the study of Achleitner *et al.* (2012) a mean shoreline length of 288 m was fished amongst the 46 lakes sampled. This is very similar to the Dutch standard of 300 m (STOWA 2002). Such sampling delivers qualitative information of the fish species present and relative estimates of fish abundance and biomass. However, Jaarsma (2007) suggests the Dutch standard corrects the catch for the efficiency of the gear used, which for electric fishing is 30% for Northern Pike *Esox lucius* and 20% for all other species across all size categories from 0-5 cm to >40 cm. The population in the habitat sampled is then calculated according to the area of the habitat in the lake, which is combined with estimates of stock in other habitats through area weighted averaging.

This otherwise attractive approach would seem to be compromised by the simple assumption of efficiency of the gear used. Whilst this would probably be broadly acceptable between lakes with a similarly narrow margin width, it is arguably very difficult to compare between a

[2] It is of note that of the ~250 SSSI lakes, all but 11 would fit into this 'small lake' category (G. Madgwick *pers comm*.)

lake with a 30 m wide margin and another with a 2 m wide margin. In effect, the comparison is being made between the abundance of fish at the *edge* of the margin that can be sampled by the gear, with the underlying assumption that the distribution of fish is consistent in different margin widths. In fact, this seems unlikely to be true as given a choice fish may tend to penetrate further into the margin where this is wide. Moreover, even if it is assumed that the edge is being sampled in the same manner across different lakes, the calculation of density in the habitat as a whole assumes the density at the edge of the margin is constant across the margin as a whole. This may be far from the case for refuging fish for example.

An alternative approach to making an assumption about catch efficiency is to enclose the area sampled within stop nets (see Perrow *et al*. 1996b) and attempt to estimate the population in a more quantitative manner by successive runs akin to sampling in streams and small rivers, by applying a depletion model that does not assume that all fish are captured (Carle & Strub 1978).

Overall, 'free' electric fishing along a margin may provide an effective relative measure of the fish abundance, biomass and community structure especially where performed in the same manner over many years (see Townsend *et al*. 1986, Perrow *et al*. 1994). However, comparison between lakes is more problematic and the data delivered would be semi-quantitative at best. In this circumstance, it seems more worthwhile to use PASE (see below), which has an underlying quantitative basis. However, exploring a large area of margin, perhaps even all of it in a small waterbody (as Perrow *et al*. 1994) with free electric fishing is likely to be fruitful as a result of the capture of large individuals difficult to sample by other means. To confer a similar advantage, PASE would have to be conducted at a large number of frequently sampled points (see below).

2.2.4 PASE

Point abundance sampling by electric fishing (PASE) works by sampling a large number of small points of known area, which theoretically provides a more statistically robust result than small numbers of large samples (Garner 1997). The known area is derived by the *a priori* use of a voltmeter to determine the distance at which a voltage gradient of 0.12V is achieved, which corresponds to the minimum effective voltage at which inhibited swimming occurs to the anode (Copp & Peňáz 1988, Lamarque 1990b, Bird & Cowx 1993). Using a large anode of 45 cm in diameter to reduce the danger zone close to the anode and the prospect of fish mortality (Novotny 1990), Perrow *et al*. (1996b) calculated an effective area of 2.4 m^2.

PASE was initially designed as a method of sampling small fish, particularly young-of-the-year (YOY), in large rivers (Nelva *et al*. 1979, Copp & Penáz 1988). Cowx *et al*. (2001) showed that fish as small as 5 mm fork length were immobilised and captured by PASE. Samples can be randomly selected, systematically sampled (providing each point is independent) or stratified across different habitats. If carried out consistently, individual samples should be comparable in space and time. Given sufficient numbers of samples, from a range of habitats, the size distributions should be representative of those from respective populations and the variance around the mean density estimate should also be small enough to provide confidence in the results (Copp 1989, Persat & Copp 1990, Perrow *et al*. 1996b, Garner 1997).

The number of points required to produce accurate estimates invariably depends on the distribution and abundance of the fishes concerned. Persat & Copp (1990) suggested that as few as 25 points may be sufficient to provide a representative picture of the fish assemblage in a large river. In a more systematic investigation of data quality of assemblage abundance, richness, structure and biotic index with increasing sampling effort in medium and large-sized rivers, Tomanova *et al*. (2013) showed that 75 points was broadly adequate. However, under certain conditions such as a high frequency of fishless points, 100 points, the maximum sampled in this study, was recommended. Tomanova *et al*. (2013) also suggested caution of the use of PASE to generate abundance data as a result of the large variation in catch between points. However, they did not attempt to quantify the relationship between the number of points sampled and measures of abundance. Conversely, when sampling YOY fish, Garner (1997) suggested that a minimum of 50 samples was required to produce reliable estimates of fish density.

PASE has been employed for a wide range of spatial and temporal investigation of fish communities (see Copp *et al*. 2005ab, Daufresne & Boët 2007, Copp 2010). PASE has been shown to provide good agreement with depletion sampling in small- and medium-sized wadeable rivers (Pretty *et al*. 2003, Laffaille *et al*. 2005) in relation to both assemblage and population size. However, in the littoral margin of shallow lakes, PASE provided significantly higher total population estimates than equivalent depletion electric fishing within stop-nets, linked to the higher estimates for the dominant small fish (YOY Perch) and the higher rate of sampling for cryptic species such as Ruffe and European Eel (hereafter Eel) *Anguilla anguilla* (Perrow *et al*. 1996b). This and other studies (e.g. Perrow *et al*. 1999b, Skov & Berg 1999, Brosseau *et al*. 2005) provide further discussion of the relative virtues of PASE compared to standard continuous electric fishing in lakes. Given that a net is swept through the point sampled irrespective of whether stunned fish are seen means that PASE may be used in situations where water turbidity is higher than desirable for good catch efficiency for more general electric fishing, and may also be subject to less operator bias.

Other than a comparison between PASE and point-abundance sampling by SCUBA in the littoral zone of a reservoir (Brosse *et al*. 2001), there has been little comparison between PASE and other sampling methods. To this end, we have recently completed the analysis of comparative sampling by both PASE and seine netting of 13 relatively small lakes of 0.71-70 ha (mean of 16 ha) incorporating some SSSI lakes in the Norfolk Broads and the West Midland Meres as well as lakes on nature reserves, country estates lakes and water parks. One lake was also sampled in different years before and after dredging to provide a total sample size of *n*=14. Whereas most lakes were shallow (<2.5 m maximum depth), three gravel pit lakes were deeper with maximum depth of ~6 m. PASE was undertaken in both littoral and limnetic zones to produce estimates of fish standing stock as ind. m^{-2} and g m^{-2} (kg ha^{-1}) in each habitat, with these combined after weighting of samples by area to produce an overall estimate. The total number of points sampled ranged from *n* = 75-549 dependent on lake area, with sampling systematically undertaken over the entire lake. Comparisons between the littoral, limnetic and combined PASE fish stock estimates with those of the limnetic zone sampled by seines (*n* = 1-14 hauls in each lake) was undertaken. As a seine set from the bank only samples a limited part of the littoral margin that is effectively free from emergent and dense submerged/overhanging vegetation that would prevent the net being hauled, it was anticipated that the estimates between seines and the littoral PASE estimates would be less similar than the estimates obtained by seines and the limnetic PASE samples.

In the shallow lakes comprising most of the sample, seine netting was typically undertaken with a 50 m long and 5 m deep seine with 6.5 mm (maximum) knotless mesh in the central 'bag'. In some circumstances, the net was extended with one or two 25 m 'wings' to produce a net of up to 100 m in length. In the three gravel pit lakes, a 100 m net of 7 m in depth with a larger 13 mm mesh was used. In the majority of cases, hauls of the net were conducted from a suitably hard bank, although in three Norfolk Broads the net was hauled by EA staff from a large (~5 m) metal pontoon, with a net skirt preventing escape of fish underneath the pontoon. In these cases, three hauls of a seine net were conducted within another encircling net of similar dimensions. The catch from the three hauls was combined, with the contents of the first haul dominating the overall catch.

Wilcoxon paired tests showed no significant difference in numerical density or biomass estimates provided by PASE and seines using both PASE in the limnetic only, littoral only or for limnetic and littoral habitats combined, despite the significant difference in PASE littoral and limnetic estimates for biomass (Table 2).

Table 2. Comparisons between density and biomass estimates derived from PASE in different habitats and seine netting, using Wilcoxon's test for paired data. P values associated with the test statistics are also shown.

Parameter	Comparison	Wilcoxon's test results	
		V	*P*
Density	PASE littoral vs PASE limnetic	70	0.094
	PASE littoral vs seine	69	0.110
	PASE limnetic vs seine	41	0.502
	PASE combined vs seine	43	0.583
Biomass	PASE littoral vs PASE limnetic	75	**0.043**
	PASE littoral vs seine	66	0.168
	PASE limnetic vs seine	49	0.851
	PASE combined vs seine	51	0.727

Moreover, linear regression on natural log transformed (Ln (x+1)) data showed significant relationships between seine and limnetic samples and seine and combined estimates for both numerical (r^2 = 0.50, p<0.01 and r^2 = 0.53, p<0.01 respectively) and biomass density estimates (r^2 = 0.44, p=0.01 and r^2 = 0.46, p<0.01) (Figures 1 & 2).

As anticipated, the highest r^2 values and thus the best relationships were between the combined PASE estimates and seines. This is reasonable given that although seines do sample part of the littoral margin as well as the limnetic zone, albeit only an unvegetated part of the margin as they must be undertaken from an area free from emergent vegetation.

Conversely, there was poor correspondence between either estimates from littoral PASE and seines for numerical (r^2 = 0.12, p=0.24) or biomass (r^2 = 0.12, p=0.24) density (Figs 1 & 2 respectively) or indeed for PASE estimates of the littoral and limnetic zones for numerical (r^2 = 0.18, p=0.16) or biomass (r^2 = 0.26, p=0.07) density (not shown in Figs 1 & 2).

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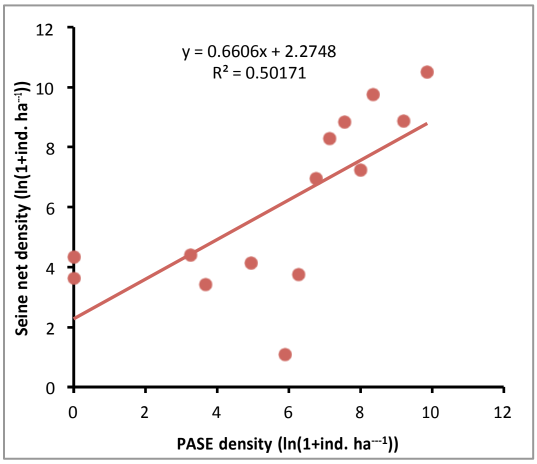
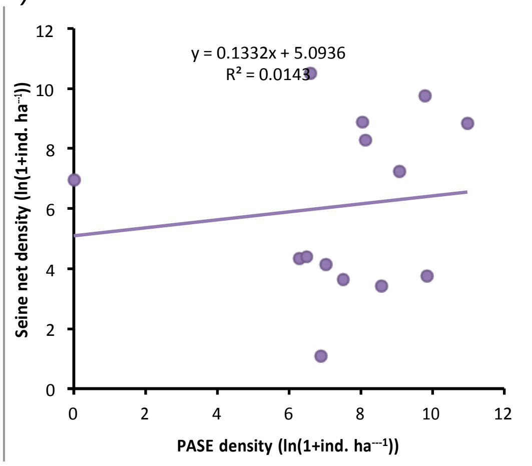
a) Seine vs limnetic PASE



b) Seine vs littoral PASE



c) Seine vs combined PASE

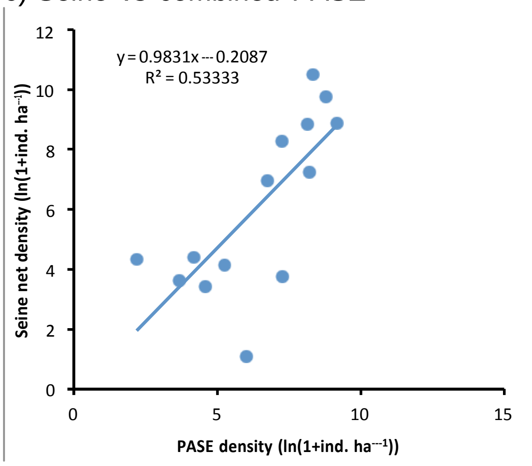
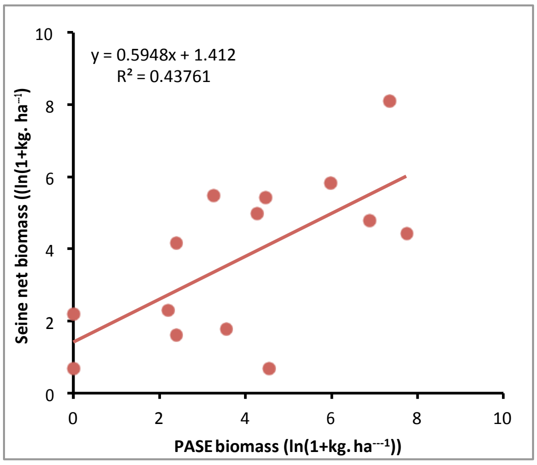
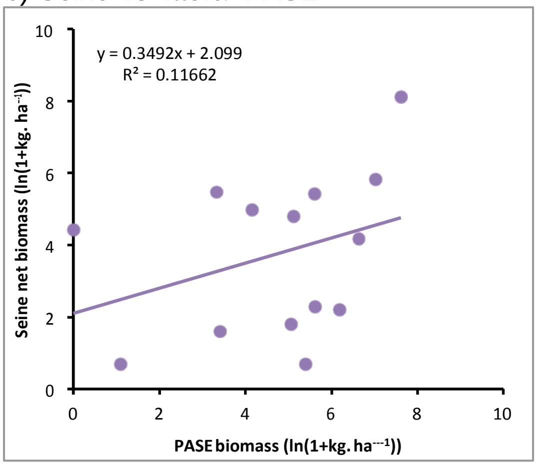


Figure 1. Linear regressions between natural logarithm transformed (ln (1+ind.ha^{-1})) density estimates derived from different sampling methods, showing: a) seine net vs limnetic PASE, b) seine net vs littoral PASE and c) seine net vs combined PASE estimate.

a) Seine vs limnetic PASE



b) Seine vs littoral PASE



c) Seine vs combined PASE

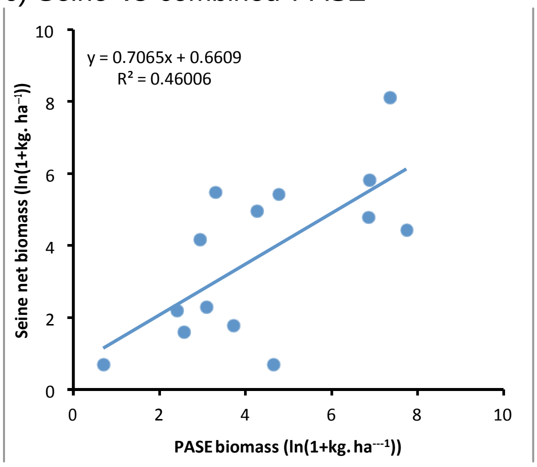


Figure 2. Linear regressions between natural logarithm transformed (ln(1+kg.ha^{-1})) biomass estimates derived from different sampling methods, showing: a) seine net vs limnetic PASE, b) seine net vs littoral PASE and c) seine net vs combined PASE estimate.

Conversely, there was poor correspondence between either estimates from littoral PASE and seines for numerical (r^2 = 0.12, p=0.24) or biomass (r^2 = 0.12, p=0.24) density (Figs 1 & 2 respectively) or indeed for PASE estimates of the littoral and limnetic zones for numerical (r^2 = 0.18, p=0.16) or biomass (r^2 = 0.26, p=0.07) density (not shown in Figs 1 & 2).

It is important to note however that estimates from limnetic or combined PASE relative to those produced by seines were not directly equivalent to each other. In the case of numerical density a density of 1 ind. m^{-2} equivalent to 10,000 ind. ha^{-1} as sampled by combined PASE corresponded to an estimate of 0.69 ind. ha^{-1} sampled by seine nets. A biomass density of 200 kg ha^{-1} equivalent to 20 g m^{-2} sampled by combined PASE corresponded to an estimate of 81 kg ha^{-1} (8 g m^{-2}) for seines. For PASE in the limnetic zone relative to seines, that is the direct equivalent sampling the same habitat, the estimates were 1 ind. m^{-2} compared to 0.42 ind. m^{-2} for numerical density and 200 kg ha^{-1} relative to 95 kg ha^{-1} for biomass density.

The relatively higher estimates of PASE relative to those obtained from seine nets may be linked to the possible underestimation of small fish, particularly young-of-the-year (YOY) by seines in relation to numerical density and by the overestimation of the biomass of large fish by PASE. In relation to the former, this may be linked to the escape of smaller individuals through seine nets (see Cowx *et al*. 2001). In relation to the overestimation of biomass, it is noteworthy that during sampling, large fish (to 7 kg or more) were captured during PASE despite the very low proportion of lake area sampled (0.1-1.5%). In other words, relatively rare, large individuals were encountered by chance during sampling of the entire surface area of the lake and potentially all, or virtually all of the habitats within it. However, capture of a large individual meant that the variance of biomass around a mean or median value was typically very large. Thus, there may be relatively low confidence in the estimate of biomass should large fish be included, with this tending towards overestimation, especially if more than one large fish is encountered.

Overall, PASE is a valuable sampling method, especially for generating quantitative estimates of the more numerically dominant smaller fish within species populations. It is also thought to be the most robust method of providing quantitative estimates in the shallow littoral margin of both generally shallow and deep lakes. Samples may be taken at a variety of locations within the littoral margin by working into it aboard a small boat or more exceptionally by wading (see Perrow *et al*. 1996c, Brosse *et al*. 2001) and the use of a long anode. The ability to sample in structured habitats compared to other methods also means that PASE is more or less the only means of generating quantitative estimates of fish populations in shallow lakes dominated by submerged vegetation. The relationship between the number of points and the accuracy of fish density estimates has not been fully investigated and is likely to vary under different conditions. Despite uncertainty, we recommend a working 'rule of thumb' to sample a minimum of 100 points wherever possible, accepting this may be limited by the very small size of the some waterbodies. For example, to maintain a spacing of 5 m between 100 points a margin length of 500 m is required, which broadly corresponds to a (circular) lake with an area of nearly 2 ha.

2.2.5 Seine netting

Seine nets have been a mainstay of commercial fishing since the time of the Phoenicians and Egyptians from the third millennium BC (Gabriel *et al*. 2005) and have been readily adapted for scientific fisheries sampling, where they remain a widely used sampling tool (Buckley 1987). In freshwaters, seines may be operated from a boat or pontoon providing a net skirt prevents the escape of fish underneath the hauling platform. More commonly, seines are hauled from the bank (beach seining).

A seine is essentially a wall of net with a float line at the top and a lead line on the bottom with a central bag (cod-end) or bunt (extra material incorporated into the build of the net), that is typically set in a broadly circular shape and then hauled by people or machine. Seines typically aim to sample the entire water column, apart from in the case of purse seines that are used in deep water to sample the upper part of the water column for more pelagic species (see 2.1. above). The mesh size of the net determines the size of fish that can be caught, with this often being smaller in the bag or bunt (often 5 mm in scientific sampling). Small mesh is however, readily clogged by plants, debris or sediment, thereby making the net more difficult to haul. In any case, seines can only be operated in water free from obstructions or snags unless these are small enough to be gathered by the net as it is hauled. Seines are not recommended for use in lakes containing submerged macrophytes, as these may be uprooted and destroyed and may anyway severely affect the performance of the net and its ability to sample fish effectively (see below). In such cases, seines may be restricted to the winter months after vegetation die-back.

Seines may theoretically be any length although a minimum length of 50 m to around 200 m is generally used. Seines smaller than this are much more limited in scope as they sample a small area of water and are generally only efficient for small fish, including YOY. Larger seines may require mechanisation to be hauled successfully. A seine should be at least 1.5 x deeper than the depth of water to be sampled to allow for billowing of the net as it is hauled without lifting the lead line from the lake bed. In essence, the balance between the resistance of the net (determined from mesh size and material carried by the net), the weight of the lead line and the speed the net is hauled is a fine one. It is all too easy to rush the hauling and for fish to escape underneath the net. Large, powerful fish may also push underneath the net or rise to the surface to attempt to leap over the float line as it is hauled. Partly for this reason, a seine may be operated within another seine operating as an encircling net, as a series of hauls to produce a combined catch or to apply a catch depletion model (see 2.2.3 above). For single haul seining STOWA (2002) suggest a constant efficiency of 80% for all sizes of fish including those >40 cm in length, assuming a suitable mesh size for the size category of fish is used.

Notwithstanding the potential for escape, seines may be more or less the best means of sampling large benthivorous or piscivorous species and thus provide the most realistic estimate of fish biomass at least in the limnetic zone. This is partly because seines sample a relatively large area of water increasing the chances of encountering large and generally relatively rare individuals. For lakes <10 ha STOWA (2002) recommend that >35% of the water surface is sampled by seines, with this proportion declining to 10% for lakes between 10-100 ha. It is better if this effort is divided between different hauls (>3) as this provides an estimate of variation around a mean value. It is also important to attempt to distribute the hauls around the lake and to ensure that a sufficient length of net is used to sample the centre of the lake rather than the edges at least in some hauls, if at all possible. This would help ensure a representative range of habitats is sampled, especially if the centre of the lake is considerably deeper than the margins. It is also critical to estimate the area contained within the net before it is hauled as accurately as possible as the shape of the set net changes the area contained within considerably.

Overall, as a large-scale method seine nets may supply quantitative estimates of all fish and especially larger individuals that may be of functional importance (e.g. benthivores or piscivores), in shallow unobstructed waters. However, the nets used must be of appropriate size (especially depth) and considerable care is required for the sampling to be efficient. Large numbers of fish may be captured, which demands careful handling and the provision of supplementary aeration within suitable holding tanks as the catch is processed. A further disadvantage is the need for a relatively large workforce, at least four persons and maybe twice that number when operating large nets.

2.2.6 Trawling

The use of trawls has only relatively recently been adapted for use in freshwaters from its large-scale origins in marine waters (Gabriel *et al*. 2005). Although trawls are often employed to sample the lake-bed and the waters immediately above it, trawls may in theory be operated at any water depth as a result of the use of buoyant floats or vanes in combination with towing speed to increase water resistance causing the net to maintain position in the water column.

Trawls are probably the most variable active gears in terms of size, shape and dimensions from fixed frame ichthyoplankton trawls designed to sample fry (perhaps 0.5 – 2 m deep by 2 m wide – see Juza *et al*. 2012), to fixed frame benthic trawls on runners with a tickler chain to disturb fish from the sediment as developed for Spined Loach *Cobitis taenia* (Perrow & Jowitt 2000) to beam trawls where the net is supported by a single bar of solid material (usually wood) across the upper edge of the net to keep the net open, to relatively large trawls that are kept open using otter boards, such as those with an effective opening of 7-10 m in width and 1-4 m in height commonly used in large, but relatively shallow Dutch lakes (e.g. Lammens *et al*. 2002). It is these latter nets that are the most useful for general assessment of fish stock in lakes.

The basic premise is that trawls are towed faster than the target fish can swim and are thus power-hungry requiring the use of relatively powerful vessels to tow the net. Lammens *et al*. (2002) used a tow speed of 1.5 m^{-2} (i.e. 5.4 km h^{-1}). The large size of trawls for general fish stock assessment also demands a large working platform to safely store and operate the net. The size of the vessel required would preclude the deployment and operation of trawls at many small-sized SSSIs, although it may be possible at large lakes where there is already an active boating fraternity with associated facilities such as a vessel ramp or wider connectivity to a large river system with boat access (e.g. the Norfolk Broads).

Trawls may not be used where there are obstructions that could snag and damage the net or where macrophyte growth is excessive. At lower density, macrophytes will simply be destroyed during sampling and could also affect the performance of the net. Water clarity can also affect performance with higher light intensity at the sediment surface being shown to significantly affect the catchability of percids such as Ruffe and YOY Pikeperch *Sander lucioperca* (Buijse *et al*. 1992). There was no effect of this parameter upon the catchability of Roach, Bream or Smelt *Osmerus eperlanus* however. For smaller fry trawls, net colour (white or black) was not important in the catchability of percids (Juza *et al*. 2012), sampling was conducted at 10-12 m and thus at relatively low light levels, although the ability of the target fish of 8-16 mm fish to avoid the net would appear to limited. Despite this, avoidance had previously been noted when the nets were used in shallower water. Adult fish also show clear avoidance of fry trawls (Juza & Kubečka 2007).

To combat any potential avoidance of trawls by fish sampling is routinely conducted at night in the Netherlands (STOWA 2002). Even so, trawls are seen to be selective according to the length of the fish sampled and the size of the net used. For the 10 m wide net described above, sampling efficiency is assumed to be 80% for fish of <25 cm in length, with this reducing to 60% for fish >25 cm. For the smaller 7 m wide net, efficiency is assumed to reduce to 30% for fish >40 cm in length.

Trawling becomes the method of choice for large lakes of >100 ha with the recommendation to sample 1-2% of the surface area in lakes to 10,000 ha. For even larger lakes, sampling 0.5-1% of surface area is thought to be adequate. In smaller lakes of 10-100 ha, trawls may still be used in some circumstances (especially where the necessary vessels can be utilised), although seines may be preferable. In these smaller waterbodies, the aim is generally to sample 2-10% of the water surface area, which is less than that suggested for seines (see 2.2.5 above).

There appears to be relatively few comparisons of trawling with other methods, although Jurvelius *et al*. (2011) showed that trawls provided estimates of the dominant fish species, namely Vendace *Coregonus albula* and Smelt in a large (110 km^2) oligotrophic Finnish lake, whereas the catches from gill-nets were too small to draw any conclusions on even species composition. Similarly, Allen *et al*. (1999) showed that shrimp trawls were more effective than traps for assessing populations of Black Crappie *Pomoxis nigromaculatus*, a percid sunfish, in two Florida lakes. Both traps and trawls captured small fish (<150 mm) but trawls sampled more adults of >250 mm.

Overall, trawling is particularly suited to large deep and shallow lakes, where there are few or no obstructions or macrophytes. The gear used may be adapted to the situation in which it is employed, but trawls at least 7 m wide fished at or near the lake bed appear to be most suitable from the general perspective of stock assessment. Trawling is typically both labour and cost intensive and with large nets can only be conducted from a relatively large vessel capable of substantial power. As such, trawling has been used rather rarely in UK lakes compared to large rivers or estuaries.

2.2.7 Hook and line

The use of hook and line during angling is a highly selective method that is not suitable for sampling the entire fish community, although if a range of gears (hook sizes and baits) were used then possibly all but very small species (e.g. Three- *Gasterosteus aculeatus* and Ten-spined *Pungitius pungitius* Sticklebacks) and individuals (e.g. YOY) could be captured. Hook and line can be a cost effective method of sampling large and/or predatory fish species in particular. It could possibly be used in conjunction with mark-recapture methods to estimate population size. Capture of known individuals in intensively managed fisheries may even provide minimum estimates of biomass density.

More indirectly, hook and line catches can be exploited through voluntary log book schemes involving local anglers. This approach has been used with great success for a number of years with Arctic Charr anglers on the large, deep lake of Windermere (Winfield *et al.* 2008), where hook and line shows high consistency with other sampling techniques for this salmonid species. However, this approach is of course entirely dependent on the cooperation of local angling stakeholders and lake owners, and as such may not be readily achieved in SSSIs where fisheries management practice may conflict, or appear to do so, with conservation objectives. Alternatively, conservation-minded user groups and owners may entirely support information sharing. In any case, considerable investment of time will be required to develop appropriate contacts and to manage the operation, including providing feedback to anglers.

2.2.8 Gill netting

Indications of relative fish density and biomass can be produced using gill nets if it is deployed in accordance with the guidance offered by European Standard EN 14757:2005. This technique is widely used around Europe and elsewhere as the main fish sampling technique in large, deep lakes (e.g. Jeppesen *et al.* 2012b, Argillier *et al.* 2013, Emmrich *et al.* 2014) and recent work has shown that when deployed with appropriate sampling effort it can produce abundance estimates which correspond strongly with those produced by hydroacoustics (Emmrich *et al.* 2012). In conjunction with hydroaocustics, following the CSM protocols for Arctic Charr and Whitefish (as Gwyniad, Schelly and Powan *Coregonus lavaretus* and Vendace) of Bean (2003a) and Bean (2003b), respectively, it has also been used with great success in large, deep lakes in the UK to deliver the CSM assessment of rare fish populations of Arctic Charr (Winfield *et al*. 2009), Gwyniad, Schelly and Powan (Winfield *et al*. 2013) and Vendace (Winfield *et al*. 2012).

However, gill netting has a major negative aspect in that it is almost always effectively destructive. Sampled fish are either killed during the netting itself, or injured by scale loss or decompression effects such that their subsequent survival is extremely low. Impacts of scientific gill netting following appropriate guidelines (e.g. Bean 2003a, Bean 2003b, European Standard EN 14757:2005) are not significant at the population level in large lakes, but they could be so in small lakes such as those intensively stocked with Carp for fisheries purposes. In shallow lakes where gill nets have to be set close to the water surface, there is also a significant danger of the unintended capture and drowning of diving birds and mammals.

As a consequence of the above issues, in England the EA has adopted a policy against the extensive use of gill nets and many other stakeholders such as angling groups and lake owners may be against their use. A similar approach is adopted in Wales by NRW, although the situation is less restrictive in Scotland under SEPA and SNH. Furthermore, limited gill netting is allowed and in fact commissioned by EA and NE in the large, deep lakes of Cumbria where it is the only sampling technique that can deliver scientifically robust samples of Arctic Charr, Schelly and Vendace. Given this background and even though gill nets would produce scientifically robust assessments in a diverse range of water bodies, our recommendations on sampling design presented below only include limited gill netting in those situations where no other biological sampling technique is feasible in deep areas and where the failure to use this technique would result in a significant data gap.

2.2.9 Traps (fykes)

Despite the extraordinary diversity (size, shape, design and construction materials) of fish traps used around the World (see Gabriel *et al*. 2005), virtually all of them operate on the ‘funnel’ or ‘maze’ principle with fish passing easily through an inviting entrance, but are then confused by blind endings within the traps and are unable to find their way out. Traps are a passive and selective technique with catches depending on a number of factors including temperature embedded in seasonal and diel variation, but also incorporating age, sex and reproductive status of the species of fish concerned as well as habitat availability (for examples of selectivity see Bagenal 1972 and Allen *et al*. 1999). The catchability of different species varies hugely with some species or groups tending to be more inquisitive or alternatively more likely to show avoidance of artificial structures. Catches are typically increased by the provision of bait within the trap, whilst invariably appeals to species with highly developed olfactory senses typically sported by scavenging catfish amongst others. Trapping provides qualitative or at best CPUE (see 2.2 above) estimates when performed in a standardised way within or between lakes.

In the UK and other parts of Western Europe, fyke nets, a traditional design for catching Eel, are often used as a low cost option to provide qualitative information on fish not easily captured by other methods. A fyke net is a series of connected hoops connected by netting with an internal series of funnels that concentrate fish in the final section of the net. A net leader of variable length connected to the first larger hoop, guides fish into the net. Fykes may be effective set perpendicular to the bank with the leader collecting fish swimming along the margin. Alternatively, fykes are traditionally set in gangs for Eels, with the leader of one issuing from the end of the net of another.

Dutch-style fykes with a large D-shaped first hoop of 1 m in height are good for larger fish such as Tench *Tinca tinca*, but their effectiveness for large fish is limited by the need to fit otter guards to prevent access by European Otter *Lutra lutra* that may drown in unguarded fykes. Workers in Norfolk have overcome this issue by fitting a large square net enclosure (a 'Bielby fyke') that projects above the water surface to the final section of the net, thereby retaining any fish but allowing a captured Otter to reach the surface to breathe.

In small lakes and ponds particularly, fykes have proved to very effective to survey species such as Crucian Carp *Carassius carassius* (Sayer *et al*. 2011). Although electric fishing (see 2.2.3 above) or PASE (see 2.2.4 above) could have been highly effective in the waterbodies concerned, this would have required a higher level of resources.

Overall, traps could be used as a low-cost option in particular circumstances to supply key information on particular species i.e. presence and possibly minimum number present, in a similar manner to visual observation (see 2.2.1 above) and angling records see 2.2.7 above). Previous experience of one of the authors shows that fykes may be used in conjunction with mark-recapture methods to estimate population size of selected species; Eel in a series of gravel pits in the case concerned. As a passive sampling method, fykes often have a relatively low catching power and so produce relatively small samples, although in particular conditions such as higher water temperatures and especially where fish are naturally aggregated (e.g. around spawning) or focussed by habitat features, they can supply surprisingly large catches.

The ease of deployment of any sort of trap adds to their value as a low effort option. This invariably reduces on larger and deeper water bodies where larger vessels and even specialist equipment may be required to set and retrieve traps.

2.2.10 Hydroacoustics

The application of hydroacoustics is particularly advanced in deep waters using vertically orientated acoustic beams, where accurate numerical and biomass density estimates may be derived. Guidance for this technique has recently been produced in the form of European Standard EN 15910:2014, which gives specific recommendations for technical settings, survey timings with respect to time of day (sampling at night is preferred) and time of year (sampling in summer is preferred) and other issues. Guidance is also given with respect to horizontally-orientated acoustic beams as required in shallow water bodies, although in the wider scientific community there is some debate regarding the efficacy of horizontal beam systems due to inherent features of their operation. Further careful evaluation of the horizontal application of hydroacoustics remains to be undertaken and as such, it cannot be recommended for use within a sampling programme requiring quantitative estimation of fish stocks.

A significant limitation of hydroacoustics is that it cannot identify the species of fish detected and some verification of the mixture of species and sizes of fish present needs to be provided by another method. Gill netting is typically employed in deep lakes and much work has been undertaken to relate gill net catch effort with hydroacoustic estimates of fish biomass (e.g. Emmrich *et al*. 2012). Where gill netting is restricted (see 2.2.8 above), catches derived from other methods could be used.

In conjunction with gill netting and following the CSM protocols for Arctic charr and Whitefish (as Gwyniad, Schelly, Powan and Vendace) of Bean (2003a) and Bean (2003b), respectively, hydroacoustics has been used with great success in in large, deep lakes the UK to deliver the Common Standards Monitoring (CSM) assessment of rare fish populations of Arctic Charr (Winfield *et al*. 2009), Gwyniad, Schelly and Powan (Winfield *et al*. 2013) and Vendace (Winfield *et al*. 2012).

On a practical level, it should be noted that fish hydroacoustic systems currently deployed on fresh waters require the use of a powered vessel to navigate consistent transects, although this vessel may be relatively small. However, Koprowski *et al.* (2013) recently described the deployment of a simple fish hydroacoustic system from a small radio-controlled boat, which has obvious attractions in the context of surveying small, shallow SSSIs where disturbance issues are an important consideration. We are currently in contact with Koprowski to evaluate further the potential use of this system in the present context.

In addition to fish applications, hydroacoustics may also be of use to produce a lake bathymetry and distribution map of macrophytes, both of which may be essential to guide the design of fish sampling using other techniques. The recent development of the BioBase system (www.cibiobase.com, Valley *et al.* 2015) offers a particularly rapid option for such an approach, including current explorations of deployment on a small radio-controlled boat. We are currently in contact with Valley to evaluate further the potential use of this system in the present context.

Overall, hydroacoustics is a valuable tool to quantify fish stock abundance and biomass and in large, deep lakes may represent the only cost-effective option. Here, vertically oriented acoustic beams may be effectively used. Whilst horizontally orientated systems have been used in shallow waters and show some promise, the experiences of workers in the field suggests that the results produced are often unreliable and further development of the technique is required before it can be recommended.

A vertically oriented hydroacoustic system is relatively expensive and requires highly trained operators to ensure its appropriate deployment and particularly the correct analysis and interpretation of resulting data. The inability of the method to distinguish between fish species may be overcome by application of the proportions of species sampled by another means, often gill netting. This is intuitively more likely to be accurate in simple fish communities containing one or two species, such as in oligotrophic systems, but it becomes more problematic to derive species-specific abundance and biomass estimates in more speciose communities that are typical of more eutrophic waters.

2.2.11 ARIS

Although in principle the ARIS system (known as DIDSON in its previous generation, www.soundmetrics.com) is simply another form of hydroacoustics, it does have a number of unique features, and as such is best considered as a separate technique. In particular, through the use of a much higher sound frequency than most other hydroacoustic systems, DIDSON and ARIS produce what is effectively a monochrome video recording of underwater features using only sound rather than light (e.g. Boswell *et al*. 2011). As a result the system can be used in complete darkness and is also effectively independent of water clarity. In terms of the present application to sample SSSI lakes, it may be deployed in turbid lakes and/or at night in the same way as might be a visual system in clear and illuminated water as discussed above. In this way, it could certainly be used to establish the presence of large-bodied fish species although quantification of their abundance would require considerable sampling effort and may only be a relative measure.

Like more conventional hydroacoustics systems, the ARIS system is relatively expensive, and as a result is not in common use. However, we understand that NE own an ARIS system and this could possibly be made available to NE contractors (G. Madgwick *pers comm*.). This is notwithstanding that the use of ARIS requires highly trained operators to ensure its appropriate deployment and particularly the correct analysis and interpretation of resulting data.

2.2.12 Proxy measures

Proxy measures to indicate fish numerical or biomass density include the nature of the zooplankton community that is structured by the density of small zooplanktivores such as Roach (e.g. Perrow *et al*. 1999a). However, it is considered unlikely that zooplankton data of sufficient resolution will be generally available from the suite of SSSI lakes to make this a viable approach. Similarly, the approach to relate various fish indices with measures of eutrophication (Argillier *et al*. 2012) relies on high quality water quality data and is also through to be too generic to be valuable, as it may be specific fish species (e.g. introduced non-native Carp) that become a particular focus of interest for SSSI lakes.

In the future, environmental DNA (eDNA) may prove able to detect and assess fish populations in lakes. The latter is currently a research area of great activity in Europe and North America (Rees *et al.* 2014, Goldberg *et al*. 2015) and one of the authors is currently involved in an exploratory project with EA and the University of Hull involving the taking and analysis of fish eDNA samples from the SSSI lakes of Bassenthwaite Lake and Derwent Water and Windermere, the fish communities of which are all also extensively studied by gill netting and hydroacoustics.

At this stage, rather than eDNA being used to determine the species composition and relative abundance of fish in SSSI lakes, it is intended that the proposed NE fish sampling project, contributes to the further development of the technique in a proposed EA-led project Thus, where conventional fish survey methods are employed water samples should also be collected to analyse for eDNA, so that comparisons can be made between conventional fish survey data and eDNA measures.

3 PROPOSED SAMPLING STRATEGY

3.1 Underlying principles

The review of sampling methods conducted confirms the accepted paradigm that different techniques have inherent strengths, weaknesses and biases. Accordingly, a number of authors (e.g. Winfield *et al*. 2009, Emmrich *et al*. 2012) have indicated that no one technique can be applied in all situations, and monitoring programmes incorporating several methods are most likely to be successful. As a consequence, the following sections provide a recommended sampling methodology with a division between shallow and deep lakes. The cut-off for a maximum depth of < 3 m and > 3 m between the two is slightly arbitrary in that all lakes deeper than 3 m will not necessarily stratify thermally and phytoplankton growth will not necessarily be controlled by nutrient availability. Similarly, phytoplankton growth in all shallow lakes will not necessarily be controlled by zooplankton grazing, as was the case in the small subset of lakes (some of the West Midland Meres) studied by Moss *et al*. (1997). Moreover, < 3 m and > 3 m maximum depth is not an obvious separation for many sampling techniques. For example, electric fishing is limited to waters of < 2-2.5 m and preferably less and seines are often used to depths of 5-6 m, although this does become more challenging. Vertically orientated hydroacoustics on the other hand, is generally considered to be effective only beyond 5 m depth due to the initial physical properties and narrowness of the acoustic beam and thus very small sampling volume for its first few metres. Consequently, there should be consideration of whether the lake to be sampled is best framed as shallow or deep, with consideration of the average and not just the maximum depth, the bed contours and substrate and the degree of likely colonisation of submerged macrophytes should these occur.

Moreover, we adopt the fundamental principle of fish stock assessment in the Netherlands and outlined by Jaarsma (2007) and encapsulated in Figure 3, that an attempt should be made to sample fish in the main habitat zones by whatever method is most suitable and then to combine the results according to the area of habitat sampled into a whole-lake estimate.

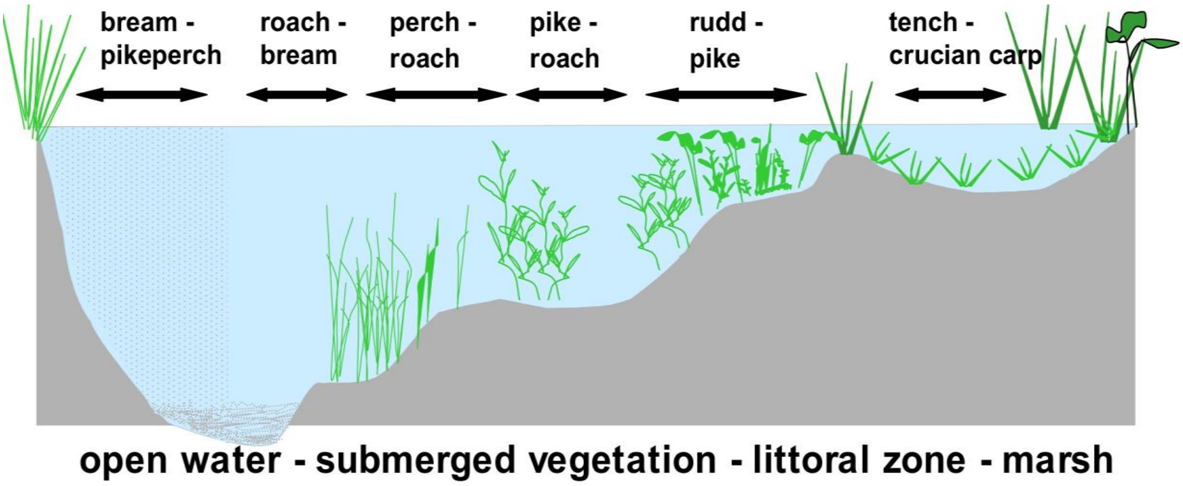


Figure 3. Generalised example of the major habitat zones and the fish species characteristic of those zones in speciose communities of shallow eutrophic systems in the Netherlands (reproduced from Jaarsma 2007).

In real terms, such a sampling model may be equally applied to both shallow and deep systems and the habitat zones may be effectively distilled into more general categories limnetic (open water and submerged vegetation in Figure 3) and littoral (littoral zone and marsh in Figure 3) as many lakes do not have such habitat diversity. There may be situations however where dense submerged, floating or floating-leaved (e.g. water lilies) macrophytes occur immediately offshore of a margin dominated by emergent or even overhanging vegetation. In such circumstances, such habitats are best viewed as being part of the littoral zone. In other situations in shallow lakes, submerged macrophytes may occur throughout the open water limnetic zone and would be incorporated within limnetic zone sampling. Moreover, in some deep lakes in particular but also in highly managed shallow lakes, the margin may be almost completely devoid of any vegetation, although it may be characterised by rocks and boulders especially in deep systems or woody debris. In some cases, a hard, artificial bank of one form or another may define the margin. Such habitats, especially if they are clearly distinguishable from those in the limnetic zone would still be classed as littoral.

3.2 Shallow lakes

3.2.1 Littoral zone

As derived from the classification in Table 1 using the entries for ‘shallow’ and ‘vegetated’ with refinement in the discussion of each sampling method, only a few methods could potentially be suitable to supply estimates of fish abundance and biomass in the littoral zone. These are electric fishing and PASE, visual observations including by snorkelling/SCUBA, cameras, ARIS, hook and line and traps. Of these, electric fishing, PASE and visual observations have more obvious potential to supply quantitative estimates. Visual observations such as that applied by Brosse *et al*. (2001), whilst not without potential, have not been applied in a wide variety of circumstances. There is also potential for observer bias and few, if any, contractors could immediately apply the method without further development with trial sampling. More problematically, visual observations cannot be used effectively in turbid conditions.

Whilst electric fishing and PASE become more limited as water clarity reduces, PASE is less constrained as the net is swept through the sampled point collecting fish even where none are seen. Simply sampling the margin using electric fishing is also not truly quantitative without the use of stop-nets (Perrow *et al*. 1996b), which then becomes very time-consuming. Coverage of the lake would then be very limited, with a tendency to sample only part of the range of available habitat types where these are variable. Such sampling is also vulnerable to patchily distributed fish stocks, with favoured areas perhaps missed by chance.

PASE on the other hand, attempts to provide a quantitative basis for the samples by defining an area of influence partly compensating for the effects of water conductivity, and is typically undertaken at points around the entire margin, unless the size of the lake becomes prohibitive. The technique is also flexible and may be undertaken by boat or even by wading (Brosse *et al*. 2001), although the former is preferred for reasons of safety.

In general then, PASE is recommended as the principal method to sample the littoral margin of shallow lakes. The same conclusion is reached for sampling deep lakes (see 3.3.1 below) with the same protocol as defined below. In order to match the recommended timing of hydroacoustic surveys in deep lakes, all PASE should be conducted concurrently with the other methods for a specific shallow or deep lakes in the general period from mid-July to mid-September as recommended by STOWA (2002).

3.2.1.1 Survey protocol for PASE

Prior to the start of the survey, the effective area around the anode must be measured in order to provide quantitative density estimates. This is achieved by recording the distance from the anode that the voltage gradient decreases to 0.12 V per cm, which is the minimum effective voltage at which inhibited swimming occurs (Copp & Peñáz 1988, Bird & Cowx 1993). During the process, the output of the box is adjusted to provide a suitable distance around the anode, in keeping with typical and desirable output from the system, that is, to stun fish effectively at a low ampage. For example, an effective stunning distance of around 50 cm from a 40 cm anode (Figure 4) would provide an effective sampling area of around 1.5 m^2. This values obtained may be validated by visual observation of the distance from which fish appear to be drawn to the anode in the waterbody being sampled. Fish clearly originating from outside the effective area or those stunned at the cathode should be discounted.

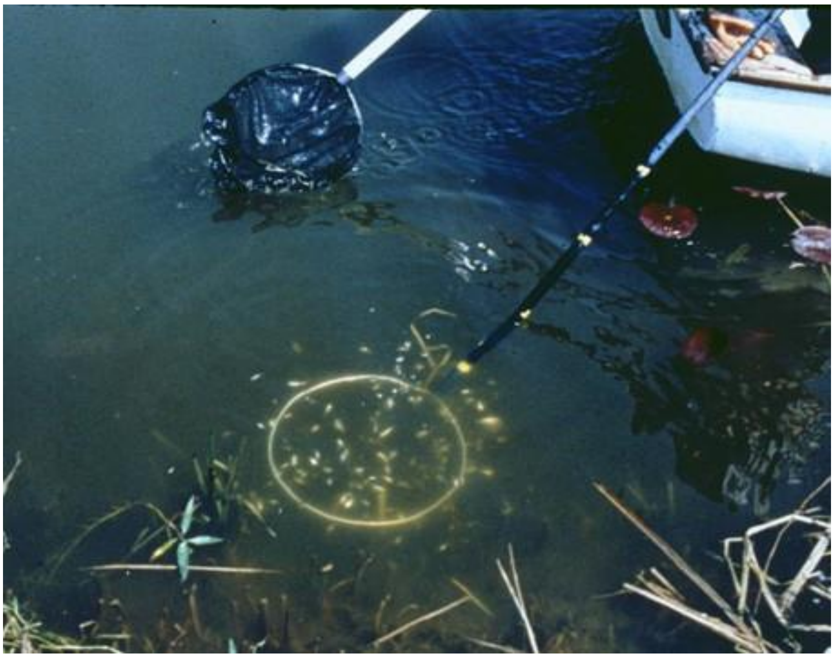


Figure 4. Example of an effective area around an anode, bearing in mind that fish will have undertaken inhibited swimming to the anode before becoming immobilised. **© Martin Perrow.**

PASE should be conducted from a suitable boat, with the electric fishing operator in sight of the person controlling the boat at all times, thereby allowing the vessel operator to apply appropriate safety procedures where required, including immediate power shut-down. A standard approach adopted over the last 20 years in the Norfolk Broads subject to long-term fisheries monitoring, has used a 3 m fibreglass dinghy operated by push-rowing from the bow and the operator fishing from the stern (Figure 5), with the ability to manoeuver rapidly to capture any stunned fish (Perrow *et al*. 1996b).

Figure 5. Example of a suitable boat and setup for PASE with the boat controlled by push-rowing and the operator in sight of the oarsperson at all times for safety reasons. © Jane Madgwick Broads Authority.

The electric fishing box and generator should be from an approved supplier and have an up to date service record. Operators should be trained in electric fishing and safety procedures and wear serviced automatic lifejackets at all times. The operator should also wear non-conductive gloves as standard. The EA recommends use of a low frequency (20-40 Hz) output (G. Peirson *pers comm*), although high frequency output (400-600 Hz) has previously been shown to produce good attraction to the anode without fatigue, which could otherwise cause injury (Lamarque 1990b). In this context, electric fishing units without adjustable frequency that produce 50 Hz as a standard output are thought to be acceptable, whereas an output of 100 Hz is generally thought to be more damaging to fish and is not recommended.

The entire littoral margin should be covered by the survey, with points sampled between 5 and 20 m apart depending on the size of the lake. The location of the sampling point should reflect the depth of the littoral margin, that is, where sections of the littoral margin exceed the reach of the anode an effort should be made to move the boat deeper into the margin in order to sample its entire width over a series of points. An anode with a minimum effective length (i.e. beyond the hand of the operator on the switch) of 2 m is required to reach ahead of the operator beyond the distance at which fish may respond to the presence of the boat. The anode ring should be a minimum of 380 mm and preferably the largest that can be safely handled in order to reduce the 'danger zone' for fish close to the anode (Novotny 1990). This is within the limitations of very conductive waters where it may be necessary to reduce the size of the anodes to keep the power demands within the abilities of the electric fishing unit and generator (Zalewski & Cowx 1990).

At each point, the anode is rapidly immersed and stunned fish gathered using a lightweight, long-handled (minimum of 2 m) net. Power should be maintained at the point for 10 seconds. The net should be swept through the area at each point, thereby collecting stunned fish even when no fish are seen, thereby reducing any observer bias and compensating for reduced water clarity. The electric fishing operator should wear polarising glasses and a shading hat or cap to improve visibility and focus.

All fish caught are identified to species level, measured to the nearest mm (fork length) and any particular characteristics of individual fish noted including any ailments or obvious parasites. Weight estimates can be calculated from length-weight regression relationships although any large specimens and Eel should be individually weighed. A large catch should be retained in a suitable container (e.g. a large plastic bin) within the boat as individuals are processed with all fish returned unharmed back into the water at the point sampled.

The location of the sampling point should be recorded on dGPS, along with an estimation of the depth of the littoral margin available to fish and the composition of the vegetation within a visualised transect of 2 m width back to the lake shore. From this, the mean width of margin is estimated which is an essential component of determining the area of littoral margin (see 3.2.3 below).

3.2.2 Limnetic zone

As derived from the classification in Table 1 using the entries for 'shallow' and 'open' with refinement in the discussion of each sampling method, a wide range of methods could potentially be suitable to supply estimates of fish abundance and biomass in the limnetic zone of shallow lakes including seines, trawling, electric fishing (especially from a 'boom' boat), PASE, visual observations, cameras, ARIS, hook and line, gill netting and traps. Of these, only seines, trawling and PASE would tend to produce quantitative or at least semi-quantitative estimates. Compared to seines, trawling is less well suited to shallow systems and becomes impractical in all but the largest systems with access for a suitably large and powerful vessel. The case study of 13 lakes presented shows PASE may produce broadly similar abundance and biomass estimates to seines (see 2.2., although there is concern of the variation around biomass values caused by the capture of larger fish. Sampling of a much larger area of water with seines (a mean of 12.5% of lake area compared to 0.6% for both littoral and limnetic points in the case study) increases confidence for the capture of large, rare individuals and species, which is fundamental to the need to estimate the stock of large benthivorous fish and, to a lesser extent, piscivores. The use of seines does however require considerably more resources in terms of personnel in particular.

3.2.2.1 Survey protocol for seine nets

Nets with 6.5 mm (maximum) knotless mesh in the collecting bag or bunt are recommended in order to sample most fish present. Experiences of the EA suggest that such a net will effectively sample fish of a standard fusiform body shape such as Roach down to ~60 mm body length (G. Peirson *pers comm*). The mesh may be larger in the 'wings' to promote the escape of fine sediment should this be present. The net itself should be a minimum of 50 m in length as large fish are likely to escape from smaller seines as they are set. In large lakes, longer nets of >100 m in length or more are required to reach the middle of the lake, unless a moveable pontoon is available. Hauling a net of the size recommended should not be conducted from a small boat from a safety perspective. When netting from a pontoon, a net skirt reaching the lake bottom must be used to prevent the escape of fish in the sampled area of the set net underneath the pontoon. Otherwise a seine net should be hauled from the bank.

Care must be taken to sample a number of locations around the lake in order to sample the range of depths and localities available, as species such as Bream occur in shoals that tend to favour particular localities. A minimum of three hauls should be conducted, with more hauls as required in order to sample >35% of the water surface in lakes <10 ha in size, with this proportion declining to 10% for lakes between 10-100 ha (STOWA 2002). It may be difficult to find suitable locations to haul a seine in some lakes and there may be a need to adapt in terms of the length and shape of net that is set. For example a shorter net of 50 m may be used in a tricky location, with a longer net set in an easier one, and where there is limited access to open water through the littoral margin, the set net may need to be a longer, more elliptical shape rather than the preferred circular shape.

Seines are set from a suitable boat, with the size and shape of the area of water enclosed accurately recorded in order to calculate abundance and biomass estimates for each haul. On-board dGPS coupled with photographs from the bank will help determine the area enclosed within the net of known length. After setting, the net is hauled by the float line at an even and steady pace by all personnel keeping the catching bag or bunt in the centre of the net (Figure 6). A minimum of four personnel are required to haul seines effectively, with this increasing to 6 or 8 personnel for larger (longer and deeper) nets, especially in soft muddy substrates where a considerable amount of sediment may be retained in a net with relatively small mesh.

Figure 6. Example of a 60 m seine-net being hauled in a turbid recreational lake with a soft bottom. Note the fourth person is taking the photograph © Martin Perrow.

When the net is approximately within 3 m of the bank or pontoon, two personnel rapidly haul in the lead line on both sides of the net keeping this as low in the water as possible to create an enclosed area. At this stage, the other surveyors hold the float line up to prevent it from submerging, especially where a large amount of fine sediment has been retained. Fish enclosed in the net can then be removed using long handled nets (Figure 7) and transferred then to large water filled drums that should be aerated with or oxygen or airstones powered by a large leisure/car battery or a generator. Larger fish should be separated from small ones. It may be necessary to remove fish from the net sequentially where the catch is large or a large amount of sediment has been retained, with the net worked to jettison sediment. different species in the rest of the catch that is bulk-weighed may then be calculated.

Figure 7. A catch of 81 Common Carp to 9.2 kg in a 60 m seine-net, prior to removal and processing. Note this is the same lake as in Figure 6 © Martin Perrow.

The final stage should involve the net being lifted onto the bank with few, if any, fish retained by this point. The welfare of fish is paramount throughout this process and critical decisions may need to be made in relation the time fish may be retained for processing. The aim should be to identify, count and measure all fish as described for PASE (see 3.2.1.1 above), although this may only be practical for all large fish and sub-sampling of smaller fish may be required in which batches are identified to species, counted and weighed. The number of individuals of in the rest of the catch that is bulk-weighed may then be calculated.

3.2.3 Estimation of fish stock

Separate numerical and biomass density estimates of the different fish species are initially calculated for both the littoral margin and open water respectively. This is achieved by using the mean density (ind. m^{-2} or ind ha^{-1} and g m^{-2} or kg ha^{-1}) by point or haul in the littoral and limnetic respectively, and multiplying by the area sampled by each technique. The relative area of each habitat sampled by each technique is then required. This is best achieved by first determining the area of the lake, which may be available or may have to be calculated using GIS. An accurate measure of the perimeter of the lake is then required using a combination of Google Earth and GIS for example. The mean width of the margin determined from the PASE multiplied by the length of lake perimeter allows the area of the margin to be determined, which is subtracted from the total lake area to also provide the area of the limnetic zone. Individual species population and total estimates for littoral and limnetic zones may then be calculated using the mean estimates for each, with these totals for each zone then combined to produce whole lake estimates. The variance around the mean estimates for each of the different zones provides some indication of the precision of the sampling. Otherwise, by the nature of the calculation, whole lakes estimates have no measure of variance associated with them.

3.3 Deep lakes

3.3.1 Littoral zone

The principles established to sample the littoral zone of shallow lakes in 3.2.1 apply equally to deep ones, and as a result PASE is to be undertaken in exactly the same way as specified in 3.2.1.1 above and incorporating any definition of littoral margin established in 3.1 above.

3.3.2 Limnetic zone

As derived from the classification in Table 1 using the entries for ‘deep’ and ‘open’ with refinement in the discussion of each sampling method, a wide range of methods could potentially be suitable to supply estimates of fish abundance and biomass in the limnetic zone of deep lakes including seine netting, trawling, visual observations, cameras, hydroacoustics, ARIS, hook and line, gill netting and traps.

Of these, only seines, trawling and hydroacoustics would tend to produce quantitative or at least semi-quantitative estimates. However, it is thought the use of seines will generally be precluded by depth, especially if this is over 6 m and trawling is unlikely to be cost-effective unless sampling concerns very large systems with access for or the existence of suitable vessels. Fish abundance, biomass and the approximate size distribution of those fish in the limnetic and profundal zones is thus best determined using vertical hydroacoustics.

In some, particularly deep SSSI lakes where the offshore fish assemblage may be taxonomically different from the inshore fish assemblage and contain unique species, a strong case can be made for the use of limited survey gill netting in deep waters, although this would be subject to obtaining appropriate permissions from EA and potentially other third parties. However, the deep lakes identified for future survey by the current project are unlikely to contain such species and it is assumed that there will be no requirement for gill netting, and this is covered below only for completeness and potential future application.

3.3.2.1 Survey protocol for hydroacoustics

Hydroacoustics with the transducer orientated vertically should be performed in accordance with the rare fish monitoring protocols of Bean (2003a) and Bean (2003b), with technical details meeting the more recent and more technically detailed general fish monitoring guidance of European Standard EN 15910:2014.

In summary, these guidelines require that surveys to be undertaken using a calibrated split- or multi-beam hydroacoustic system operating at a sound frequency of between 38 kHz and 1.8 MHz. The survey should be carried out over a series of pre-planned transects of either systematic parallel or zig-zag design from an appropriate survey vessel bearing in mind potential access issues at some relatively small or remote lakes. CEH use a 4.8 m rigid-hulled inflatable boat (RIB) that needs to be deployed from a trailer into the water, although it is also possible to successfully use a hydroacoustic system on considerably smaller vessels. The vessel should be powered with a low noise, preferably four-stroke or electric (if feasible) motor. Cruise speed should be a maximum of 10 km h^{-1}.

Night-time surveys should be conducted whenever possible because at such time fish are typically more effectively recorded by hydroacoustics. Time of year also has a significant effect on the results of hydroacoustic surveys and so should be standardised, preferably to the summer months of July and August as recommended for the UK by Bean (2003a) and Bean (2003b).

Figure 8. A hydroacoustics system deployed on a 4.8 m RIB. Sound is projected and recorded by a transducer mounted on a vertical pole to the side of the vessel, with a GPS receiver mounted at its top to provide positional information. The system's surface unit is visible as a grey case in the centre of the vessel, with a controlling laptop computer on top of it. The system is powered by a single 12 V battery visible in a blue casing towards the stern of the vessel. © Ian Winfield.

3.3.2.2 Survey protocol for gill netting

Any survey gill netting accompanying hydroacoustics to verify the species and sizes of fish present should be performed in accordance with the rare fish monitoring protocols of Bean (2003a) and Bean (2003b), with technical details of net design also conforming to the more technically detailed general fish monitoring guidance of European Standard EN 14757:2005. This entails using benthic and pelagic versions of the Norden survey gill net as described by Appelberg (2000). The benthic version is bottom-set and is approximately 1.5 m deep and 30 m long, with 12 panels of equal length having bar-mesh sizes 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm, respectively. The pelagic version, which is set floating from the water surface, is approximately 6.0 m deep and 27.5 m long, with 11 panels of equal length having bar-mesh sizes 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm, respectively. However, the levels of sampling effort recommended by European Standard EN 14757:2005 are generally unacceptable in the UK and so it is instead recommended that nets are deployed at each lake in a minimum pattern of three inshore nets, three offshore bottom nets and three offshore surface nets. Winfield *et al*. (2009) and Winfield *et al*. (2013) provide examples of such UK deployments for Arctic Charr and Vendace respectively.

With the exception of any large salmonids or eels still in good condition which should be measured (fork length, mm) before being released alive, all fish caught should be removed from the nets and killed, where practical by overdose with 2-phenoxy-ethanol, and then provisionally identified and enumerated for each net before being frozen at -20 ◦C to await future processing in the laboratory. At a later date and after being partially thawed from storage, all fish should be definitively identified, enumerated, measured (fork length, mm), weighed (total wet, g) and sexed before hard parts such as scales, opercular bones or otoliths are removed for potential subsequent age determination.

3.3.3 Estimation of fish stock

The principles established in 3.2.3 above with different estimates from littoral and limnetic zones to be combined into an overall estimate also hold for deep lakes where sampling is conducted by PASE in the littoral margin and hydroacoustics survey fish the limnetic zone. Like PASE, estimates of fish numerical and biomass density derived from hydroacoustic estimates are typically presented as mean values with 95% confidence limits based on densities recorded on individual transects or some other sampling horizontal unit. The difference to 3.2.3 above is that in order to establish the contribution of the different species to the hydroacoustic estimates, the relevant contribution of the different species will have to be determined from their contribution to each density estimate from PASE samples.

Care must be taken with this approach however, as the assemblage in the different habitats may be very different, especially if the littoral zone is heavily vegetated with emergent plants in particular. For example in the study of Volta *et al*. (2013) in Lake Montorfano in Italy (51 ha in area with a maximum depth of 6.8 m) Bream dominated catches in open water (62%) whilst Pumpkinseed *Lepomis gibbosus* dominated the catch in the dense reed-dominated littoral zone (85%), where only few bream were captured (1.1% of catch). In particular, it may have to be assumed that littoral specialists such as Pike are not present in the limnetic zone or at least in lower numbers. However, the fact that larger Pike are not tied to vegetated, often littoral habitat, may also mean that biomass estimates do not have to be adjusted.

3.4 Overview of the survey programme

The recommended survey programmes for both shallow and deep lakes adopt the principle of the use of separate sampling methods in the littoral and limnetic zones, with the results combined by respective area of these habitats to produce whole-lake estimates for each species and overall.

PASE is to be used in the littoral zone of both shallow and deep lakes, with the limnetic zone sampled by seines in shallow lakes and by vertical hydroacoustics in deep ones. Should abundant macrophytes be present in a shallow lake it may be advisable to wait until near the end of the overall survey period of mid-July to mid-September to see if macrophyte abundance is naturally reduced to an acceptable level to allow the use of seine netting. It is also possible that total (or nearly so) cover of emergent and overhanging marginal vegetation, abundant underwater obstructions and possibly even excessive fine sediment precludes the use of seine nets. In all cases, then PASE may be used to sample the entire lake. Gill netting may be required as a supporting tool in the limnetic of large, deep systems where it is suspected the assemblage in the littoral and limnetic zones are highly likely to be different and the partitioning of hydroacoustic density estimates by the relative proportion of the different species in the littoral catch cannot be justified.

In addition to the main methods, useful supplementary information may be supplied by anglers' catches, which can even be used to estimate abundance and especially biomass of large benthivores or piscivores, especially where a known number (or good estimate) of individuals is involved. However, the availability, quality and feasibility of such a source of information is likely to be highly lake-specific and so it is not possible to be prescriptive other than to note that a species list for the angling catch should be compiled as a universal first step. However, for deep lakes it is acknowledged that in most cases even such qualitative data is likely to be biased towards species of the littoral or limnetic zones due to spatial patterns of angling effort.

Any visual observations made during sampling or by stakeholders may also be incorporated in some circumstances. In some cases, information from traps, ARIS and cameras could also be used to supplement the results from the main survey methods. However, apart from the use of fykes, which could occasionally be included in a sampling programme, the use of cameras or ARIS or specific underwater observations using snorkelling or SCUBA will require specific dedicated effort. It would seem unlikely there will be an opportunity for this to be undertaken unless there are specific reasons to do so, such as the trial of a system in relation to a management technique, which could conceivably include the provision of fish refuges or an investigation of the efficacy of a fish barrier.

Moreover, it was intended that during fisheries sampling by the current project an attempt would be made to gather samples for a planned e-DNA project led by the EA. There are as yet no agreed standards for such water collection, but it is recommended that as a minimum 2L samples are taken from mid-water depths at three locations in each of the inshore and offshore zones of the lake. The sampling device should be sterilised between samples and the water filtered immediately on return to the laboratory and the filtrate put into appropriate storage.

4 PROSPECTIVE SITES

4.1 Rationale for site selection

The tender specification for the current project suggested that a rationale for the selection of sites to be sampled at a later stage (2015/16) would be developed. However, the small amount of time available for the current project coupled with the relative lack of detailed information in the database for SSSI lakes supplied meant that this was not practicable. Rather, it was agreed that a short-list of sites could be derived from the views of the steering group at the inception meeting held on NE offices in Peterborough on 11th December.

The steering group for the project comprised Project Manager Genevieve Madgwick (NE/EA Lake Restoration Specialist), Ruth Hall, Stephen Arnot, Christopher Evans and Rob Cathcart of NE, and Graeme Peirson, Research Scientist (Fisheries) for the EA. The majority were in face-to-face attendance with Ruth Hall in telephone conference, although Rob Cathcart was unfortunately unable to attend. Martin Perrow and Ian Winfield represented the contractors undertaking the project for NE.

Although it was anticipated that a draft short-list of sites could be derived from the 'hands on' knowledge and understanding of key SSSI lakes of the steering group, at the inception meeting, this proved not to be possible. Instead, it was agreed that Gen Madgwick would consult regional staff with responsibility for, and knowledge of, the SSSI lakes in the database. During this consultation period of several weeks, information on a number of lakes was gathered, with particular focus on fish as the potential factors influencing current (failing) condition status.

Thus, as summarised in subsequent communication from Dr Peirson, the question to be answered by the project was:

"*Could fishery activity be contributing to unfavourable condition in these specific sites, for which prior information suggests this is likely*?"

Rather than the more general question:

"*Are the fish biomasses present in SSSI lakes impacting on favourable condition, specifically macrophyte communities?*"

For the latter, as a general principle, a range of lakes of varying condition status and fish stocks would require investigation, which was thought to be difficult with the limited time and budget available to the next phase of the project. To answer the former question, the primary purpose of sampling selected sites would be to see what is really there, bearing in mind the limitations of previous attempts to do so. Sampling would then be accompanied by an analysis to determine if fishery activity was really driving poor status.

4.2 List of sites

A total of 27 SSSI lakes with standing water interest were put forward, with all apart from one (Skelsmergh Tarn) in unfavourable condition (Table 3). Fish were thought to be responsible for the poor condition of twelve sites (44%), with the condition of a further two (7%) possibly linked to fish, although in fact, no definitive information was available for any site.

All lakes are however recreational fisheries that are known to have been stocked with fish, or suspected to have been stocked in the past as a result of the presence of non-native species, especially Carp, which were an important part of angling interest. Skelsmergh Tarn is of particular interest as a result of a current application to stock further coarse fish (Rudd). The majority (24) are coarse fisheries, with three trout fisheries. It is of note however that 12 sites, 11 of which fall within the series of lakes known as the West Midland Meres do have at least some fisheries information gathered in the period from 1987-2010 (Table 4).

The lakes selected vary in size from <0.5 to 75 ha, and with a maximum depth from 1-15 m, although the depths of two potentially deep reservoirs (Blackbrook and Cropston) is not listed in the database. Accordingly, 16 of the 27 lakes (59%) are classed as shallow.

Table 3. The SSSI lakes selected for sampling in the next phase of the project with the intensity of shading indicating priority sites (*n*=6), preferred sites (*n*=14) and possible sites (*n*=7).

SSSI lake details			Depth			Condition Assessment			Fishery			General notes
Lake	Region	Area (ha)	Max (m)	Mean (m)	Cat.	Status	Year	Fish	Rights	Key fish	Survey	
Skelsmergh Tarn	2	0.48	1		Shallow	F	2010		Kendall & District Angling Club	Tench		Permission to stock Rudd has been sought. Site under HLS Scheme.
Fenemere	6	9.4	2.2		Shallow	U-NC	2013	Y	Grinshill & Fenemere	Carp, and pike	Y	Management Plan (APEM) in place: fish exclosure experiment recommended. Poor water quality and macrophyte growth.
Bomere Pool	6	10	15.2	5.1	Deep	U-R	2010	Y	Fishing club?	Carp	Y	Fish management and stocking policy required. Site under HLS Scheme.
Cole Mere	6	27.1	11.9	3.3	Deep	U-R	2010		Syndicate	Large carp present		Thought to support a low fish stock.
Crose Mere	6	15	9.3	4.8	Deep	U-NC	2013	Y	Ellesmere Angling Club	Carp	Y	Angling club has 250 members. Large carp present and stocks could have contributed to macrophyte decline. Fish management and stocking policy required.
Netherwood Lakes (Lake 50)	7	2.5	<3		Shallow ?	U-D	2009	Y	Private owner	Carp		Poor water quality. Suffered predation from otters. Abundant *Elodea* sp.
Overwater	2	22	9.6	3	Shallow ?	U-NC	2010		Over Water Hall Hotel	?		Investigation into diffuse water pollution implemented. High fishing intensity indicates fish stocking.
Big Mere	4	4	2.4		Shallow ?	U-R	2011		Private owners	?		No longer a commercial fishery. Carp and Well's catfish reported to be present in 1990's.
Watch Lane Flash	4	7.5			Shallow	U-NC			Elworth Angling Society	Carp		High fishing intensity and contains carp, bream and 'silver' fish. Rumoured to have catfish. Poor water quality with few macrophytes, potentially brackish.

Tabley Moat	4	4							Lymm Angling Club	?		Angling club present and has high fishing intensity.
Tabley Mere	4	16	4.4		Deep				Lymm Angling Club	?	Y	Management Plan (APEM) in place: No more additional stocking. Lower fishing intensity than at Tably Moat. Fish densities considered to be at acceptable levels High nutrient levels and impacted by blanket weed.
Maer Pool	6	4.8			Shallow	U-R	2010		Maer Estate	Carp	Y	Management Plan (APEM) in place: remove carp, monitor tench populations and no further stocking. High nutrient levels due to diffuse water pollution.
Marton Pool	6	10.9	8		Deep	U-R	2011	Y	Marton Pool Holiday Home & Lodge Park	Pike	Y	Management plan required.
White Mere	6	23.5	14.2	4.4	Deep	U-R	2009	Y	Ellesmere Angling Club	General	Y	Management Plan (APEM) in place. Fish not attributed to impact on the site condition. Angling club has 250 members.
Oss Mere	6	9.5	3		Shallow ?	U-D	2010	Y	Prince Albert Angling Society	General	Y	Management Plan (APEM) in place: improve habitat to promote native fish population. Fish kills have been recorded. Microchalk applied to reduce nutrients (angling club) and investigation into effects of angling bait conducted (CEFAS).
Mallard Lake (Lake 52)	7	10	<3		Shallow ?	U-D	2009		Mallard Flyfishers Ltd	Trout	Y	Annual stocking of rainbow trout. Wiltshire Wildlife Trust have a management plan to retain the fishery and deliver biodiversity and SSSI remit. License requires the angling club to submit estimates of catch versus stocking. Unfavourable due to abundance of Elodea sp. with species present.
The Lake	13	4			Shallow	U-R	2013	Y	Lulworth Estate	Carp	Y	Carp (250 fish of 1-2.5lb), tench, chub, rudd, roach and crucian carp (< 3000 fish) stocked in 2002. Turbid when fish density is high. Macrophytes have declined. Site under HLS Scheme.

												Sparsholt College have undertaken removals of carp and other species, Numbers of carp are likely to remain.
Dry Pond	14	0.3			Shallow	U-NC	2009		Horsham & District Angling Associations	Carp		Fished but no stocking data.
Folly Pond	14	0.5			Shallow	U-NC	2009		Horsham & District AA	Carp		Tench introduced in 2015. No fish density / baseline data.
Chapel Mere		6.5	2.4		Shallow							Limited fishing intensity. No stocking of fish. Fish populations believed to not be an issue.
Comber Mere	4	51.5	12			U-NC	2012		Syndicate & guests	General		Impacted through diffuse water pollution. EA questionnaire carried out to gain a better understanding of the fish stocks.
Blackbrook Reservoir	5	32.3				U-NC	2014	Y	Blackbrook Fly Fishing Club	Trout		Trout stocked annually but no information available on the extent of stocking. Potential impacts of stocking unclear.
Cropston Reservoir	5	52.9				U-NC	2010	Y	Cropston Angling Club	Trout and pike		Brown and rainbow trout present. Judicial review regarding water transfers and potential influence on water quality. Potential impacts of stocking unclear.
Aqualate Mere	6	75.3	1.4	0.6	Shallow	U-NC	2013	Y	Syndicate	Pike (roach x bream hybrids)	Y	Potential for biomanipulation trial exclosure following dredging. Syndicate of 100 members, fishing intensity relatively low and number of boats restricted.
Betley Mere	6	9.3	1.8		Shallow	U-NC	2009	Y	Syndicate	Carp	Y	Trial biomanipulation instigated in 2015. High densities of small cyprinids believed to be responsible for poor water quality and low macrophytes. Carp also present. Syndicate of 30 fisherman – reduced fishing intensity.
Berrington Pool	6	2.5	12		Deep	U-R	2010	P	Berrington Angling Club	Carp	Y	Small club. Carp believed to stocked illegally, and anglers required to remove any carp caught. No evidence that this is occurring. Club and owner not receptive to carp removal. Fish survey requested and aim for fish removal.
Boundary Pond	14	<1			Shallow	U-R	2009	P	Horsham & District AA	Carp		Fish stocked by angling club.

Table 4. The SSSI lakes selected for sampling in the next phase of the project with most recent fisheries survey information from the period 1987-2010. Survey methods and the resultant density, biomass and species composition are shown. Species known or thought to be present but sampled are shown in parentheses.

Lake	Method	By	Date	Density	Biomass	Species composition
Bomere Pool	Echo-sounding	NRA	1987	-	-	Tench, Bream, Pike, Roach, Carp, Perch, Rudd, Eel, Brown Trout and Chub
Fenemere	Seine net & hydro-acoustic	APEM	2009	2.24 ind. $1000m^{-3}$	247 kg ha^{-1}	Eel, Bream, Perch, Pike, Roach, Roach x Bream hybrid, Rudd and Three-spined Stickleback
Crose Mere	Gill net	Manchester Metropolitan University	1995	-	-	Perch, Roach, Pike and Eel (Carp)
	Echo-sounding	NRA	1987	Low	-	
	Seine net	Sport & Leisure Fisheries / Liverpool University	1980's	-	-	Pike, Perch and Roach
The Lake	Seine net?	Sparsholt College	2010	-	-	Carp, Tench, Eel and Three-spined stickleback
Maer Pool	Hydroacoustic, seine net and electric fishing	APEM	2009	-	484.7 kg ha^{-1}	Tench, Perch, Pike and Carp
Marton Pool	Gill nets and echo-sounding	Manchester Metropolitan University	1995	-	-	Roach, Perch and Pike (Carp)
Oss Mere	Hydroacoustic, seine net and electric fishing	APEM	2009	-	61.6 kg ha^{-1}	Perch, Bream, Pike and Rudd
White Mere	Gill nets	Manchester Metropolitan University	1995	-	-	Roach, Perch, Ruffe and Pike

	Hydroacoustic and seine net	APEM	2009	4.6 ind. 1000m^{-3}	146 kg ha^{-1} (horizontal survey) 217 kg ha^{-1} (vertical survey)	Perch and Pike
Tabley Mere	Seine net and electric fishing	APEM	2009			Roach, Three-spined Stickleback, Bream, Perch, Carp and Roach x Bream hybrid
Aqualate Mere	PASE and seine net	ECON	2004	0.01 ind. m^{-2} (PASE) 0.01 ind.m^{-2} (seine)	6.9 g m^{-2} (PASE) 14.4 g m^{-2} (seine)	Bream, Roach x Bream hybrid, Roach, Pike, Eel, Three-spined stickleback, Bullhead and Perch
Berrington Pool	Gill net	Manchester Metropolitan University	1995	-	-	Perch, Roach, Bream, Tench and Pike
	Echo-sounding	NRA	?	-	-	-
Betley Mere	Hydroacoustic and seine net	APEM	2009	8.99 ind. 1000m^{-3}	335 kg ha^{-1}	Bream, Roach, Perch, Pike, Silver Bream, Tench, Roach x Bream hybrid and Three-spined Stickleback (Carp)

The majority of sites (17 – 63%) are West Midland Meres, with two within the Cotswold Water Park complex of lakes, and others ranging from Cumbria in the north to Sussex and Dorset in the south. The lack of selected lakes within the Norfolk Broads is the only notable omission.

In keeping with this distribution, four of the six priority sites are West Midland Meres, with one in the Cotswolds and one in Cumbria. Similarly, the meres contribute 10 of the 14 preferred sites. The two reservoirs in the series, both of which are stocked with trout are included within the 7 possible sites

5 CONCLUSIONS

The review of fisheries survey methods concluded that a combination of methods is best employed to provide quantitative estimates of fish numerical and biomass density in SSSI lakes. In addition, survey effort should be partitioned between littoral and limnetic zones in both shallow (< 3 m) and deep (>3 m) systems. PASE was selected as the best method to sample the littoral zone in both shallow and deep systems, with seine nets used to survey the limnetic zone in shallow lakes, whereas vertically-orientated hydroacoustics is the preferred method to sample the limnetic (and profundal) zone in deep lakes. In both cases, estimates for the littoral and limnetic zones were to be combined to produce a single whole-lake estimate for both numerical and biomass density.

A series of 27 lakes, the majority of which (96%) were in unfavourable condition with potential for this to be linked to previous fish stocking as a result of recreational angling interest, were selected for survey in the next phase of this project by NE staff. 'West Midland Meres' formed the majority (63%) of the suite of selected lakes, although the geographic coverage of the lakes was wide from Cumbria in the north to Dorset in the south. The lakes selected ranged from <1 – 75 ha in size.

The ultimate aim of survey was to determine if fishery activity was contributing to unfavourable condition in the selected sites, with a first step to establish the current fish stock. In fact, 44% of the sites had some previous fisheries survey information, although in most cases this was limited or at least suspected to be less than definitive.

It was thought to be unrealistic that all 27 sites could be sampled in the next phase of the project with an initial budget of £45,000. As a result, 6 'priority' and 14 'preferred' sites, that is 20 sites in total, was taken to be a maximum number of lakes that could possibly be sampled in the short term. The available information suggests that 13 (65%) of these 20 lakes may be classed as ‘shallow’, with 6 classed as ‘deep’ (30%) and one as yet undetermined.

The detailed sampling protocol for PASE, seine netting and vertical hydroacoustics provided in this report underpins a tender specification for the upcoming survey phase of the project. This specification is provided as a separate document accompanying this report. The specification also requests that tenderers state the means by which information gathered at individual lakes will be formulated into individual lake management plans and into some form of overarching analysis. However, as agreed at the steering group meeting of 11th December, the next phase of the project will not now include recommendations for fish removal trials and further investigation of the 200 kg ha^{-1} stocking limit, as was initially intended. These aspects will instead form the focus of future related pieces of work, with analysis of any relationships between fish density or biomass and macrophytes and perhaps other quality parameters undertaken with a larger dataset from a range of shallow and deep lakes.

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