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The population dynamics and conservation of Arctic charr, Salvelinus alpinus; lessons from Llyn Padarn, North Wales

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The population dynamics and conservation of Arctic charr, *Salvelinus alpinus*; lessons from Llyn Padarn, North Wales

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Date: 30/04/2022

'Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy. Rwy'n cadarnhau fy mod yn cyflwyno'r gwaith hwn gyda chytundeb fy Ngoruchwyliwr (Goruchwylwyr)

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ABSTRACT

Salmonids, such as Arctic charr (*Salvelinus alpinus*), are of high socio-economic and ecological value, but are in widespread decline because of anthropogenic disturbance, especially near their southern range edge in Wales. It is therefore essential to understand the threats these species face and identify appropriate management actions to secure persistence of populations across space and time.

To investigate deep-water thermal-oxygen habitat, I collated disparate data sets for lakes across the British Isles. Presence-absence models found the temperature at the 7 mg/l dissolved oxygen isocline (TDO7) was a strong predictor of Arctic charr occurrence. TDO7 values of 10, 12.5, and 15 °C are suggested as boundaries for classifying lakes as offering excellent, good, moderate, or poor-quality habitat. In stratified lakes where most populations reside, spatial and temporal analysis identified that elevated nutrients (total phosphorus and dissolved organic carbon) are associated with a lower suitability of thermal-oxygen habitat. At Welsh lakes there has been a significant trend toward stronger thermal stratification driven by warmer summers between 2007 and 2020. Significant declines in hypolimnetic oxygen were detected at two out of four Welsh lakes, whereas hypolimnetic temperatures have been stable. Llyn Padarn had the lowest oxygen concentrations of 31 lakes assessed.

The survival of eggs is critical to understanding the population dynamics of salmonids. However, studies of Arctic charr spawning habitat and reproductive success have been rare. Chapter 3 adds important new information to the sparse literature, including the timing and duration of fry emergence, the characteristics of spawning habitats, and data showing significant temporal variation in fry production at Llyn Padarn. The suitability of swim-up traps for sampling emergent fry was demonstrated and a new and apparently productive spawning site was discovered, indicating greater resilience to ongoing disturbances at the second spawning site.

In cases where an intractable environmental disturbance is causing population decline, supplementation may be the only action available to prevent imminent extinction. However, the efficacy of supplementation has been questioned by many; in particular, there are concerns regarding the impacts on population genetics. Chapter 4 involved analysis of population dynamics and genetics between before and approximately two generations after supplementation at Llyn Padarn. The results showed the census and effective population size has increased in recent years, and that this increase has been largely driven by supplementation,

rather than improvements in natural recruitment. Microsatellite markers did not detect a significant difference in genetic diversity between before and after supplementation or between wild and stocked adults. Likewise, both stocked and wild adults obtained a similar body condition and length, indicating an equal capability to exploit the wild environment. Thus, the supplementation programme has been successful in preventing seemingly imminent extirpation whilst maintaining favourable genetics. However, the reproductive contribution of stocked fish (i.e., fitness) remains unknown.

An important demographic parameter for conservation is the relative contribution of life-stage vital rates to population growth rate (λ). All other things being equal, management interventions should focus on life-stages with a large effect on λ . Analysis of a demographic model for Padarn charr indicated fry and juvenile survival are most important for λ and that larger adults (> 280 mm) are relatively unimportant (Chapter 5). However, comparisons of models constructed for 41 populations (Chapter 6) showed the importance of life-stages can vary substantially according to population life-history traits associated with climate and ecological niche. Accordingly, consideration needs to be given to the phenotypic life-history state of the population being managed.

The study represents a comprehensive investigation of topics pertinent to the conservation biology of Arctic charr and provides important information for managers at Llyn Padarn, and lakes with cold-adapted fish populations more generally.

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Chapter 1. Introduction

Declines in freshwater fish over recent decades are among the most dramatic measured for any vertebrate taxa (Ricciardi and Rasmussen, 1999; Winemiller, 2005; Darwall *et al.*, 2018). The status of salmonids is particularly precarious as they generally require pristine habitat, and are thus highly vulnerable to anthropogenic disturbance (Jeppesen *et al.*, 2012; Sinnatamby, Cantin and Post, 2020; Smialek, Pander and Geist, 2021). The observed and expected loss of salmonid populations is of great concern to human society as they provide vital ecosystem services, including food production through aquaculture and fisheries, and recreation in the form of sport fishing (Cowx, 2015; Darwall *et al.*, 2018; Liu, Bailey and Davidsen, 2019; Winfield, Berry and Iddon, 2019). Furthermore, their contribution to the ecological functioning and biodiversity of the depauperate habitats they typically occupy is recognised, if not yet fully appreciated (Adams *et al.*, 2007; Reist *et al.*, 2013). It is therefore essential to understand the threats these species face and identify appropriate management actions to secure persistence of populations across space and time. As illustrated by reference to the target species, Arctic charr (*Salvelinus alpinus*), such considerations rely on a sound scientific evidence base, several aspects of which are presented in the current study.

ECOLOGY AND DISTRIBUTION OF ARCTIC CHARR

Arctic charr are a cold-adapted salmonid with a circumpolar distribution and the most northerly of all freshwater fish (Klemetsen *et al.*, 2003). Populations compose a monophyletic group with a common anadromous ancestor that colonised freshwater habitats toward the end of the last glacial maximum ~10,000 to 15,000 ybp (Brunner *et al.*, 2001). At northern latitudes, many populations continue to exhibit an anadromous life-style; breeding in freshwater and migrating to forage in coastal waters each summer (Jørgensen and Johnsen, 2014). Further south, a migratory strategy is no longer pursued, with populations generally residing in oligotrophic, upland lakes (Klemetsen *et al.*, 2003). Isolated populations in small head water streams have been described at high latitudes in North America (McCart and Craig, 1973; Bain, 1975).

A notable feature of lake resident Arctic charr is the range of intraspecific diversity exhibited both between and within locations. The most famous example of within lake variability is that of Thingvallavatn, Iceland, where four different ecotypes are found: (1) a limnetic type that feeds on zooplankton in the lakes pelagic zone, (2) a larger piscivorous type that feeds on small fish including juvenile charr, (3) a large-bodied benthic type feeding on snails, and (4) a small

benthic type that retains juvenile characteristics throughout life (dwarf form) and forages among the interstices of cobbles in the shallow littoral zone (Sandlund et al., 1992). In addition to dietary and habitat preference, ecotypes vary in life-history traits (longevity, fecundity, growth, and age at maturity), breeding habits (time and location of spawning) and morphological characters, such as skin colouration, mouth shape, fin size, and gill raker number (Sandlund et al., 1992). Since the diversity of Thingvallavatn charr was first described, several similar polymorphic lakes have been identified, including Loch Rannoch in Scotland (Adams et al., 1998); lakes Skøvatn, Tårnvatn, and Skogsfjordvatn in northern Norway (Smalas, 2013; Kjaer, 2018), and most recently, a large lake in Greenland supporting five resident ecotypes and an anadromous form (Doenz et al., 2019). Such systems are considered among the best examples of early stage adaptive radiation, and are thought to have emerged because of the variety of ecological niches and absence of competing species in large and relatively young postglacial lakes (Schluter, 1996; Sparholt, 2000; Doenz et al., 2019). The range of ecological niches present unique selection pressures that favour certain traits. For example, it is proposed that the morphological traits of small benthic types are beneficial for exploiting feeding opportunities in shallow cobbled areas and for evading the predators that are present at higher densities than in other lake zones (Sandlund et al., 1992; Jonsson, 2000). Mouth shape of ecotypes is often divergent, reflecting different prey types (Jonsson and Jonsson, 2001). While other species of postglacial fish can display high levels of intraspecific variability (e.g., *Coregonus* spp.), it is considered that the phenomenon arises most frequently and dramatically in Arctic charr (Klemetsen, 2013).

A question of great interest is whether ecotypes within waterbodies are reproductively isolated populations or phenotypes sharing a single gene pool (Alexander and Adams, 2000; Jonsson and Jonsson, 2001). In some populations, polymorphism appears to be ontogenetic with no reproductive isolation or genetic differentiation among different forms (Nordeng, 1983). Piscivores may be individuals that grow rapidly and thus attain a size where predation on small fish becomes anatomically possible (Sparholt, 1985). In other populations, ecotypes (or morphotypes) appear to be reproductively isolated, with membership to a particular population determined to some extent genetically (Simonsen *et al.*, 2017; Doenz *et al.*, 2019). Reproductive isolation leading to genetic polymorphism may arise in sympatry because of differences in time and location of spawning (Child, 1984; Østbye *et al.*, 2005). Alternatively, populations may once have been separated by a physical barrier or have colonised the lake at different times during de-glaciation (Frost, 1965; Alexander and Adams, 2000).

The British Isles, composed of England, Wales, Scotland, and Ireland, lies at the southern limit of Arctic charr distribution. The majority of populations (~ 295) are located in the lochs of Scotland (Maitland and Adams, 2018). Approximately 50 populations are to be found in Ireland (Igoe et al., 2003), whilst England and Wales have just 8 and 3 extant natural populations, respectively (Adams and Maitland, 2007; McCarthy, 2007). Because of the mild climate relative to higher latitudes, most populations are found in deep lakes that provide cool hypolimnetic waters during the summer months when temperatures higher up the water column are unsuitably warm (Graham and Harrod, 2009). With the exception of a few lochs in Scotland (Adams et al., 1998; Maitland and Adams, 2018), the range of ecological variability within lakes does not match that found in Arctic regions, and this is likely because populations live within multispecies lakes (Jonsson and Jonsson, 2001). In particular, brown trout (Salmo trutta) tend to dominate the littoral zone, leaving the pelagic zone as the only productive habitat available for Arctic charr (McCarthy, 2007; Morrissey-McCaffrey et al., 2019). However, whilst most populations are planktivorous, significant differences in morphology, colouration, genetics, and life-history are observed among lakes (Partington and Mills, 1988; Igoe and Hammar, 2004; Adams and Maitland, 2007). Such variation is likely to have arisen because of micro-evolutionary processes and several millennia of isolation in allopatry (Bush and Adams, 2007; Maitland and Adams, 2018).

THREATS TO ARCTIC CHARR IN THE BRITISH ISLES

Globally, Arctic charr are a widespread species and listed as unthreatened on IUCN red list. However, locally, and in particular toward the southern limit of the species range, Arctic charr are highly threatened and in decline (Jeppesen *et al.*, 2012). In the most recent review of the status of Arctic charr in the UK, Maitland et al. (2007) note that in Scotland at least 12 populations are now extinct, as are 4 of 12 native populations in England and 1 of 4 native populations in Wales. In Ireland, a millennial review by Igoe *et al.*, (2003) identified at least 15 populations confirmed to have become extinct in the last century. Long-term population monitoring campaigns are few, but where available, the data generally show declines in population size in recent decades (Winfield *et al.*, 2010). Despite these obvious concerns regarding the future persistence of Arctic charr in the British Isles, the species currently receives little national legal protection (Maitland *et al.*, 2007). There are a small number of sites where Arctic charr are a protected feature of a Site of Special Scientific Interest (SSSI), meaning activities must not cause unacceptable damage to the population or habitat (Thomas, Griffiths and Hall, 2010). Both Maitland et al. (2007) and Igoe *et al.*, (2003) list eutrophication, climate change and introductions as environmental factors which collectively threaten the continued survival of the Arctic charr in Britain and Ireland. Acidification, heavy metal contamination, exploitation, aquaculture, and disturbance to spawning habitat are additional localised pressures affecting some populations. Below I describe the nature of these threats, but do not cover acidification, heavy metal contamination, and aquaculture, which are not currently pertinent to the study system of the thesis (Llyn Padarn). I direct the reader toward Maitland *et al.*, (2007) for further information on these topics.

Eutrophication

Eutrophication is the process of nutrient enrichment that increases a waterbodies algal biomass, and is a ubiquitous concern across the world's freshwater habitats (Smith and Schindler, 2009; Quinlan et al., 2021). Whilst eutrophication can be a natural process that occurs as a lake matures in age, anthropogenic activities, such as the fertilisation of agricultural land and municipal sewage discharge, are largely responsible for the dramatic shifts in nutrient loads that have occurred since industrialisation (Smith and Schindler, 2009). When algae and other organic matter decompose on the lakes bottom, the effect is to remove oxygen from the water (Knoll et al., 2018; Quinlan et al., 2021), and it is this process that presents the main threat for fish species such as Arctic charr that utilise deep-water thermal refugia during summer. Like other salmonids, Arctic charr are highly oxyphilic, requiring approximately 7 mg/l of dissolved oxygen for optimal physiological functioning (Davis, 1975). Thus, increased algal decomposition through eutrophication reduces the volume of suitable deep-water habitat and may force fish to ascend the water column to occupy more oxygenated, but warmer habitat (Molot et al., 1992; Dillon et al., 2003; Jacobson, Stefan and Pereira, 2010). The settlement of algal detritus can also degrade littoral and deep-water spawning habitats by infilling the interstitial spaces where Arctic charr tend to deposit their eggs (Miller et al., 2015). Finally, increases in productivity can change lake ecology in a manner that allows native and non-native competitors and predators to establish or increase in abundance (Jacobson et al., 2017; Morrissey-McCaffrey et al., 2019).

Climate change

Anthropogenic climate warming has changed the composition of fish communities in lake ecosystems, with further and more dramatic changes expected in this century if global air temperatures rise as predicted (Sharma *et al.*, 2007; Graham and Harrod, 2009; Jeppesen *et al.*,

2012; Hansen *et al.*, 2017). It is estimated the surface temperature of lakes increases by 70 to 85 % of the increase in air temperature (Schmid, Hunziker and Wüest, 2014; Butcher *et al.*, 2015), which has corresponded to an average warming in surface waters of ~0.27°C per decade during the late 20^{th} and early 21^{st} century (Kraemer *et al.*, 2015; O'Reilly *et al.*, 2015). These trends are of particular concern for cold-adapted fish at their southern range edge, such as Arctic charr in the British Isles. In analysis of long-term monitoring data for eleven populations, Winfield *et al.*, (2010) found a significant positive relationship between the observed population decline and vulnerability to climate change based on water body latitude, altitude and mean depth. Declines may have been caused by impacts of warmer temperatures on Arctic charr physiology or phenology. Alternatively, warming may have triggered ecological changes at these lakes that are unfavourable for Arctic charr.

Arctic charr are regarded as one of the most cold-adapted of all salmonids, being capable of growth at temperatures of just 0.3° C (Graham and Harrod, 2009). Comparison of growth rate collected along a north-south gradient in Europe suggest growth reaches a maximum between 15 and 17° C and ceases at ~21–22° C (Larsson, 2005). Studies of upper lethal temperatures of juveniles from northern and southern areas of the species European distribution show very similar results and indicate that lethal temperatures are in the region of 23° C (Baroudy and Elliot, 1994; Whitfield and Elliott, 2002). Netting surveys at different lake depths in Ireland found adult fish occupied regions of the lake where summer temperatures ranged from 11.4 and 15.6° C (Morrissey-McCaffrey et al., 2019). In an experimental set-up, Larsson (2005) found preferred temperatures selected by Arctic charr from Sweden were between 10.8 and 11.8° C, which was lower than the optimal growth temperature. Similarly, Mortensen, Ugedal and Lund (2007) observed a population in northern Norway to prefer a temperature of 11.5 to 11.8° C. Eggs are likely to have a lower temperature tolerance. In a review of the available literature, Kelly et al., (2020) report that studies consistently identify egg mortality dramatically increases when temperatures exceed 8.5° C. Monitoring data for two lakes in England and Ireland show that current maximum and mean winter temperatures are on average ~ 1 °C warmer compared to early the 1980s and the 8.5 °C threshold has been frequently exceeded in recent winters (Kelly et al., 2020). Warmer temperatures may also effect egg development before spawning, resulting in reduced egg viability (Jobling et al., 1995). Finally, climate warming is likely to cause phenological changes which could have consequences for survival rates of early life stages (Skoglund, Einum and Robertsen, 2011). In particular, it is

expected that warming will delay the time of spawning and shorten the incubation time of eggs (Jonsson and Jonsson, 2009).

Because Arctic charr are understood to utilise deep-water refugia to shelter from warm summer temperatures near the surface, a critical question is how climate change will effect these habitats and the associated charr populations (Graham and Harrod, 2009). Whilst surface warming over recent decades has been almost ubiquitous across lakes, trends in deep-water zones have been more inconsistent, which can be attributed to the complex process of thermal stratification (Butcher et al., 2015; Kraemer et al., 2015; Richardson et al., 2017). In most deep lakes at mid to high latitudes, thermocline formation between spring and late summer greatly reduces mixing between surface and bottom waters, resulting in a stratified profile consisting of a warm surface layer that closely reflects summer atmospheric temperature and a cooler deep layer (hypolimnion) with temperatures similar to spring (Stefan et al., 1996; Boehrer and Schultze, 2008; Richardson et al., 2017). Therefore, the effect of climate change on deep-water temperature will depend greatly on how climate change impacts thermal stratification (Kraemer et al., 2015). Climate change could increase wind strength and storm frequency which may disrupt stratification to cause deep-water zones to warm at a quicker rate than expected (Butcher et al., 2015; Martinez-Alvarado et al., 2018). Alternatively, and more likely, atmospheric heating and reductions in average wind strength will generate stronger and more stable thermoclines, meaning temperatures in the hypolimnion may change at a slower rate than the lake surface (Butcher et al., 2015; Deng et al., 2018; Woolway and Merchant, 2019). Some lakes that are currently mixed could become stratified, and in such cases, summer deepwater temperatures may in fact become cooler than present (Woolway and Merchant, 2019). Lake turbidity is expected to increase according to some climate change predictions, which might result in a shallower thermocline and thicker hypolimnion (Solomon et al., 2015). However, whilst a shallower and stronger thermocline may have some beneficial outcomes for thermal refugia habitat, low circulation between bottom and surface waters can have a deleterious impact on deep-water oxygen, especially in the presence of eutrophying and turbidifying nutrients such as phosphates and dissolved organic carbon that increase respiration rates in lakes (Solomon et al., 2015; Deng et al., 2018; Jane et al., 2021). In combination, lake warming, stronger stratification, and elevated nutrients may act to reduce the volume of thermal refugia habitat for Arctic charr, thereby lowering population carrying capacity (Figure 1).



Figure 1. Diagram illustrating the reduction in optimal thermal-oxygen habitat for Arctic charr at stratified lakes caused by heating from the surface and hypoxia rising from the lake bottom.

Non-native species

The introduction of species to waterbodies previously un-inhabited by them (i.e., non-native species) can cause significant change in fish community structure and ecosystem functioning (Morrissey-McCaffrey et al., 2019). In recent decades, non-native species have proliferated across freshwater habitats due to introductions by people (intentional and accidental) and changes to habitat caused by anthropogenic activities (Britton, Gozlan and Copp, 2011; Anderson et al., 2015; Greenhalgh et al., 2022). For Arctic charr, there is particular concern regarding the spread of Eurasian perch (Perca fluviatilis) and cyprinid species, such as roach (Rutilus rutilus), which occupy ecological niches that might otherwise be utilised by Arctic charr (Corrigan et al., 2011). Species distribution models for lakes in Ireland have found Arctic charr probability of occurrence and abundance is significantly reduced when perch or roach are present, although this may reflect changes to lake habitat that are favourable for perch and roach and unfavourable for Arctic charr, rather than direct competition for resources (Connor et al., 2019; Morrissey-McCaffrey et al., 2019). Both perch and roach are most successful in mesotrophic to eutrophic lakes and prefer warmer temperatures than Arctic charr (Connor et al., 2019; Morrissey-McCaffrey et al., 2019). Thus, climate warming and eutrophication are likely to increase their range and local abundance.

Introduced species that potential predate on Arctic charr at different life-stages include pike (*Esox lucius*), ruffe (*Gymnocephalus cernuua*), Eurasian perch, and signal crayfish

(Pacifastacus leniusculus). Pike are a large ambush predators with similar environmental tolerances as Eurasian Perch (Connor et al., 2019). They are known predators of juvenile and adult Arctic charr, in particular at the time of spawning when Arctic charr move into shallow water areas where pike prefer to hunt (Frost, 1965; Mills and Hurley, 1990). Species distribution models for lakes in Ireland and Sweden have shown that the probability of Arctic charr presence is greatly reduced in lakes with populations of pike (Hein, Öhlund and Englund, 2012; Connor et al., 2019). The impact of predation by perch is not well understood, but inspection of the stomach contents at lakes in Ireland found Arctic charr were only a small proportion of the diet (Morrissey-McCaffrey et al., 2019). Perch are not a large species, especially when occupying low productivity lakes (Mills and Hurley, 1990), and therefore it is likely that only small juvenile charr are eaten. Ruffe are voracious predators of fish eggs, although studies have been focused on Coregonus spp., rather than Arctic charr (Adams and Tippett, 1991; Etheridge, Bean and Adams, 2011). Unlike Coregonus spp. which are broadcast spawners (Etheridge, Bean and Adams, 2011), Arctic charr often make some effort to bury their eggs beneath the substrate surface (Frost, 1965), which may provide some protection from ruffe. The signal crayfish has spread rapidly across freshwater habitats in the British Isles since its introduction in the 1970s (Greenhalgh et al., 2022), but the potential threat to the region's Arctic charr populations has not been assessed. At Lake Vattern in Sweden, field experiments identified that signal crayfish consumed ~ 40% of Arctic charr eggs at littoral spawning sites (Setzer, Norrgard and Jonsson, 2011).

Whilst not considered non-native species, there is concern regarding the apparent increase in the abundance of fish-eating birds at several lakes in the British Isles, with some arguing that populations should be managed to protect vulnerable salmonid populations (Winfield, Crawshaw and Durie, 2007). Cormorants (*Phalacrocorax* spp.) are very efficient diving birds and Arctic charr are known to be component of their diet (Frost, 1965). At Haweswater in the English lake district, the proliferation of cormorants was identified as a significant threat to the population of *Coregonus lavaretus* which occupy similar habitat to Arctic charr (Winfield, Crawshaw and Durie, 2007). Other fish eating birds, such as Mergansers, may predate juvenile fish in shallow water but adult fish are too large (Marquiss and Carss, 1994). Certain species of diving duck can consume large numbers of eggs (Frost, 1965).

Spawning habitat disturbance

In common with other salmonids, Arctic charr, utilise loose rocky substrates to deposit their eggs, but are perhaps more cosmopolitan in terms of choice of spawning habitat. These habitats include inflowing and outflowing rivers, the shallow littoral zone, and the profundal zone, with substrate particle size ranging from pea-sized gravel to cobbles over 200 mm in diameter (Table 1). Eggs require highly oxygenated waters and must not become smothered by fine sediments, and thus spawning sites in lakes may be associated with groundwater upwellings, inlet or outlet tributaries, islands, or bed forms in deeper waters (Riley et al., 2019). Egg laying does not typically involve excavation of deep nests, or redds, as is the case for *Salmo* spp., but the female may use her tail to cut a small divot in the substrate which is then covered in a haphazard manner after the eggs have been fertilised (Frost, 1965). This being said, there are a very few cases where spawning behaviour has been described. At sites where large cobbles are present the eggs may passively fall into the deep interstitial spaces (Milner, 1985; Low et al., 2011). It is understood that Arctic charr return to the site of birth to breed, which may be due to olfactory imprinting, or alternatively, strain-specific pheromones may ensure members of a population navigate to the same spawning site (Frost, 1965; McCubbing, Bayliss and Locke, 1998; Nordeng and Bratland, 2006).

Location	Depth (m)	Spawning substrate	Location	Reference
Llyn Peris	0.75 -2	Slate, granules (4-2 mm), pebbles (64-4 mm), and cobbles (256 -64 mm). No weed growth and little encrusted algae.	Littoral	Butterworth (1980)
Llyn Padarn	1-2	Slate and clean homogeneous coarse gravel substratum. Median particle size of 52 mm.	River	Milner (1985)
Llyn Cwellyn	1.5 –7	A wide range of bed material was present, including fine gravel (median particle size 3 mm), coarser gravels (< 50 mm diameter), and cobbles 100-200 mm in diameter.	Littoral	Milner (1985)
Three Irish lakes	0 - 1.24	Course mineral substrate. Geometric mean ranged from 35 to 100 mm.	Littoral	Low <i>et al</i> . (2011)
Windermere	1-3	Stony. Particle size ranged from pea sized gravel to head sized stones.	Littoral	Frost (1965)
Windermere	9.5 – 28	Predominantly walnut and fist sized stones.	Profundal	Frost (1965)
Windermere	1-3	Stony. Predominantly walnut and fist sized stones.	River	Frost (1965)
Loch Mealt	1-2	Spawning only in a stony bay free of silt. The rest of the lake had a substrate of ooze	Littoral	Barbour (1984)
Loch Builg	1-2	Clean gravel of thumbnail to potato size, interspersed by small boulders	Littoral	Barbour (1984)
Loch Doine	12	Clean gravel of thumbnail to potato size, interspersed by small boulders	Littoral	Barbour (1984)
Ennerdale	< 1 m	Fine pea sized gravel	River	McCubbing, Bayliss, & Locke (1998)

Table 1. Some characteristics of Arctic charr spawning sites in the British Isles.

Disturbance of spawning habitat is a broad term used here to describe any physical disturbance that reduces the availability and suitability of spawning habitat. The sediment regime and flow conditions in rivers are likely to be very important for maintaining suitable substrate composition, and therefore, modifications to the river channel, riparian zone, or catchment land use may have negative effects on spawning habitat and the survival of early life-stages (Geist and Dauble, 1998; Railsback *et al.*, 2013). In particular, deposition of fine sediments can suffocate eggs and alevins and may physically impede alevin movement and emergence (Crisp,

1988; Mari et al., 2016; Sear et al., 2016). Flooding and associated high flow velocities can cause eggs and alevins to be washed out from the substrate. Washed-out eggs may suffer mortality because of mechanical shock and become more vulnerable to predators (Crisp, 1990; Fitzsimons et al., 2007). Studies by Crisp (1990) in an artificial stream found brown trout eggs before the eyed stage suffered 45 - 65 % mortality after drifting 10 m downstream, whereas no detectable change in survival occurred for eggs that had reached the eyed stage. Studies directly measuring mortality in washed-out salmonid alevins could not be located, but given low mobility at this stage of the life-cycle, it seems likely that mortality is increased due to exhaustion or higher risk of predation (Jensen and Johnsen, 1999; Grimardias, Faivre and Cattaneo, 2012). Jensen and Johnsen (1999) detected a strong correlation between elevated discharge during the alevin period and year-class strength of Atlantic salmon (Salmo salar). However, the correlation might not represent a direct causal link between discharge and alevin mortality, rather flooding may have affected survival of fry after emergence by removing suitable nursery habitat (Lobón-Cerviá and Rincón, 2004). Barriers preventing breeding adults from accessing spawning grounds, such as weirs, dams and culverts, are probably not a significant concern, as the migrations made by river spawning Arctic charr are generally very short (Walker, 2007), but there may be issues at certain locations (e.g., at Llyn Padarn). The primary disturbances threatening littoral sites are likely to be infilling of interstitial spaces through sedimentation and water abstraction at reservoirs causing eggs to become dewatered (Milner, 1985; Low et al., 2011; Winfield et al., 2013; Miller et al., 2015). Littoral sites close to the mouth of inflowing rivers may be vulnerable to the same physical disturbances as spawning sites in rivers.

Exploitation

There are relatively few Arctic charr lakes in the British Isles where the species is specifically targeted by anglers, although they may be caught inadvertently on many waters by people fishing for brown trout (Maitland *et al.*, 2007). Angling for Arctic charr is quite a specialist activity as many lakes are remote and a boat is required to access the deep waters where the fish reside. Thus, the threat posed by angling may be limited at most locations, although even low levels of exploitation could have a substantial impact on populations occupying small waterbodies or populations that are declining due to other anthropogenic disturbances (Maitland *et al.*, 2007).

Subsistence, commercial, and sport fisheries are common in Scandinavia and North America, with anadromous fish, which can attain a large length of ~ 1 m, being particularly valuable (Hegge, Dervo and Skurdal, 1991; Gallagher and Dick, 2010) . In some cases fisheries have become unsustainable and contributed to population declines or extirpation (Gallagher and Dick, 2010; Lukin, 2013). However, in the British Isles, commercial fisheries are few and now only operate on a small scale. The most notable examples are Windermere and Coniston water in the English Lake District, where a technique known as plumb lining, a type of angling, is employed (Maitland *et al.*, 2007; Winfield, Berry and Iddon, 2019). Historically the fishery used seine nets but in the middle of the 19th century the catch became unsustainable, with temporarily disastrous results (Winfield, Berry and Iddon, 2019). This precipitous decline came at a time when trade in this species was flourishing and both the local population and the tourist trade were increasing (Winfield, Berry and Iddon, 2019). In Wales, Arctic charr at Llyn Padarn and Llyn Cwellyn were once removed in a large numbers and were valued for their good eating, especially by the local miners (Ellidge, 1976).

CONSERVATION AND MANAGEMENT

Conservation management seeks to reduce, remove, or mitigate the threats affecting a population. Below I summarise the possible interventions available to local managers to address threats to Arctic charr. Climate change is not covered, since addressing climate change requires global action. Faced with seemingly inevitable warming in the coming decades, it will be vital that managers consider increasingly local pressures in-order to maximise persistence of Arctic charr populations.

Deep-water hypoxia

The primary nutrients that cause eutrophication and consequent deep-water hypoxia are nitrates and phosphates, and therefore reducing inputs of these nutrients is the best approach to protect deep-water thermal refugia habitat (Smith and Schindler, 2009; Paerl *et al.*, 2020; Quinlan *et al.*, 2021). A third nutrient, dissolved organic carbon (DOC), can also cause oxygen to decrease but the mechanisms are different from eutrophying nutrients: first, DOC increases turbidity which reduces the light available for photosynthetic organisms that add oxygen to lakes; and second, mineralisation of DOC by heterotrophs removes oxygen from lakes (Solomon *et al.*, 2015; Knoll *et al.*, 2018). Of these three nutrients, phosphates probably have the greatest influence on oxygen because they are generally the limiting nutrient for primary production in lakes (Smith and Schindler, 2009). However, the recent increase in DOC at many northern

lakes and rivers is a concern that is often overlooked (Worrall *et al.*, 2004; Stanley *et al.*, 2012; Solomon *et al.*, 2015).

Nutrients arrive at lakes from two types of source: point sources and diffuse sources (Nielsen et al., 2012). Point sources of phosphates and nitrates are mostly associated with municipal and industrial wastewater and include discharges from sewage works and wastewater from intensive animal farms. The main diffuse sources are often livestock and land run-off from fertilised agricultural land in the catchment. DOC sources are mostly diffuse, with increases being associated with changes in land use, such as forestry activities, as well as climate (Stanley et al., 2012). Point-source issues are relatively easy to fix (e.g., redirection of sewage outlets or increased treatment of effluents), whereas reducing diffuse sources is more complex, since these are influenced by nutrient cycling processes and complex nutrient transport pathways (Nielsen et al., 2012). Typical catchment-wide interventions to reduce phosphates and nitrates include preventing livestock access to rivers, restoration of riparian buffer zones, and changes to farming practices, in particular the use of fertilisers (Kleinman et al., 2015). Catchments that are badly affected by eutrophication can be designated as Nitrate Vulnerable Zones (EU Nitrates Directive) which places restrictions on fertiliser use (Hatton-Ellis, 2016; Carvalho et al., 2019). There are numerous case studies where management has been successful in reversing eutrophication trends, and the benefits for cold-adapted fish, in terms of increased population size, have been demonstrated (Elliott et al., 1995; Jeppesen et al., 2005; Gerdeaux, Anneville and Hefti, 2006).

Controlling non-native or pest species

Eradication of non-native fish is rarely possible, especially in large lakes, but suppression efforts can be successful (Britton, Gozlan and Copp, 2011). The physical removal (cropping) of non-native fish from a water body is a control method that uses capture techniques (e.g., netting, trapping, and electric fishing) to remove non-native species from the water body. If sufficient numbers can be captured, this approach is generally effective in suppressing population abundance and reducing their recruitment (Britton, Gozlan and Copp, 2011). To ensure maximum efficiency and cost-effectiveness, it may be wise to utilise the capture techniques that target the life-stage which is most important for population growth rate (Morris, Shertzer and Rice, 2010; Cox *et al.*, 2013) Regarding species of particular concern for Arctic charr, there are cases where perch and cyprinids have been suppressed by cropping, although the lakes were considerably smaller than those typically occupied by Arctic charr (Ludgate and

Closs, 2003; Neilson *et al.*, 2004). Clearly, in lakes where non-natives are not currently present, it essential that measures are taken to prevent and detect their arrival (Britton, Gozlan and Copp, 2011; Morrissey-McCaffrey *et al.*, 2019). Where lake conditions have become favourable for non-natives (e.g., because of eutrophication) then returning the lake to normal conditions may reduce population size. At several alpine lakes in Switzerland and France with Coregonid populations, re-oligotrophication has seen a decrease in the abundance of perch and cyprinids (Gerdeaux, Anneville and Hefti, 2006).

There are variety of techniques available that may reduce the impact of fish-eating birds. These include people walking around the lake to scare birds away; automated scarers, although birds may eventually become habituated; shooting to scare; cutting down roosting trees for cormorants (but illegal during breeding season); habitat modifications to provide more cover for fish; and finally, where fish eating birds are causing serious damage to fisheries a license to kill a limited number of birds as a reinforcement to scaring may be granted (Giles et al., 2004; Winfield, Crawshaw and Durie, 2007). These interventions can only be undertaken when there is clear evidence of impact on the fishery and are likely to have varying degrees of success (Giles et al., 2004; Winfield, Crawshaw and Durie, 2007). A good case study is Haweswater, where a variety of scaring techniques were tried to reduce pressure on local Coregonus lavaretus population. The results of the trials found that frequent disturbance by humans combined with some tree cutting was the only effective technique (Winfield, Crawshaw and Durie, 2007). Over a four-month period 27 visits were made to scare cormorants at roosting sights which prevented the birds from breeding. Whilst the interventions prevented breeding, and thus may have reduced future population size, the number of adults during the period of scaring did not decrease. Foraging pressure on the whitefish population is thus unlikely to have been reduced in the short-term. Culls are probably the only method that will have an immediate impact, but are contentious since the target species are protected by law (Birds Directive) and are a natural component of lake ecosystems (Winfield, Crawshaw and Durie, 2007). Winfield, Crawshaw and Durie (2007) make a compelling argument that scaring and culling can only be justified once all other threats to the fish population have been addressed.

Restoration of spawning and nursery habitat

Restoration of spawning and nursery habitat are common practical conservation measures for many salmonid species (Hendry *et al.*, 2003; Wheaton, Pasternack and Merz, 2004), although examples specifically targeting Arctic charr could not be found in the literature. Spawning

ground interventions typically aim to improve the suitability of the substrate or manage flow, and include manually cleaning the substrate of fine sediments, installation of gravel traps to stabilise spawning gravels, substrate supplementation, channel engineering to alter flow conditions, and modifying riparian and catchment land use to manage sediment inputs (Hendry and Cragg-Hine, 1997; Hendry *et al.*, 2003). Each of these interventions may be suitable for stream spawning Arctic charr, dependent on the specifics of the situation. Spawning grounds in lakes are not so affected by flow or movement of substrate, but could potentially benefit from catchment management, gravel cleaning (if sites have experienced sedimentation through by eutrophication), and substrate supplementation, if the availability of suitable substrate is a limiting factor for the population. The complexity of patterns in flow dynamics and sediment transport mean spawning habitat restorations are often unsuccessful in the medium to long-term (Kondolf, 2000).

Habitat use by Arctic charr in the months following alevin emergence are poorly understood, which limits any assessment of the possible practical interventions that could be implemented to improve habitat and survival at this stage of the life cycle. Observations reported by Frost (1965) indicate fry do not linger long at spawning grounds. Therefore improving habitat complexity and bankside cover at river spawning sites, which are measures recommended for Salmo spp. (Hendry and Cragg-Hine, 1997), are unlikely to be beneficial. Klemetsen et al., (1989) propose young Arctic charr may initially take up residence in the profundal zone before moving higher up the water column as they grow. Some observational support is provided by hydro-acoustic surveys in the English Lake district which have detected large numbers of small fish residing in deep-water zones (pers. comms. Ian Winfield, Centre for Ecology and Hydrology). If it is indeed the case that fry utilise the profundal zone, then deep-water hypoxia may be a particular concern at this stage of the life cycle. Fry at Thingvallavatn are found in the shallow littoral zones (Sandlund et al., 1988), but this is perhaps because surface temperatures are lower than in the British Isles and fewer competing species are present. Finally, fry may occupy the same pelagic habitats as older individuals. However, fish < 1 year are rarely recorded in pelagic netting surveys (e.g., Frost, 1965; Butterworth, 1980; Partington and Mills, 1988), although this is likely because the mesh size has been too large.

Fisheries management

As discussed earlier, commercial and recreational exploitation of Arctic charr is not common in the British Isles, meaning there are few case studies where fisheries management has been implemented. At Windermere regulations were introduced when overfishing became an issue. These regulations include a closed season (1st October to 14th of March), a minimum size limit (200 mm), and a ban on the use of seine nets (Winfield, Berry and Iddon, 2019). Currently it is not considered that the fishery is having a negative impact on the population (Winfield, Fletcher and James, 2007). Catch and release is a growing conservation strategy for recreational fisheries (Brownscombe et al., 2017). Catch and release does not necessarily require that no fish can be taken, rather measures such as bag limits, minimum size, and use of barbless hooks mean that a large proportion of fish with a high reproductive value are returned, and in a better condition to survive (Giles et al., 2004). However, a possible issue with catch and release for Arctic charr is that fish caught by anglers suffer barotrauma to their swim bladders due to being lifted quickly from depth. Such reports are merely anecdotal, and scientific literature indicting that barotrauma is a particular issue at recreational Arctic charr fisheries could not be located. Plum lined lures at Windermere are normally not deployed at a depth > 20 m and undersized fish are returned alive, although survival of released fish has not been assessed (pers. comms. Ian Winfield, 2022). Given that many Arctic charr populations are highly threatened, there is a reasonable case that angling is simply not sustainable or ethical. A byelaw is currently under consideration in Ireland that will outlaw angling or killing of Arctic charr from any waterbody (Evans, 2021).

Supplementation and translocation

Supplementation and translocation programmes have been implemented for several populations of threatened cold-adapted fish in the British Isles. These are crisis measures to be undertaken when populations are facing extinction due to intractable ecological issues (Adams and Maitland, 2007; Kissel *et al.*, 2014). Supplementation involves adding hatchery-reared individuals to the native population. Translocation seeks to establish a back-up population in another lake that could, in theory, be used to supplement the native population in the future. Both can be controversial, and the efficacy of existing programmes is not always rigorously assessed.

Perhaps the earliest example of Arctic charr conservation translocation in the British Isles is the Llyn Peris population which were transferred in the 1970s to a nearby lake (Ffynnon Llugwy) prior to extirpation caused by the construction of the Dinorwig pumped storage hydroelectric power station (McCarthy, 2007). However, whilst a reasonable-sized population continues to persist, individuals exhibit a stunted growth pattern (McCarthy, 2007), suggesting characteristics of Ffynnon Llugwy were not suitable to maintain the Llyn Peris phenotype. Moreover, subsequent stocking at Ffynnon Llugwy using Arctic charr from other local lakes has meant the once unique Peris genotype has now been lost (Beck, 2014). More recently the fry of Llyn Padarn Arctic charr were released at nearby Llyn Crafnant when severe algal blooms were impacting the lake. A total of 5000 fry were stocked between 2010 and 2012 but the efficacy of the programme was not assessed. Recent eDNA sampling at Llyn Crafnant in 2018 did not detect Arctic charr (pers. comms. Mat Seymour). In England, fry from Ennerdale Water were translocated to Kielder Water but there is no scientific literature on the programme. Loch Doon charr were translocated in 1986 and 1990 to two nearby reservoirs and in both cases breeding populations appear to have become established (Adams *et al.*, 2014). Numerous translocations of rare *Coregonus spp.*, a similar fish in several ways to Arctic charr, have been undertaken with a high success rate (Thomas *et al.*, 2013; Adams *et al.*, 2014).

In the case of Llyn Padarn and Ennerdale, supplemental breeding programmes utilising native brood stock have been implemented alongside translocations. The process involves capturing wild breeding fish and then stripping eggs to be artificially fertilised with the seamen from males. The fertilised eggs are then reared in an aquaculture facility (hereafter, hatchery) before being released at the lake at between approximately 3 and 6 months old. The method is considered preferable to utilising a hatchery or non-native brood stock, as was often the approach in the past (Savary et al., 2017). Breeding from wild native brood-stock should maintain locally-adapted phenotypes and, by utilising a different brood stock in each year, reduce the risk of inbreeding and erosion of genetic diversity (Selly, Hickey and Stevens, 2014). However, some of the problems associated with captive brood stocks may still arise when using wild brood stock. First, stocked fish may have lower genetic diversity than the wild population if the number of parents is small or there is a parental bias, meaning adherence to correct breeding protocols is essential (Machado-Schiaffino, Dopico and Garcia-Vazquez, 2007; Selly, Hickey and Stevens, 2014). Second, hatchery and wild environments differ by many aspects including fish density, spatial and temporal heterogeneity of physical environment, intra- and interspecific interactions, and food availability, and consequently, conditions in the hatchery do not necessarily select the fittest genotypes for the wild (Milot et al., 2013). Hatchery conditions can also induce plastic phenotypic responses that may result in environmental carryover effects on life-history or behavioural traits which are not beneficial once released in the wild (Milot et al., 2013). Alternatively, stocking of juveniles may supress wild recruitment because of advantages gained from hatchery conditions. In particular, greater

food availability in the hatchery means stocked juveniles are often considerably larger than their wild counterparts, and this may offer a considerable competitive advantage, even if underlying adaptive fitness to the environment is lower (Kostow, 2009). Finally, non-random sampling or artificial breeding can cause unintentional selection on particular traits. The disruption of natural mate choice and hence of sexual selection may have important genetic implications for the next generation (Fleming and Petersson, 2001; Anderson *et al.*, 2013; Perry *et al.*, 2019). In summary, heritable and phenotypic traits selected by artificial breeding or hatchery conditions that are disadvantageous in nature may result in low fitness of stocked fish and have long-lasting evolutionary impacts on the fitness of wild-stocked populations through hybridisation (Milot *et al.*, 2013).

DEMOGRAPHIC MODELS AND SENSITIVITIES

As outlined in the preceding sections, Arctic charr are faced by a variety of threats which impact different stages of the life cycle. Resources for conservation are not unlimited, meaning managers must prioritise interventions that will have the greatest effect on population viability. Since a fundamental objective of conservation is to increase the populations mean fitness, measured as the population growth rate (λ), it is useful to understand the relative importance of life stages for λ in order that management and research efforts can be focused accordingly (Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Wang *et al.*, 2017). To illustrate, if survival of eggs is the most important life stage for λ and survival of mature adults the least important, then it would be recommended that management efforts are directed toward protecting or enhancing spawning habitat and reducing predation on eggs, rather than reducing angling pressures on adults. Of course, this is rather simplistic, since there are many other factors that must be taken in to account when developing a management strategy (Manlik, Lacy and Sherwin, 2018). The importance of life stages for λ is one of several considerations.

Population projection matrices (PPMs) and associated sensitivities are the predominant method for investigating the importance of life-cycle stages for λ (Benton and Grant, 1999; Manlik, Lacy and Sherwin, 2018; Doak *et al.*, 2021). PPMs provide a transparent mathematical framework for organising lifetime mortality and reproductive schedules, which together determine the rate at which a population grows or declines (Caswell, 2001). A PPM is a type of structured demographic model composed of discrete stages which classify individuals in the population according to some demographic trait, typically age or life-cycle stage (e.g., egg, juvenile, or adult). Stages are linked by transitions which determine the contribution that individuals in one stage will make to another stage over a given time period (the projection interval). In an age-structured PPM, these transitions are the probability of an individual surviving to the next age class and the number of offspring produced (Figure 2). The reproduction transition often includes several underlying vital rates. For example, in Arctic charr the number of offspring produced by a mature female over a 1-year projection interval will be the product of fecundity, egg survival, alevin survival, and the survival of fry until the first birthday. Simple deterministic PPMs can be constructed from a static life-table documenting population vital rates at a single time point (i.e., age specific maturation, survival, and fecundity schedules), which are often the only type of demographic data available for populations of fish (Vélez-Espino, Fox and McLaughlin, 2006; Wang *et al.*, 2017).



Figure 2. Life cycle graph illustrating an age structured matrix projection model (PPM) for an Arctic charr population reaching maturity at age 3.

Sensitivities quantify the importance of life-stages by calculating the change in λ caused by perturbation to each life-stage vital rate (Caswell, 2001). Two types of sensitivities analysis can be employed: (1) non-proportional sensitivities which calculate the change in λ caused by an equal and absolute-sized perturbation to each life-stage vital rate; and (2) proportional sensitivities (elasticities) which calculate the change in λ caused by an equal and proportional-sized perturbation to each life-stage vital rate. Unlike non-proportional sensitivities, the elasticities of vital rates always sum to unity, meaning their relative contribution to λ can be

compared among PPMs with a different number of stages (Benton and Grant, 1999). Furthermore, elasticities allow comparison of the sensitivity of λ to survival and fecundity that are measured on different scales; the former being constrained between 0 and 1 and the latter having no upper constraint.

Patterns in the relative importance of life stages for λ are an emergent property of a populations life-history traits, such as longevity, age at maturity, and brood size (Cole, 1954; Oli and Dobson, 2003; Vélez-Espino, Fox and McLaughlin, 2006). Since these life-history traits are often highly variable among Arctic charr populations and the different lacustrine ecotypes and migratory forms (Venne and Magnan, 1989; Vøllestad and L'Abée-Lund, 1994; Tallman, Saurette and Thera, 1996), it is possible the relative importance of life-stages for λ varies substantially across the species, meaning populations may not respond equally to disturbances or management interventions affecting certain life-stages. For example, a population with a growth rate that is more sensitive to fry survival should respond more favourably to supplementation than a population whose growth rate is less sensitive to fry survival. Therefore, in order that management can be optimised at the population level to conserve important intraspecific diversity (Adams *et al.*, 2007; Bush and Adams, 2007; Reist *et al.*, 2013; Mee *et al.*, 2015), life-stage sensitivities of λ should be assessed and characterised across the range of life-history variation exhibited by Arctic charr.

LLYN PADARN ARCTIC CHARR

The Arctic charr in Wales, named locally as the 'Torgoch', or red belly, is an iconic wildlife species of the region with significant cultural importance (Ellidge, 1976). Llyn Padarn, which is a moderate-sized (98 ha) glacial lake situated at low altitude (105 m), supports one of only three remaining natural populations in the country, the others being Llyn Cwellyn and Llyn Bodlyn (McCarthy, 2007). A fourth population once found a short distance upstream of Llyn Padarn in Llyn Peris was extirpated by the construction of a pumped storage hydro-electric power station that began operating in the early 1980s (McCarthy, 2007). Four other lakes in the region, Ffynnon Llugwy, Llyn Cowlyd, Llyn Dulyn, and Llyn Diwaunedd, are known to support established introduced populations (McCarthy, 2007). Each population is located in the northwest of the country in the mountainous region of Snowdonia.

The Llyn Padarn Arctic charr population is one of the best studied in the British Isles. The earliest investigation was undertaken by Powell (1966) who focused mainly on diet and parasites. Later, and shortly before construction of the power station, Butterworth (1980)

collected information on the life-history of Llyn Peris and Llyn Padarn populations and Child (1977) compared genotypic relationships between the two lakes. Following the construction of the power station Milner (1985) undertook a SCUBA survey of spawning areas in the lakes newly modified inflowing river, the Afon y Bala. Most recently, O'Toole (2010) and Beck (2014) investigated variation among Arctic charr populations in Wales, the former focusing on morphology and the latter showing that each natural population is genetically distinct. In addition to academic research, population status has been monitored at varying time intervals since the 1990s by the Welsh environment agency (Natural Resources Wales, NRW).

Physical, biological, and chemical characteristics of the lake, such as bathymetry, hydrology, plankton, and nutrients, are also well described, and frequently assessed by NRW to meet national water quality monitoring requirements for the EU Water Framework Directive (WFD). Bathymetric surveys show a mean and maximum depth of 15.9 and 27 m, respectively. There are two basins, with the deepest being located toward the south-eastern end of the lake and extending over a relatively small area. Other species of fish include brown trout (*Salmo trutta*), minnow (*Phoxinus phoxinus*), stickleback (*Gasterosteus aculeatus*), eel (*Anguilla anguilla*), Atlantic salmon (*Salmo salar*), and perch (*Perca fluviatilis*). Perch are not native to the lake and are believed to have arrived within the last couple of decades (pers. comms. Walter Hanks, NRW). It is not known how they arrived. The lake is classified as having good ecological status according to WFD criteria (Hatton-Ellis, 2016).

Similar to most populations in the British Isles, Llyn Padarn Arctic charr are pelagic zooplanktivores which spawn in the late autumn to early winter (McCarthy, 2007). However, some aspects of their life-history make the population somewhat distinct. First, recent surveys have shown Padarn charr reach an unusually large length of up to 38 cm, which is substantially larger (~45 %) than when measured in the 1970s (McCarthy, 2007). The increase in length could be due to a faster growth rate or a change to life-history traits (e.g., delayed age at maturity) in response to disturbances or environmental changes at the lake (McCarthy, 2007; Johnston and Post, 2009). Second, spawning occurs in the inflowing river (Afon y Bala), although this behaviour only arose after construction of the power station prevented access to the historical spawning areas that were located where the Afon Hwch once entered Llyn Peris (Figure 3). River spawning in the British Isles has only been recorded in two populations from the Lake District and a handful from Scotland (Frost, 1965; McCubbing, Bayliss and Locke, 1998; Walker, 2007).



Figure 3. Lynn Padarn and the location of current (Afon y Bala) and historic (Llyn Peris) spawning sites according to Milner (1984) and Butterworth (1980), respectively. Fish access to historic spawning grounds was prevented following construction of the power station at Llyn Peris.

Despite being a protected feature of the Llyn Padarn Site of Special Scientific Interest (SSSI) the status of the Arctic charr population is precarious. Monitoring surveys that began in 1997 identified a rapid decline in the number of breeding age fish (Thomas, Griffiths and Hall, 2010; Winfield *et al.*, 2010). The causes of the decline are not fully understood, but one major contributing factor is likely to be hypoxia in the lake. Analysis of diatom species in sediment cores showed that Llyn Padarn has become increasingly productive since the mid-1800s, culminating in a severe blue-green algae bloom in 2009 (Bennion *et al.*, 2010). An assessment of lake habitat undertaken by NRW (Thomas, Griffiths and Hall, 2010) found oxygen concentrations in the hypolimnion were below the 3 mg/l threshold that is considered unsuitable, and possibly lethal, for Arctic charr and salmonids generally (Davis, 1975; Jones, Winfield and Carse, 2008). Municipal waste water, rather than diffuse catchment sources, have probably caused eutrophication (Hatton-Ellis, 2016). The sediment cores showed a marked change in the diatom community occurred in the 1950s when the local Llanberis sewage treatment works began operating (Bennion *et al.*, 2010). Investigations of the sewage network

found effluent from local industry (Siemens Healthcare Diagnostics Ltd) was causing a substantial increase in phosphorus entering the system (Thomas, Griffiths and Hall, 2010).

A second factor implemented in the population decline is spawning habitat disturbance. The only confirmed breeding grounds in the Afon y Bala are extremely vulnerable to flooding. The spawning area is located a short distance downstream from the confluence of two highly responsive mountain rivers, the Afon Peris and Afon Hwch (Figure 4). The arrangement is not natural, rather a consequence of redirecting the Afon Peris and Afon Hwch when the power station was constructed. The Afon Peris now flows through a tunnel before joining the Afon Hwch. At the confluence of these two rivers is a weir that prevents Arctic charr from migrating further up the Hwch, but access to the tunnel is possible. The stretch of the Afon Hwch directly above the spawning area is a deep canalised man-made channel, and thus water velocities can be substantial during spate events (Mould and Todd-Burley, 2015). In addition, the power station discharges large volumes of generating water into the Afon y Bala after periods of heavy rainfall. Hydrological modelling commissioned by NRW showed severe flood events and power station discharges will mobilise the spawning gravels where Arctic charr are anecdotally understood to deposit their eggs (Mould and Todd-Burley, 2015). There are also concerns regarding elevated temperatures in the river which occur when the power station discharges. Prolonged temperature spikes of up to 11.5 °C have been recorded which may greatly increase egg mortality (Thomas, Griffiths and Hall, 2010; Kelly et al., 2020). Reproductive success might also have been affected by eutrophication and algal blooms. It was suspected, but never confirmed, that littoral areas of the lake were once utilised for breeding (Powell, 1966; Butterworth, 1980). Such sites, if they existed, may have been lost or degraded due to sedimentation by algal detritus (Miller et al., 2015). Discharges from the sewage treatment works occur downstream of the Afon y Bala spawning area but may have impacted any suitable habitat in the lower reaches and mouth of the river.



Figure 4. Disturbances impacting the Afon y Bala spawning site.

Additional threats other than anoxia and spawning habitat disturbance are perch, fish eating birds, and angling. The arrival of non-native perch is a concern, and their continued persistence may be facilitated by changes in lake productivity. However, the size of the perch population and its impact on the Arctic charr population is unknown. Fish-eating birds are present (cormorants and Mergus spp.), and anecdotally it is reported these species may have become more common. Llyn Padarn is one of the few lakes in the British Isles where Arctic charr are targeted by anglers. Records collected by the angling club (Seiont, Gwyrfai and Llyfni Fishing Society) begin in 1997, and show several hundred Arctic charr were removed by anglers in some years (maximum 600). Historically, these numbers may have been higher as Arctic charr were once widely eaten locally (Ellidge, 1976). Such levels of fishing activity were perhaps sustainable before the impacts of eutrophication and changes to the spawning habitat, but the available data are not adequate to assess this. In recent years the numbers of Arctic charr taken by anglers has greatly reduced, with no fish landed between 2010 and 2016. This trend probably reflects the decline in the population size and a consequent reduced interest among anglers in specifically targeting Arctic charr by plumb lining (angling data do not record fishing effort). However, angling records for Arctic charr are not necessarily accurate, especially since some fish may be taken by non-members without a permit (pers. comms. Robin Parry, Seiont, Gwyrfai and Llyfni Fishing Society, 2022). Finally, because Llyn Padarn is only moderately

deep and located at a low altitude and latitude, the impact of climate change will be perhaps greater than at most other lakes in the British Isles (Winfield *et al.*, 2010).



Figure 5. Lifecycle of Llyn Padarn Arctic charr. Fish reach maturity at around 3 to 4 years old (Butterworth, 1980) and spawn on gravel substrates in the lakes inflowing river, the Afon y Bala. Eggs hatch after approximately 75 days and alevins emerge around 1 month later (Baroudy and Elliott, 1994). After the alevin stage, habitat use is poorly understood: fry probably enter the lake shortly after emergence where they may take up residence in either the littoral zone, profundal zone, or pelagic zone (Frost, 1965; Sandlund *et al.*, 1988; Klemetsen *et al.*, 1989). Older juveniles and adults forage for zooplankton in the pelagic zone.

To halt the population decline, several management interventions have been undertaken. The most substantial has been a supplemental breeding programme organised by NRW since 2009. Each year fyke nets have been set at the Afon y Bala spawning grounds to obtain gametes from wild fish. Eggs are fertilised in a manner to maximise the number of parental crosses and then transported to a hatchery facility. In the first two years fry were stocked at nearby lake (Llyn Crafnant) that was intended as a refuge population that could supplement Llyn Padarn in the future. After 2011 fry have been released only at Llyn Padarn. To address eutrophication and consequent anoxia the water company (Welsh Water) have undertaken to improve wastewater treatment. Phosphate stripping was first introduced in 1995 (Bennion *et al.*, 2010), with further more intensive work undertaken subsequent to a severe blue-green algae bloom in 2009. These works have involved more chemical treatment measures to strip nutrients and improvements

to the sewer system to reduce incidents and severity of storm discharges (NRW, 2022). Inputs of phosphates from Siemens Healthcare Diagnostics Ltd have also been removed from the system. A long-planned spawning habitat remediation project involving re-engineering the canalised river channel to reduce flow velocity at the spawning grounds was implemented in the autumn of 2021, although reportedly not as intended (pers. comms. Katrina Marshall, NRW, 2022). Some smaller scale interventions have been made over previous decades to improve substrate suitability, including adding substrate, deployment of large boulders to manage flow and sediment dynamics, and clearing accumulations of gravel from the Peris diversion tunnel to prevent their deposition onto the spawning grounds during spates (Mould and Todd-Burley, 2015). Beyond the requirement for a rod license and membership of the angling club, fishing for charr is not regulated except for a club rule limiting anglers to four fish taken per day (pers. comms. Robin Parry, Seiont, Gwyrfai and Llyfni Fishing Society, 2022). A central goal of NRW is to manage natural resources sustainably in manner that benefits local economies and communities, and therefore, a complete ban on angling is not considered desirable (pers. comms., Huw Jones, NRW). The supplementation programme was paused in 2020 in the hope that attempts undertaken to improve habitat will result in a naturallyviable population.

OBJECTIVES OF THE THESIS

The thesis contains five research chapters that address three general objectives: (1) assess the status of the Arctic charr population at Llyn Padarn and habitat at the lake; (2) evaluate the efficacy of management interventions implemented by local managers and stakeholders (supplemental breeding and improvements to waste water treatment), and (3) investigate the relative importance of life-stages for population growth (sensitivities analysis) to guide prioritisation of management interventions. The first two research chapters are focused on two primary threats suspected to be limiting population viability at Llyn Padarn, these being (1) deoxygenation and warming of the hypolimnion and (2) disturbance to spawning habitat. Chapter 2 leverages environment agency monitoring data to characterise the deep-water thermal-oxygen habitat of cold-adapted fish in the British Isles (Arctic charr and *Coregonid* spp.) and model temporal analysis focuses on Llyn Padarn and three other Welsh lakes with native populations of cold-adapted fish (Arctic charr and *Coregonus lavaretus*). Chapter 3 concerns the reproductive ecology and reproductive success of Arctic charr at Llyn Padarn and includes surveys investigating the location of spawning sites and density of emergent fry

using a novel design of swim-up fry trap. Chapter 4 utilises a variety of longitudinal data for Llyn Padarn Arctic charr (abundance, body condition, and population genetics) to assess conservation status and the efficacy of the supplemental breeding programme. The final two research chapters are focused on the relative importance of life-stages for population growth. In chapter 5 I develop a PPM for the Llyn Padarn population using the available demographic information and calculate the sensitivity of population growth rate to changes in life-stage vital rates. In chapter 6 I collate literature data to construct slightly simplified PPMs for 41 diverse populations of Arctic charr to characterise intraspecific variability in the relative importance of life-stages for population growth. The final discussion chapter considers specifically the implications of the findings for the conservation and management of Arctic charr at Llyn Padarn. The research represents a comprehensive investigation of important topics pertinent to the conservation biology and management of Arctic charr, and cold-adapted postglacial fish more generally.

Chapter 2. The thermal-oxygen habitat of cold-adapted fish in the British Isles: niche range, temporal and spatial patterns, and the influence of anthropogenic stressors

INTRODUCTION

Warming and deoxygenation driven by anthropogenic climate change and nutrient enrichment has occurred in lakes across the globe (O'Reilly et al., 2015; Jenny et al., 2016; Knoll et al., 2018; Jane et al., 2021), and represents a significant threat to oxygen-sensitive, cold-adapted fish at their southern range edge (Graham and Harrod, 2009; Jeppesen et al., 2012). These species are understood to require access to cool, deep-water refugia habitat during the summer period of maximum thermal stress (Dillon et al., 2003; Graham and Harrod, 2009). However, in the presence of elevated nutrients, such as phosphates and dissolved organic carbon, respiration rates of heterotrophs in deep-water zones may exceed oxygen production by autotrophs, resulting in oxygen depletion, and in extreme cases, hypoxia or anoxia (Knoll et al., 2018; Quinlan et al., 2021). Thus, in combination, deoxygenation rising from the lake bottom and warming from the surface act to squeeze suitable thermal-oxygen habitat (Dillon et al., 2003; Jacobson, Stefan and Pereira, 2010). Importantly, this squeeze and its implications for sensitive fish populations is unlikely to be equal between lakes due to differences in climate, morphometric, and physico-chemical properties that will influence the current suitability of thermal-oxygen habitat and the severity, and perhaps direction, of future change (Jacobson, Stefan and Pereira, 2010; Richardson et al., 2017; Kelly et al., 2020). Identifying which types of lakes are likely to retain suitable habitat and which are most vulnerable will be necessary to prioritise conservation and identify effective management actions.

Stratification is perhaps the most significant property of lakes influencing deep-water thermaloxygen conditions (Stefan *et al.*, 1996). In mid and high latitude regions, deeper lakes will often develop a thermocline between spring and early autumn which reduces circulation of surface and bottom waters, creating a stratified profile consisting of a warm epilimnion on top a cool hypolimnion with temperatures similar to the spring (Stefan *et al.*, 1996; Boehrer and Schultze, 2008). In contrast, water column temperatures of mixed lakes are homogenised and closely follow seasonal changes in air temperature (Stefan *et al.*, 1996; Boehrer and Schultze, 2008). Consequently, stratified lakes are more likely to offer cool deep-water habitat in summer than un-stratified lakes. Moreover, stratification may slow the effect of climate warming relative to mixed lakes due to a positive feedback effect between air temperature and thermocline strength and stability (Richardson *et al.*, 2017; Woolway and Merchant, 2019). Deep-water zones could become cooler in mixed lakes if they become stratified (Woolway and Merchant, 2019), although basin morphometry may prevent a switch in mixing regime for certain lakes, in particular those that are shallow (≤ 10 m) or have a large surface area to depth ratio (Stefan *et al.*, 1996).

Whilst stratification generally improves thermal habitat, low circulation between bottom and surface waters can have a deleterious impact on deep-water oxygen, particularly in the presence of high nutrient loads. Phosphates and nitrates arriving from anthropogenic sources, such as sewage discharge and run-off from agricultural land, increase algal biomass, which through decomposition, increase biological oxygen demand (i.e., eutrophication) (Quinlan et al., 2021). Dissolved organic carbon (DOC) has the same effect on oxygen demand when mineralised by microbes but strong light attenuating properties mean DOC also influences thermal conditions and mixing dynamics (Solomon et al., 2015). Shallower, steeper thermoclines and a cooler average water temperature are predicted to arise in high DOC environments, with positive outcomes for thermal habitat (i.e., a thicker, cooler hypolimnion) but negative outcomes for oxygen habitat if a stronger and more stable thermocline reduces mixing between the hypolimnion and oxygenated surface waters (Solomon et al., 2015). Furthermore, DOC can inhibit oxygen production by plants and algae by reducing the availability of light for photosynthesis (Solomon et al., 2015). Increases in DOC have been detected at numerous mid to high latitude lakes in recent decades, a process which has been termed 'brownification' (Worrall et al., 2004; Brothers et al., 2014; Couture et al., 2015; Knoll et al., 2018). Because nutrient inputs can be reduced by catchment improvements (e.g., riparian restoration and changes in land-use practices), their management is perhaps the most effective intervention available at a local level to mitigate habitat squeeze caused by climate warming (Jones, Winfield and Carse, 2008; Jacobson, Stefan and Pereira, 2010; Stanley et al., 2012).

Investigations across lakes with different climate, morphometric, and physico-chemical properties can develop our understanding of the mechanisms influencing thermal-oxygen conditions (eg., Dillon *et al.*, 2003; Jacobson, Stefan and Pereira, 2010). Yet although surveys across space are useful, they may fail as predictive tools if change across time alters mechanism or invokes new ones (Clark *et al.*, 2010). The potential for cooler but more hypoxic conditions to arise in deep-waters because of a positive interaction between air temperature and stratification strength is one example (Richardson *et al.*, 2017; Woolway and Merchant, 2019), as is brownification, which has been linked to air temperature and rainfall increases driven by

climate change and may have a complex non-linear relationship with thermal-oxygen habitat (Worrall *et al.*, 2004; Stanley *et al.*, 2012; Solomon *et al.*, 2015). Thus, temporal analyses are particularly valuable.

In the British Isles, four species of cold-adapted fish are found near to their southern range edge: Arctic charr (Salvelinus alpinus) and three species of Coregonus (Coregonus lavaretus, C. autumnalis and C. abula). Arctic charr is more common, although the majority of populations are located in the cooler northern and mountainous regions of Scotland (Maitland et al., 2007). Further south, populations are fewer and often declining, with extinction rates especially high in Ireland (Igoe et al., 2003; Adams et al., 2007; Connor et al., 2019). Coregonus spp. are the rarest freshwater fish in the region, with only 13 natural populations remaining (Harrod et al., 1999; Winfield et al., 2013; Winfield, Fletcher and James, 2016). With the exception perhaps of northern Scotland, summer thermal conditions are thought to be near the tolerable limit for these four species, and a significant factor contributing to the decline and extirpation of some populations (Maitland et al., 2007; Winfield et al., 2010; Winfield, Fletcher and James, 2016). Despite these concerns, deep-water thermal-oxygen habitat has only been assessed in detail for a small number of lakes, and these analysis have tended to be focused toward lakes known to have poor quality habitat (e.g., Elliott and Bell, 2011). Temporal analyses of trends have been restricted to just two lakes in the English Lake District (Jones, Winfield and Carse, 2008; Elliott and Bell, 2011). Consequently, little is known regarding the range of thermal-oxygen habitats occupied, or how habitat suitability varies across lakes in response to climate and nutrient loads.

Data limitation has previously prevented such a large-scale analysis of deep-water habitat in the British Isles. Measuring thermal-oxygen conditions of the water column is not a trivial exercise, requiring boat access and specialised instruments that measure temperature and oxygen concentration at depth intervals down to the lake bottom (depth profile). Alternatively, expensive *in situ* monitoring buoys can be deployed to perform these tasks remotely. In the UK, the longest running monitoring programme is a survey of twenty lakes in the English Lake District, known as the Lakes Tour, which has been carried out in six years between 1984 and 2010 (Maberly *et al.*, 2011). A second programme, UKLEON, consists of a network of monitoring buoys that collect high frequency measurements of water column conditions at eleven locations selected to represent the range of lake types found in the UK. A third monitoring programme, which commenced in ~ 2007, is undertaken by national environment agencies to meet the assessment requirements of the European Union Water Framework
Directive (WFD). These depth profile data are managed by five independent environment agencies (Republic of Ireland and the four constituent nations of the UK) and have not been collated into a single useable data set or been analysed beyond their intended purpose of characterising the typology of waterbodies. Compared with the Lakes Tour and UKLEON programmes, the WFD monitoring campaign has sampled a greater number of lakes, and crucially, more lakes with cold-adapted fish populations.

Here, WFD monitoring data are collated and leveraged to increase understanding of the thermal-oxygen habitat utilised by Arctic charr and *Coregonus* spp. at their range edge in the southern British Isles. Specifically, the objectives were to (1) characterise the thermal-oxygen niche range of cold-adapted fish and investigate the importance of thermal-oxygen habitat in explaining their contemporary distribution; and (2) compare the relative quality of thermal-oxygen habitat between lakes and regions and assess the role of stratification, nutrient load, and air temperature in driving temporal and spatial patterns. Data that were suitable for temporal analysis were only available for a handful of lakes. Welsh lakes with natural cold-adapted fish populations had the most complete and consistent time series, and therefore temporal analysis were only undertaken for this region. The results are discussed in the context of population vulnerability to climate change and nutrient enrichment.

METHODS

Compilation of lake monitoring data

Physico-chemical data collected for the purposes of statutory monitoring requirements (e.g., WFD assessment) were acquired through formal requests to the government environment agencies of England (EA), Wales (NRW), Scotland (SEPA), Northern Ireland (NIEA), and the Republic of Ireland (EPA). Aside from depth profiles for temperature and dissolved oxygen (DO), the specific parameters of interest were surface dissolved organic carbon concentration (DOC) and surface total phosphorous (TP). Additional data for Lake District lakes collected prior to WFD implementation were supplied by the Centre for Ecology and Hydrology (CEH). Methods of measuring depth profiles included sondes manually deployed from boats and automated *in situ* monitoring buoys. In cases where several depth profiles had been collected on the same day (either at the same location or at multiple locations) then the profiles were merged. Depth profiles should ideally extend to the deepest point of the lake where oxygen depletion is generally most severe, but this had often not been achieved (mean sampling depth was 73% of lake maximum depth).

After quality control, data requests yielded a total of 3468 depth profiles for 192 lakes, including 33 with extant natural Arctic charr populations and 8 with *Coregonus* populations. Only 3 lakes from Scotland had depth profile data, compared to 33 for England (1984 – 2018), 21 for Wales (2003 – 2020), 18 for Northern Ireland (2013 – 2017), and 118 for the Republic Ireland (2001 – 2020). Quality control of depth profiles involved checking for data points with implausible values (temperature < 0 °C or > 30 °C or dissolved oxygen < 0 mg/l or > 40 mg/l); removing profiles where the sampling depth interval was > 5 m; and lastly, removing profiles where the frequency of depth sampling points was < 3. To generate a dataset with consistent depth resolution within and among lakes, each temperature and dissolved oxygen profile was linearly interpolated at 0.1 m depth intervals. Inspection of the distributions for TP and DOC values did not identify any erroneous data points from, for example, recording error. Data compilation and all analysis described hereafter were undertaken using the statistical programming language R (R Core Team, 2018) unless otherwise stated.

Spatial patterns in deep-water thermal-oxygen habitat

Models were developed to empirically describe the effect of nutrients (TP and DOC), air temperature, and maximum depth on the suitability of thermal-oxygen habitat for cold-adapted fish in stratified and un-stratified lakes. The metric selected to characterise thermal-oxygen habitat was the temperature at the 7 mg/l isocline (TDO7), illustrated in Figure 1 (Jacobson, Stefan and Pereira, 2010; Jane et al., 2021). Oxygen concentrations below 7 mg/l are generally considered sub-optimal for adult salmonids (Davis, 1975), meaning TDO7 represents the coolest water available where DO concentrations are not expected to impede physiological functions. Increases in lake heating and hypoxia place an upward pressure on TDO7 from the lakes' surface and bottom, respectively. Atmospheric longwave radiation (measured as air temperature) and solar radiation warm surface waters which are circulated deeper into the lake via mixing processes (Stefan et al., 1996). Maximum depth was selected as a predictor variable because of its central role in regulating the penetration of solar radiation and the mixing dynamics of lakes (Stefan et al., 1996; Boehrer and Schultze, 2008). Also, deeper lakes inevitably have larger deep-water zone than shallow lakes, meaning mid-water zones are less vulnerable to oxygen depletion (Jacobson et al., 2017). TP was selected as it the most important nutrient regulating primary productivity (Quinlan et al., 2021). Average TP for each lake was calculated as the median for spring months (March, April, and May) across all sampling years. Spring months were chosen because TP prior to stratification generally has a greater effect on primary production than does TP later in the year (Molot *et al.*, 1992). DOC was calculated as the median for all months sampled across years.

Climate reanalysis data from the UK Met Office (HadUK data set, Hollis *et al.*, 2019) and Met Eireann (MEERA data set, Whelan, Hanley and Gleeson, 2017) were used to calculate the average surface air temperature (TA °C) at each lake. The MEERA climate analysis provides interpolated monthly surface air temperature at a 2.5 km grid square resolution for the Republic of Ireland. Data were supplied in GRIB file format for years 2010 to 2018 and were converted to a separate text file for each month using the programme ecCodes in Linux. Mean temperature for each grid square resolution) are available from 1862, but to be consistent with Republic of Ireland data, average air temperature was calculated using yearly averages from 2010 to 2018. Depth data were retrieved from the CEH lakes portal database for UK lakes and from a database supplied by the EPA for Republic of Ireland lakes. Maximum depth was changed to the maximum profile depth for a small number of lakes where the latter exceeded the former.

To account for inconsistencies in profile depth relative to lake maximum depth, measurements below two thirds of the lake maximum depth were removed before calculating TDO7. If DO exceeded 7 mg/l throughout the profile then TDO7 was assigned as the temperature at two thirds maximum depth (Jacobson, Stefan and Pereira, 2010). If DO always exceeded 7 mg/l but the profile did not reach two thirds of maximum depth the profile was excluded from the analysis. Only profiles measured in the summer to early autumn (June 1st to October 15th) were selected for the models. After selecting profiles in the defined time-period and removing those without TDO7, DOC or TP values, the data set included 1309 profiles for 101 lakes (Figure 2). Of these lakes, 69 had at least one profile where minimum DO was \leq 7 mg/l. TDO7 values were averaged (median) for each lake prior to modelling. The median day when lake profiles were sampled was day 224 (10th August). Spearman's rank correlation indicated there was no significant relationship between sampling day and TDO7 (n = 101, *rs* = -0.01, *p* = 0.62).

Generalised additive models (GAMs) were used to capture the non-linear relationships between thermal-oxygen habitat (TDO7) and some of the predictor variables (Jacobson, Stefan and Pereira, 2010; Solomon *et al.*, 2015). The mgcv package was used for the calculation of the GAMs (Wood, 2004) with smoothing terms selected using restricted maximum likelihood (REML) estimation (Marra and Wood, 2011). The multivariate GAMs described the response of thermal-oxygen habitat to four independent predictor variables: TP, DOC, maximum depth

and average air temperature. To understand how lake mixing affected relationships between TDO7 and predictor variables, models were investigated separately for strongly stratified and weakly stratified lakes. Strength of thermal stratification (STS) was calculated as the maximum temperature gradient over a 1 m depth change (Yu, Tsuno and Hidaka, 2010), with lakes below or equal to the median value for STS ($0.33 \text{ °C} \cdot \text{m} - 1$) classified as mixed (N = 52) and those above the median as stratified (N = 51). Spearman's rank correlation was undertaken for predictor variables to aid interpretation of model outputs (Appendix 1).



Figure 1. Example of a depth profile measuring dissolved oxygen (blue line) and temperature (red line). The point where the green vertical line intersects the temperature axis is the temperature at the 7 mg/l dissolved oxygen isocline (TDO7). The dashed horizontal line shows the depth of the thermocline delineating the epilimnion (above) from the hypolimnion (below).

Thermal-oxygen habitat niche modelling

Presence-absence models using logistic generalised linear regression were developed to investigate the probability that cold-adapted fish populations occur over a range of thermaloxygen habitats measured in mid to late summer (15th July to 15th of September). Predictor variables were selected to represent the quality of deep-water thermal-oxygen habitat (TDO7, mean hypolimnetic oxygen, and mean hypolimnetic temperature) and the size of the lake and deep-water refugia habitat (lake volume and thickness of the hypolimnion, respectively). The hypolimnion was delineated using the meta.depths function (slope = 0.1, seasonal = FALSE) from the rLakeAnalyzer package which calculates the bottom depth of the metalimnion (Winslow et al., 2019). If the range of temperatures in the profile is less than 1 °C, the meta.depths function does not return values for the metalimnion (that is, the profile is not considered stratified). In these cases, mean dissolved oxygen and temperature were calculated from the whole profile. Profiles that did not attain two thirds of the lakes maximum depth were excluded. Thickness of the hypolimnion was measured as the distance in meters between the top of the hypolimnion (Figure 1) and the lakes maximum depth and was set as 0 m for unstratified profiles. Lake volume was estimated from surface area and maximum depth (CEH Lakes Portal and EPA databases) using the approx.bathy function (method = cone) from the rLakeAnalyzer package (Winslow et al., 2019). Logistic regression predicted probabilities of 1 or 0 for lake volume which was fixed by normalising the distribution of volume by natural log transformation. Where multiple profiles were available for a lake then average (median) values for the relevant explanatory variables were used for the presence-absence models. The location of extant natural (i.e., not translocated) Arctic charr and Coregonus populations was surveyed from peer review literature (Harrod et al., 1999; Igoe et al., 2003; McCarthy, 2007; Winfield et al., 2013; Winfield, Fletcher and James, 2016; Maitland and Adams, 2018) and reports by Inland Fisheries Ireland (Fisheries Ireland Publications, 2021). After removing man-made lakes, the data set consisted of 31 lakes containing cold-adapted fish populations (Arctic charr n = 24, Coregonus n = 6, Coregonus and Arctic charr n = 1) and 73 where coldadapted fish were either reported as extinct or had never been recorded (Figure 2). The median profile sampling day was 226 for lakes with cold-adapted fish populations and 227 for lakes without. Presence-absence models were investigated for each predictor variable separately and together and the most parsimonious model then selected using the stepAIC function (direction = both) from the MASS package (Venables and Ripley, 2002). This method chooses the most parsimonious model (minimum AIC) by calculating the likelihood of the data given each model

and penalizing additional parameters with added constants (Hein, Öhlund and Englund, 2012). Models with AIC values within two units of the minimum are considered comparable alternatives. Both a generic model for all cold-adapted fish species and a separate model including only Arctic charr presence-absences were developed. *Coregonus* lakes were not included as absent lakes in the Arctic charr model as the species compete for the same ecological niche and only coexist at one lake in the British Isles (Haweswater). A model for *Coregonus* was not investigated because of the small number of presences and because *Coregonus* lakes included three different species (*C. laveretus*, *C. autumnalis*, and *C. albula*). Model predictions for probability of occurrence were used to rank current suitability of habitat by region (North Wales, the English Lake District and Ireland) for each of the 31 lakes with cold-adapted fish. The term niche is used here to describe the habitat characteristics of lakes occupied.



Figure 2. Location of 104 lakes used for modelling deep-water thermal-oxygen habitat niche of cold adapted fish and 101 lakes used for GAMs investigating spatial variation in thermal oxygen habitat.

Temporal trends in thermal-oxygen habitat

Temporal trends in deep-water thermal-oxygen habitat (TDO7 and hypolimnetic DO and temperature), thermocline characteristics (strength and depth of hypolimnion), and environmental forcing variables (spring TP, annual DOC, summer air temperature, and spring air temperature) were investigated for Welsh lakes with natural Arctic charr (Llyn Padarn, Llyn Cwellyn, and Llyn Bodlyn) and Coregonus populations (Llyn Tegid). The late summer period was selected (August 1st to 7th of September) since this was when most profiles had been collected. Where multiple profiles were available for a single year (mostly Llyn Padarn) then median values were calculated for thermal-oxygen habitat and thermocline structure. Tests for monotonic temporal trends in variables were undertaken globally and for each lake using the non-parametric Mann-Kendall test from the rkt package (Marchetto, 2021). The Mann-Kendall from the rkt package is suitable for data with missing years and can include an optional variable for sample location (i.e., lake in the global analysis) and a confounding variable to be partialed out, in this case the median day of profile sampling. The partialing of day was not necessary for trends in TP, DOC and air temperature. Local average summer air temperature (June, July, August) and spring air temperature (March, April, May) for each year when depth profiles were sampled was retrieved at 1 km grid square resolution from the UK Met Office HadUK data base (Hollis et al., 2019).

Relationships between temporal patterns in thermal-oxygen properties of lakes (thermal-oxygen habitat and thermocline characteristics) and environmental forcing variables were investigated using multivariate linear regression implemented separately for each lake. Predictor variables were included in multivariate models when theory expected a direct effect on the dependent variable. Accordingly, the regression for hypolimnetic oxygen included nutrients (DOC and TP), thickness of the hypolimnion (TH), and strength of thermal stratification (STS); the regression for hypolimnetic temperature included water clarity (measured as DOC), air temperature, STS and TH; the regression for TDO7 included air temperature, DOC, TP, STS and TH; and lastly, the regression for STS and TH included air temperature and water clarity. Day of profile sampling was included as an additional explanatory variable in all models. Independent variables were natural log transformed. Spearman's rank correlation did not indicate collinearity of predictor variables for either of the four lakes (rs < 0.75). The most parsimonious model was identified using forward and reverse stepwise selection (Venables and Ripley, 2002). In the analyses described above (Mann-Kendall temporal tends and linear regression), Bonferroni correction of *p* values for multiple

tests would have been too conservative given the number of lakes (4) multiplied by the number of variables (9).

Time series sample sizes for each lake are given in Table 1. Profiles at Llyn Padarn were collected from a boat up until 2011 when an automated monitoring buoy was installed at a marginally deeper location. The depth profile sampling location at Llyn Cwellyn was changed in 2012 when a deeper region of the lake was discovered. The DOC and TP sampling station at Llyn Tegid was moved in 2016 from the outflow to a location on the lake's bank. Maximum profile depth varied between lakes and between years (69 % to 100% of lake maximum depth). To maintain the longest possible time series, depth profiles were truncated to the top two thirds of the lake water column as described for previous analyses. The thermocline was detected in all years except for Llyn Bodlyn in 2008, 2010, 2014, and 2015. In these cases mean dissolved oxygen and temperature were calculated from the whole profile and hypolimnetic thickness assigned a value of 0 m. To allow for natural log transformation when including zeros, a value of 1 was added to hypolimnetic thickness for all lakes when used as predictor variable for linear regressions.

Lake	Depth profile	Missing years			
		Air temperature	DOC	TP	
Llyn Padarn	2003, 2005, 2007,	2020	2003, 2005, 2020	2003, 2005, 2020	
	2009 - 2020				
Llyn Cwellyn	2007 - 2019				
Llyn Bodlyn	2007 - 2015,		2019	2017, 2019	
	2017 - 2019				
Llyn Tegid	2007, 2008 –			2019	
	2019				

Table 1. Time series information for Welsh lakes with cold-adapted fish populations.

DOC = dissolved organic carbon; TP = total phosphorous.

RESULTS

Spatial patterns in thermal-oxygen habitat

Summer to early autumn TDO7 values of the sampled lakes ranged from 6.56 - 20.7 °C. Values were significantly lower in stratified lakes (MD = 12.79 °C) compared with un-stratified lakes (MD = 15.99 °C; Wilcox test, p < 0.001). Results of the GAM models (Table 2) showed that lake depth and air temperature were the best variables for capturing variation in TDO7 among weakly stratified lakes, with shallower lakes in warmer climates generally offering lower

quality thermal-oxygen habitat (i.e., higher TDO7 values, Figure 3). Both variables were similar in terms of the variation explained but the effect of depth was substantially higher than air temperature (Table 2), with the latter exhibiting a convex relationship with TDO7 in the multivariate GAM (Figure 3). TDO7 increased in more eutrophic lakes (i.e., lakes with higher TP) but TP was not a significant term in the multivariate GAM, which might have been explained by a moderate negative correlation for TP and depth (rs = -0.48, p < 0.001). DOC was not important for mixed lakes, whereas in stratified lakes DOC was associated with a significant increase in TDO7 and had a stronger effect than air temperature (Table 2). Increases in TDO7 occurred steeply between DOC values of 0.7 to \sim 5 mg/l, after which the relationship flattened. A significant linear increasing relationship was found for TP and TDO7 (Table 2, Figure 3). Air temperature was indicated to have a weaker effect in stratified lakes than mixed lakes and was not a significant term in the multivariate GAM for mixed lakes. This might be explained by a moderate positive correlation between air temperature and TP (rs = 0.50, p < 0.001). There was a steep downward trend in TDO7 up to a depth of ~50 m, after which the curve became shallower, although the sample size of these deep lakes was small. The multivariate model for stratified lakes was substantially better at explaining variation in TDO7 than the model for un-stratified lakes (deviance explained = 84.7% and 54.9% respectively).

Table 2. Generalised additive models (GAMs) estimated degrees of freedom (df) and significance of smooth terms (F statistic and approximate *P* value) for the response of thermal oxygen habitat (temperature at 7 mg/l dissolved oxygen, TDO7) to dissolved organic carbon (DOC), maximum depth (Z_{Max}), average surface air temperature 2010 - 2018 (TA) and total phosphorus (TP) in mixed and stratified lakes. Multivariate models were significant for both lake typologies (p < 0.001).

Stratification	Variable	Univariate GAM			Multivariate GAM			
		Df	F	DvE	df	F	DvE	
Mixed	ТР	1.62	4.001 *	14.8 %	1.001	0.177 ^{ns}		
(N = 51)	DOC	1	1.845 ^{ns}	3.63 %	1	2.352 ^{ns}		
	Z _{Max}	1	27.1***	35.6 %	1.215	14.343***		
	ТА	3.09	5.268 **	32.8%	1.482	9.335 **	54.9 %	
Stratified	TP	2.206	10.83 ***	38.7%	1	5.19*		
(N = 50)	DOC	2.45	7.24 ***	33.7%	2.34	9.67***		
	Z _{Max}	2.38	26.03 ***	61.5%	1	41.752 ***		
	ТА	2.367	3.735 *	21.4 %	1	3.275 ^{ns}	86.3 %	

DvE = deviance explained. ns = non-significant, * p < 0.05, ** p < 0.001. *** p < 0.001



Figure 3. Multivariate generalised additive models (GAMs) estimated response of oxythermal habitat (temperature at 7 mg/l dissolved oxygen, TDO7) to dissolved organic carbon (DOC), maximum depth, TP (total phosphorus) and average surface air temperature 2010 - 2018 (TA) in stratified lakes (panel a) and weakly stratified lakes (panel b). The y axis of each plot is on a scale where zero equals the mean effect on TDO7 of the variable and shaded area represents ± 2 standard errors.

Thermal-oxygen niche of cold-adapted fish

Cold-adapted fish exhibited a large niche range in terms of lake TDO7 (6.64 - 17.36 °C), hypolimnetic dissolved oxygen (5.65 - 11.1 mg/l), and hypolimnetic temperature (7.48 - 17.62 °C), and occupied lakes both with and without a hypolimnion (Table 3). Logistic regression showed the probability of cold-adapted fish presence increased in well-oxygenated lakes with a large hypolimnion and cool temperatures (Figure 4). Coefficients of the slope were significant for all candidate predictor variables that were investigated (p < 0.05). AIC indicated TDO7 and the natural log of lake volume explained the most variation in population occurrence (TDO7, AIC = 98; volume, AIC = 96), followed by hypolimnetic thickness (AIC = 105). Hypolimnetic temperature and oxygen were relatively poor in explaining variation (AIC = 114 and 123,

respectively). For the models of Arctic charr presence-absence, TDO7 captured more variation than volume (AIC = 83 and 91) and the AIC of hypolimnetic thickness was closer to volume relative to the generalised cold-adapted fish models (AIC = 92). Hypolimnetic temperature and oxygen were again the poorest performing predictors (AIC = 99 and 108, respectively). For data including both Arctic charr and *Coregonus* populations, stepwise procedures identified the combination of TDO7 and volume as the most parsimonious model (TDO7: Z = -3.575, *p* < 0.001; ln volume: Z = 3.872, *p* < 0.001, AIC = 79). Likewise, TDO7 and volume were selected when modelling only Arctic charr presence-absence, but in this case, TDO7 had a stronger effect than volume (TDO7: Z = -3.626, *p* < 0.001; ln volume: Z = 3.183, *p* < 0.01, AIC = 73).

TDO7 values of 10.34, 12.67 and 15 °C marked the 0.75, 0.5, and 0.25 probability that coldadapted fish were present in lakes (Figure 4). These values reduced marginally for the Arctic charr model (10.18, 12.39, and 14.59 °C for the 0.75, 0.5 and 0.25 probability of occurrence, respectively). Predicted probability of occurrence using the generalised model (TDO7 + lake volume, Arctic charr and *Coregonus* presence-absence) indicated lakes with cold-adapted fish in the Ireland and North Wales offer the least suitable habitat (MD = 0.41 and 0.60 probability of occurrence respectively), with the English Lake District having more favourable habitat by some distance (MD = 0.94, Table 3). Pairwise Wilcoxon tests with Bonferroni correction showed the differences between countries in probability of occurrence were significant when comparing England and Ireland (p < 0.05), but not for other comparisons. Thermal-oxygen, morphometric, and physico-chemical characteristics of the sampled lakes with cold adapted fish are summarised in Table 3.



Figure 4. Presence-absence models describing the response of cold-adapted fish populations to the quality of deep-water thermal-oxygen habitat (TDO7 and mean hypolimnetic temperature and dissolved oxygen) and the size of habitat and deep water refugia (natural log lake volume and thickness of the hypolimnion). Solid vertical lines mark the x axis value at 50% probability of occurrence. Right and left dashed vertical lines mark the 25% and 75 % probability of occurrence, respectively. Lake sample size was 104 (present = 31, absent = 73).

Lake and region	Species	TDO7	HDO (mg/l)	HT (°C)	TH (m)	Volume	Maximum depth (m)	DOC (mg/l)	TP (µg/l)	TA (°C)	Poccur
		(C)				(KIII)	deptil (III)				
Windermere N. (12)	AC	6.64	8.99	7.48	48.9	0.056	62	1.55	16	9.46	0.98
Ullswater (11)	С	8.16	8.32	8.84	47.3	0.06	62	1.52	13.3	9.08	0.98
Thirlmere (1)	AC	6.8	9.97	8.2	32.9	0.017	49	2.2	4.91	8.72	0.97
Wastwater (13)	AC	7.36	11.20	8.28	58.8	0.023	76	0.70	2.75	8.58	0.97
Coniston Water (13)	AC	8.45	8.63	9.27	41.6	0.029	56	1.58	13.8	9.27	0.96
Haweswater (11)	AC C	9.3	9.28	10.3	64.8	0.031	74	2.51	7.9	8.27	0.94
Ennerdale Water (13)	AC	8.26	10.10	9.52	25.4	0.014	42	1.10	4.3	9.15	0.94
Crummock (13)	AC	8.15	9.63	9.39	27.3	0.012	43	1.04	5.6	9.18	0.94
Windermere S. (12)	AC	14.03	6.96	9.46	26.5	0.31	42	1.81	19.14	9.46	0.67
Buttermere (13)	AC	10.87	8.19	11.72	14.4	0.003	29	0.75	7.4	9.32	0.64
Derwent Water (15)	С	15.16	7.44	14.98	9.9	0.13	22	1.68	25.9	9.73	0.39
Bassenthwaite (14)	С	15.86	7.85	16.01	8.9	0.012	21	2.08	15.9	9.67	0.31
		8.3	8.81	9.43	30.1	0.02	46.4	1.57	10.7	9.22	0.94
Lake District		6.8 – 15.7	7 – 10.9	7.67 – 15.7	9.19 - 63.2	0.005 - 0.05	21.3 – 75	0.71 - 2.4	3.1 - 24.1	8.35 – 9.71	0.35 - 0.98
Lough Muckross (4)	AC	8.40	9.78	10.02	53.3	0.02	73	4.02	8.5	9.66	0.95
Lough Leane (3)	AC	11.27	7.03	12.23	28.1	0.13	60	5.89	11.1	9.88	0.94
Lough Neagh (1)	С	15.34	8.65	15.54	28.9	1.27	30	7.49	107.3	10.08	0.93
Lough Mask (7)	AC	16.40	9.39	16.77	0	0.5	58	NA	7.4	9.83	0.81
Lough Derg (11)	С	16.80	9.10	16.91	0	0.5	36	9.52	17.2	9.72	0.77
Lough Melvin (14)	AC	15.93	9.20	15.97	0	0.1	45	13.32	26.1	9.22	0.65
Lough Inchiquin (2)	AC	12.26	6.68	12.57	25.3	0.003	37	NA	8.42	8.93	0.5
Lough Caragh	AC	15.8	5.77	14.3	30.44	0.02	40.4	5.08	9.9	9.93	0.4
Lough Doo (17)	AC	14.65	9.71	14.59	0	0.008	46	3.53	5.3	7.93	0.37
Lough Talt (9)	AC	13.85	9.44	15.18	0	0.004	39	NA	NA	8.40	0.36
Lough Currane (4)	AC	17.36	9.14	17.63	12.9	0.04	35	NA	8.9	10.87	0.33
Lough Derryclare (5)	AC	16.30	9.28	16.65	0	0.008	31	5.28	5.9	9.55	0.21
Lough Shindilla (18)	AC	15.90	9.21	16.14	0	0.002	22	6.33	6.2	9.16	0.1
Lough Ardderry (17)	AC	16.50	9.15	16.81	0	0.001	14	13.51	7.6	9.22	0.06
Lough Kindrum (8)	AC	16.20	9.47	16.20	0	0.001	12	NA	NA	9.54	0.06
		15.9	9.2	16	0	0.21	37.1	6.11	8.52	9.55	0.41
Ireland		9.41 – 17.2	6.09 - 9.75	10.8 - 17.4	0 - 45.3	0.001 - 1	13 - 68.8	3.64 - 13.5	5.54 - 83	8-10.6	0.06 - 0.95
Llvn Cwellvn (14)	AC	8.44	8.76	9.32	21	0.004	36	1.58	7.8	9.01	0.86
Llyn Tegid (14)	С	11.75	6.55	10.76	22.7	0.019	40	4.13	17.7	9.34	0.80
Llyn Padarn (123)	ĂC	13.03	5.65	12.31	11.4	0.003	27	1.25	7.8	10.29	0.39
Llyn Bodlyn (12)	AC	12.82	9.32	13.17	7	0.0004	20	1.91	9	8.06	0.15
North Wales		12.3	7.65	11.5	16.2	0.003	31	1.74	8.44	9.17	0.60

Table 3. Characteristics of lakes with cold adapted fish (AC = Arctic charr, C = *Coregonus* spp.) used for modelling thermal-oxygen niche with summary statistics by country (median and 95 % confidence intervals). Lakes are ordered highest to lowest according to predictions of probability of occurrence (P_{Ocurr}) using logistic regression with TDO7 and natural log of lake volume as independent variables.

Numbers in parenthesis are the sample size for depth profiles. TDO7 = temperature at the 7 mg/l DO isocline; HDO = mean hypolimnetic oxygen; HT = mean hypolimnetic temperature; TH = thickness of the hypolimnion; DOC = dissolved organic carbon, TP = spring total phosphorous; STS = strength of thermal stratification; TA is the average air temperature.

Temporal trends in thermal-oxygen habitat at Welsh lakes

Mann-Kendall trend tests (Table 4, Figure 5) showed average hypolimnetic dissolved oxygen (HDO) decreased between 2007 and 2019 at Llyn Cwellyn, Llyn Tegid, and Llyn Bodlyn, with the trend being significant for Llyn Cwellyn and Llyn Tegid, and when analysed globally (i.e., for lakes combined). HDO was stable (tau = 0.00, p = 0.946) at Llyn Padarn (2003 – 2020). The strength of thermal stratification increased in all lakes (tau = 0.20 - 0.42) which was significant in the global analysis. Thickness of the hypolimnion decreased at Llyn Tegid, Llyn Padarn, and Llyn Bodlyn, but not significantly. A significant global trend in thickness of the hypolimnion. Deep-water temperature metrics (hypolimnetic temperature and TDO7) were unchanging except for Llyn Tegid where a significant increase in TDO7 was detected (tau = 0.45). Regarding air temperature (TS), and trends for both variables were significant in the global analysis (tau = 0.31 and 0.31 respectively). Increases in TS and DOC were strongest at Llyn Cwellyn (tau = 0.43 and 0.46 respectively). In contrast to summer air temperatures, spring temperatures did not exhibit a monotonic trend, and neither did TP.

Table 4. Mann-Kendall statistics testing the hypotheses of a monotonic temporal trend in thermaloxygen habitat (HDO, HT, TDO7), thermocline structure (TH, STS), and environmental forcing variables (DOC, TP, TS, TSP) for lakes with cold-adapted fish populations in north Wales.

Variable	Llyn	Bodlyn	Llyn (Cwellyn	Llyn Padarn		Llyn Tegid		Global	
	Tau	Р	Tau	Р	Tau	Р	Tau	Р	Tau	Р
HDO	-0.18	0.07	-0.48	0.023*	0.00	0.946	-0.72	0.002*	-0.3	0.005*
HT	-0.18	0.103	-0.1	0.721	-0.06	0.891	-0.21	0.767	-0.12	0.398
TDO7	-0.09	0.197	-0.15	0.564	0.06	0.655	0.45	0.047*	0.06	0.67
TH	-0.24	0.303	0.53	0.012*	-0.42	0.224*	-0.27	0.48	0.24	0.947
STS	0.21	0.32	0.3	0.191	0.42	0.056*	0.36	0.206	0.33	0.005*
DOC	0.23	0.35	0.46	0.037	0.41	0.086	0.12	0.631	0.31	0.005
TP	-0.06	0.85	0.33	0.12	-0.22	0.37	0.34	0.16	0.11	0.33
TS	0.33	0.14	0.43	0.048	0.12	0.58	0.42	0.064	0.31	0.003
TSP	-0.09	0.731	-0.07	0.792	-0.05	0.826	0.03	0.945	-0.04	0.66

P values for depth profile variables are after partialing out the effect of sampling day and are in bold for values < 0.05. * indicates that *p* was significant at the 0.05 level without partialing out the effect of sampling day (not implemented for DOC, TP, TS, and TSP). HDO = median hypolimnetic dissolved oxygen concentration; HT = median hypolimnetic temperature; TDO7 = temperature at the 7 mg/l oxygen isocline; TH = thickness of the hypolimnion; STS = strength of thermal stratification; DOC = dissolved organic carbon; TP = total phosphorous; TS = summer air temperature; TSP = spring air temperature.



Figure 5. Temporal trends in deep-water thermal-oxygen habitat (HDO = average hypolimnetic dissolved oxygen, HT = average hypolimnetic temperature, TDO7 = temperature at the 7 mg/l oxygen isocline), thermocline structure (TH = thickness of the hypolimnion, STS = strength of thermal stratification), nutrient load (TP = summer total phosphorous), dissolved organic carbon (DOC), spring air temperature (TSP = average spring air temperature March – May) and summer air temperature (TS = average summer air temperature June – August) for the four lakes with natural cold-adapted fish populations in Wales. Mann-Kendall statistics (Table 4) identified significant global trends (i.e. across lakes) for HDO (tau = -0.3), STS (tau = 0.33), DOC (tau = 0.31) and TS (tau = 0.33).

At Llyn Cwellyn, linear regression (Table 5) showed declining temporal trends in HDO were driven by the increases in DOC, TP and thickness of the hypolimnion. TP and DOC had the same relationship with HDO at Llyn Tegid, but in this case, HDO increased with thickness of the hypolimnion. At Llyn Bodlyn, the shallowest lake and the only one not to stratify in each year, hypolimnetic thickness and strength of stratification were selected as predictor variables and both had a downward effect on HDO. Similar effects of stratification strength on HDO were identified for Llyn Padarn and Llyn Tegid (model not significant for Llyn Padarn). Regarding deep-water thermal habitat (HT), warmer spring air temperatures increased deepwater temperature at all lakes except for Llyn Tegid, whereas summer temperature was either not selected as a predictor variable or, in the case of Llyn Bodlyn, had a downward effect on hypolimnetic temperature. Stronger stratification strength resulted in cooler deep-water temperatures and was significantly associated with summer air temperature in three out of four lakes, with spring air temperatures not shown to be as important for this variable. Higher values for DOC were indicated to cause weaker stratification at Llyn Cwellyn and Llyn Tegid. Regarding hypolimnetic thickness, warmer springs reduced hypolimnetic thickness whilst warmer summers had the opposite effect. Significant models for TDO7 were identified for Llyn Tegid and Llyn Bodlyn. TP was the only forcing variable selected for Llyn Tegid and caused TDO7 to increase. The model for Llyn Bodlyn indicated lower TDO7 values occurred in years with cool summers, warm springs, and high concentrations of DOC. Unexpectedly, increases in TP reduced TDO7.

Lake	Dependent variable	Stepwise selected explanatory variable(s)	R^2 , <i>p</i> -value
Llyn Bodlyn	HDO (10)	TH (-3.03*); STS (-1.36) 9.77 -0.67 ln(TH + 1) -0.31 ln(STS)	0.59, < 0.05
	HT (11)	TS (-1.12); TSP (1.87); STS (-1.53); DOY (2.99*) -54 -2.82 ln(TS) + 1.3 ln(TSP) -0.17 ln(STS)	0.63, < 0.05
	TDO7 (10)	TS (-1.10); TSP (3.88*); TP (-2.99*); DOC (-3.41*); DOY (5.21*) -141.43 -3.25 ln(TS) + 4.64 ln(TSP) -2.08 ln(TP) -9.15 ln(DOC) + 29.45 ln(DOY)	0.82, < 0.05
	STS (11)	TS (2.27*)	0.29, < 0.05
	TH (11)	TS (1.69); DOC (3.85*); DOY (-3.59*) $549.67 + 27.93 \ln(TS) + 18.69 \ln(DOC) - 114.45 \ln(DOY)$	0.71, < 0.01
Llvn	HDO (13)	DOC (-5 11*) TP (-4 41*) TH (2 91*)	0.76. < 0.001
Cwellvn	1120(15)	$2.38 + 3.32 \ln(TH + 1) - 1.12 \ln(TP) - 3.16 \ln(DOC)$	
- ··· J	HT (13)	TSP (t = 1.78); STS (-2.22)	0.38, < 0.05
	TDO7 (13)	STS $(t = -1.49)$	0.21, 0.06
	STS (13)	TS (t = 4.07^*); DOC (t = -1.54) - $11.63 + 4.85 \ln(TS) - 0.41 \ln(DOC)$	0.55, < 0.01
	TH (13)	TS (t = 3.87^*); TSP (t = -1.41) -66.78 + 36.41 * ln(TS) -4.27 ln(TSP)	0.61, < 0.01
Llyn	HDO (11)	STS (-1.56)	0.12, 0.15
Padarn	HT (11)	TSP(t = 1.77); STS (t = -2.16)	0.37, 0.06
	TDO7 (11)	TH (1.52); TP (1.22); DOC (1.24)	0.35, 0.16
	STS (11)	TS ($t = 2.62*$)	0.37, < 0.05
	TH (11)	TSP ($t = -1.54$)	0.12, 0.15
Llyn Tegid	HDO (11)	DOC (-2.03); STS (-2.015); TP (-3.82*); TH (-2.43) 36.6 -5.05 ln(TH +1) -2.65 ln(TP) -1.23 ln(STS)	0.71, < 0.05
	HT (12)	STS (t = -2.89*); DOY (t = -2.48) 107.63 -1.34 ln(STS) -17.97 ln(DOY)	0.62, < 0.01
	TDO7 (11)	TP (2.87*); DOY (-2.99*)	0.46. < 0.05
	STS (12)	TS ($t = 1.6$); DOC ($t = -1.92$)	0.32, 0.07
	TH (12)	TSP (t = -1.46); DOY (t = -3.16^*)	0.5, < 0.05

Table 5. Multivariate linear regression for the response of thermal-oxygen habitat and thermocline structure to temporal variation in forcing variables at the four lakes with natural cold-adapted fish populations in Wales.

HDO = hypolimnetic dissolved oxygen concentration; HT = hypolimnetic temperature; TDO7 = temperature at the 7 mg/l oxygen isocline; TH = thickness of the hypolimnion; STS = strength of thermal stratification; DOC = dissolved organic carbon; TP = total phosphorous; TS = summer air temperature; TSP = spring air temperature. Numbers in parenthesis after dependent variables are the number of years included in the model. Parenthesis after independent variables are the t-value for the model term (* p < 0.05). Significant models are highlighted in bold. All independent variables were natural log transformed and selected using stepwise AIC. Formulas are provided for models with R^2 values > 0.5.

DISCUSSION

Thermal-oxygen niche

The results demonstrate the importance of cool, well-oxygenated waters for the occurrence of Arctic charr and Coregonus spp. in the British Isles and identify critical niche ranges for thermal-oxygen habitat and the relative quality of habitat between lakes and regions. The approach of indexing temperature at a given oxygen isocline (in this case 7 mg/l) first proposed by Jacobson, Stefan, & Pereira, (2010), provided a single metric that integrated the effect of deep-water oxygen depletion on the minimum accessible water temperature, and was the best thermal-oxygen habitat variable for explaining population occurrence. Physiological studies have demonstrated that an oxygen concentration of $\sim 7 \text{ mg/l}$ is a critical threshold for salmonids, with exposure to lower values for durations of more than a few hours inducing stress responses, and values below ~ 3 mg/l considered lethal (Davis, 1975). The 7 mg/l threshold is also designated by the WFD as the minimum threshold indicating good ecological status for lakes with salmonid populations (JNCC, 2015). Some support for the significance of the 7 mg/l value was provided by the infrequency of cold-adapted fish in lakes with average hypolimnion oxygen concentrations < 7 mg/l (13% of populations, see Table 3 and Figure 4). TDO7 could be included in a framework for assessing and monitoring thermal-oxygen habitat at lakes with native populations, and for identifying translocation sites to establish back-up populations. Values of 10, 12.5, and 15 °C (0.75, 0.5, and 0.25 probability of cold-adapted fish occurrence, respectively) are suggested as possible boundaries for classifying lakes as offering excellent, good, moderate, or poor-quality habitat.

The rationale for TDO7 as an explanation for population occurrence is that salmonid populations will not persist if forced by unsuitably warm temperatures to spend prolonged periods below the 7 mg/l oxygen isocline. However, salmonid species will vary to some degree in their critical oxygen requirements. Indeed, it has been suggested that Arctic charr and *Coregonus* spp. are amongst the most tolerant salmonids to low oxygen (Baroudy and Elliot, 1994; Jones, Winfield and Carse, 2008; Thomas *et al.*, 2013). Therefore, during the summer period of maximum thermal stress, both may have the capacity to shelter in deeper more oxygen depleted waters where temperatures are cooler than at the 7 mg/l isocline. The selection here of the 7 mg/l isocline was forced by the necessity to standardise profiles by truncating sampling depth to two thirds of each lakes' maximum depth, which meant lower oxygen concentrations were infrequently detected. However, as indicated by Jacobson, Stefan, & Pereira, (2010), temperatures at different oxygen isoclines are generally strongly correlated, and therefore

variation in TDO7 among lakes and over time likely reflects variation in temperature at lower oxygen isoclines. Truncating depth profiles to remove the deepest regions may be viewed as a limitation since it meant potentially cooler habitat was not recorded for lakes where the 7mg/l isocline was not detected. However, the deepest regions of lakes typically represent a very small proportion of lake volume, for example, bathymetric maps for Windermere north basin show just 6% of lake volume is below two thirds of maximum depth (Ramsbottom, 1976). Therefore, the thermal conditions at such depths may have little influence on the likelihood of lakes supporting cold-adapted fish. Moreover, temperatures are generally homogenised in mixed lakes and in the hypolimnion of stratified lakes (Boehrer and Schultze, 2008).

Outcomes of presence-absence models can be affected by incorrect classification of population occurrence, i.e., lakes categorised as present when the population is in fact extinct or lakes wrongly classified as absent because the population is un-discovered or incorrectly believed extinct (Mackenzie, 2005). Regarding the possibility that lakes without recorded populations of cold-adapted fish were incorrectly classified as absent, there is a high degree of confidence that the classifications are correct owing to the status of each lakes as a WFD waterbody, which necessitates characterisation of fish communities (Champ, Kelly and King, 2009). Extinctions are less certain due to the difficulty in detecting populations that might be very small. As an illustration, the Coregonus population at Bassenthwaite Lake was considered extinct up until 2013 - 2014 when surveys captured a small number of individuals for the first time in 12 years (Winfield, Fletcher and James, 2016). In Ireland, there is some uncertainty regarding the current status of Arctic charr at Lough Currane (listed as status unknown by Igoe et al., (2003)) and also Lough Ardderry, which did not feature in the review of Irish Arctic charr distribution by Igoe et al., (2003) but is referenced in an Inland Fisheries Ireland report (Inland Fisheries Ireland, 2010). A further consideration is whether extinct populations should have been classified as present if the cause of extinction was attributed to a disturbance not related to thermal-oxygen habitat. An example is Lough Dan, which was found to have good thermaloxygen habitat (TDO7 = 10.07 °C) but the Arctic charr population was extirpated in the late 20th century due to acidification (Maitland *et al.*, 2007). However, in such circumstances generally, one cannot be certain that thermal-oxygen habitat was not a secondary causal factor, although it appears this was not the case for Lough Dan.

A second important consideration of presence-absence modelling is whether all of the sampled habitats are accessible to the species (Radinger and Wolter, 2015). Cold-adapted fish are understood to have colonised lakes in the British Isles from the sea *via* river networks toward

the end of the last glacial maximum, with limited dispersal occurring subsequently as the anadromous phenotype of Arctic charr and *Coregonus* spp. became extinct (Igoe and Hammar, 2004; Maitland *et al.*, 2007). Given the substantial geomorphological changes that occurred during and since the last glacial period, including the disappearance of river systems (Glasser *et al.*, 2004; Kearsey *et al.*, 2019) and waterfall formation (Glasser *et al.*, 2004; Zaprowski and Floyd, 2005), we cannot know if a presently un-occupied lake without a clear route to sea was once accessible and colonised, or indeed, if lakes existed in their current state. Examples of headwater lakes or rivers with fish populations that are now isolated by natural barriers to upstream migration (e.g. waterfalls) are numerous (May *et al.*, 2017), and include Arctic charr (e.g., Bain, 1975).

The multivariate presence-absence models showed volume was of similar importance for the occurrence of cold-adapted fish as TDO7. A more voluminous lake will have a greater amount of thermal refugia habitat than a less voluminous lake with the same hypolimnetic thickness and should have the capacity to support a larger population. Larger population size generally infers a greater resilience to environmental disturbance and a longer period of decline is required before extinction is reached (Sgrò, Lowe and Hoffmann, 2011). Greater volume may also facilitate coexistence with competitors, such as *Salmo trutta*, *Perca fluviatilis*, and *Rutilus rutilus* (Connor *et al.*, 2019; Morrissey-McCaffrey *et al.*, 2019).

Niche ranges and probability of occurrence derived from presence-absence models provide useful measures for classifying current suitability of thermal-oxygen habitat and relative vulnerability of populations to eutrophication, brownification, and climate warming. The results indicate populations in the English Lake District are generally the most likely to maintain healthy populations owing to low TDO7 values and large lake volumes. Eight out of the twelve sampled lakes had probabilities of occurrence > 0.90, a value that was only matched outside of the region by three other lakes (Lough Muckross, Lough Leane, and Lough Neagh, Ireland). Bassenthwaite Lake had the lowest quality habitat, and as described earlier, the population's status is highly precarious (Winfield, Fletcher and James, 2016). Windermere south basin had the highest TDO7 for an Arctic charr lake in the UK (14.03 °C) and the population has experienced significant declines relative to the north basin (Winfield *et al.*, 2010) where thermal-oxygen habitat was the highest quality of all sampled lakes (TDO7 = 6.64 °C). Low average hypolimnetic oxygen concentrations in the south basin indicate high TDO7 values were a consequence of a shallow 7 mg/l oxygen isocline. In Wales, Llyn Padarn and Llyn Bodlyn both offer poor quality habitat (0.15 and 0.39 probability of occurrence,

respectively). In the case of Llyn Bodlyn, low habitat quality is a consequence of the lake's small volume and shallow depth. In contrast, and similar to Windermere south basin, low probability of occurrence at Llyn Padarn appears driven by oxygen depletion causing TDO7 to rise above the median temperature for the hypolimnion. Also in common with Windermere south basin, the Llyn Padarn population has declined dramatically in recent decades. Llyn Cwellyn was indicated to be the most likely Welsh lake by some distance to maintain a viable Arctic charr population. However, temporal analysis identified deoxygenation may be underway, although the most recent values for hypolimnetic oxygen remain above the 7 mg/l threshold (Figure 5). Irish lakes are noteworthy because the majority were found not to have a hypolimnion, which is often assumed to be a requirement for lakes to support cold-adapted fish (Graham and Harrod, 2009). These un-stratified lakes generally had the highest TDO7 values and lowest probability of occurrence, especially in lakes where volume was small. However, in contrast to stratified lakes such as Windermere south basin and Llyn Padarn, the high TDO7 values could be attributed to the mixing of warm surface waters, rather than oxygen depletion (Table 3). Arctic charr extinction rates are particularly high in Ireland (Igoe et al., 2003), which may be a consequence of the low-quality thermal habitat alone, or more likely, in combination with other disturbances (Connor et al., 2019; Morrissey-McCaffrey et al., 2019). However, unlike Wales and the Lake District where all lakes with cold-adapted fish were sampled, only 28 % of the ~45 extant Irish Arctic charr populations featured in the data set (Igoe *et al.*, 2003). Therefore, the sample is not necessarily representative of thermal-oxygen conditions across the country.

In addition to current deep-water thermal condition, several additional factors may influence the likelihood of a population persisting in a warmer climate. First, lakes may be differently affected in the future by additional stressors that could increase with climate change to exacerbate impacts. The focus here has been on oxygen depletion driven by TP and DOC, but competition from introduced non-native fish, whose prevalence is expected to increase as lakes warm, will likely accelerate population decline and extirpation (Connor *et al.*, 2019; Morrissey-McCaffrey *et al.*, 2019). Second, phenotype variation between populations of cold-adapted fish is high (Adams and Maitland, 2007; Bush and Adams, 2007), and some of these phenotypes may be better adapted to cope with temperature increases than others (Zillig *et al.*, 2021). Finally, differences in habitat quality between lakes might not be maintained if mechanisms regulating deep-water thermal-oxygen habitat are altered by climate warming, or if relationships between air temperature and habitat are not the same across lakes, which is discussed further in the following sections.

Spatial patterns in thermal oxygen habitat

Thermal-oxygen habitat was significantly affected by TP, DOC, air temperature, and lake depth. However, how and if these factors affected TDO7 differed according to stratification status. Cooler deep-water temperatures in stratified lakes suggests they are more likely to maintain viable cold-adapted fish populations than mixed lakes if thermal-oxygen habitat is squeezed. Moreover, air temperature had a weaker effect on TDO7 among stratified lakes compared to mixed lakes, indicating they might be less responsive to future climate warming. This finding is similar to temporal analysis of 215 lakes in North America by Richardson *et al.*, (2017), who found that the rate of lake warming between 1975 and 2012 was significantly higher in mixed lakes. As expected, deeper lakes had lower TDO7 values. Water cools as it is circulated to greater depths and a thinner deep-water zone in shallow lakes will mean that the 7 mg/l oxygen isocline (if present) is closer to the surface (Jacobson, Stefan and Pereira, 2010).

It was hypothesised that DOC could reduce TDO7 by increasing light attenuation and stratification strength, but might also have the opposite effect for three reasons: (1) photosynthetic oxygen production is inhibited; (2) mineralisation of DOC by heterotrophs consumes oxygen; and (3) stronger stratification may cause oxygen depletion (Mulholland, 2003; Brothers *et al.*, 2014; Couture *et al.*, 2015; Solomon *et al.*, 2015; Knoll *et al.*, 2018). No significant effect was found for mixed lakes, whereas for stratified lakes, TDO7 increased with DOC up to values of ~ 5 mg/l, indicating the oxygen reducing effect exceeded any cooling effect. Above the 5 mg/l value, TDO7 varied little with DOC, indicating the two opposing effects of DOC were in equilibrium (Figure 3). Therefore, brownification at stratified lakes that are already turbid may have limited impact on the quality of thermal-oxygen habitat compared with clear lakes.

Total phosphorous is generally strongly positively correlated with primary production which drives eutrophication and consequent oxygen depletion (Molot *et al.*, 1992; Jacobson, Stefan and Pereira, 2010; Quinlan *et al.*, 2021). Accordingly, it was expected phosphorous would increase TDO7 by causing a shallower 7 mg/l DO isocline, especially in stratified lakes. Support regarding mixing status was provided by the multivariate GAM which showed a significant upward effect of TP on TDO7 for stratified lakes, whereas the effect was weaker and not significant for mixed lakes. The findings suggest preventing or reducing nutrient

enrichment will be a more worthwhile management intervention for stratified lakes than mixed lakes.

The significance of stratification for thermal-oxygen habitat indicates that Irish populations occupying mixed lakes are most threatened by climate warming. However, as indicated in the temporal analyses here, and by Richardson et al., (2017) and Woolway and Merchant (2019), a possible response of lakes to a rise in air temperature is that stratification strength will increase, which could conceivably cause deep-waters of currently mixed lakes to become cooler. The cold-adapted fish lakes sampled in Ireland are deep enough to stratify (Table 3), but other aspects of basin morphometry, as well as wind, cloud cover, hydrology, and water clarity, may currently be preventing stratification (Stefan *et al.*, 1996; Boehrer and Schultze, 2008). Except for morphometry, climate change could affect these variables in a manner that increases stratification strength.

Temporal patterns in thermal-oxygen habitat

Hypolimnetic oxygen concentration declined in three of the four lakes in Wales with natural cold-adapted fish populations. At Llyn Tegid, the decline in dissolved oxygen resulted in a significant increase in TDO7 despite a slight decrease in hypolimnetic temperature. Similar declines in deep-water oxygen have been reported in other analyses at a variety of lakes across the globe (Nelligan *et al.*, 2019; Jane *et al.*, 2021). It was hypothesised that decreases in dissolved oxygen would be driven by increases in nutrients and the formation of deeper and stronger thermoclines. Linear regression (Table 5) provided significant support at three of the lakes: Llyn Bodlyn, where oxygen was lowest in years with the deepest and strongest thermoclines; Llyn Cwellyn, where increases in DOC and TP drove the declining trend in dissolved oxygen; and Llyn Tegid, where each of the predicted effects were detected. As has been previously outlined, DOC may decrease dissolved oxygen by generating stronger thermoclines, inhibiting photosynthesis, or increasing biological oxygen demand. However, contrary to theory, higher DOC reduced stratification strength at Llyn Cwellyn and Llyn Tegid, suggesting the role of DOC in driving dissolved oxygen concentrations at these lakes is biological, rather than physical.

Despite increases in summer air temperature, hypolimnetic temperature was unchanging. Thermocline steepness increased across the four lakes and linear regression indicated steeper thermoclines developed in warmer summers, which protected deep-water refugia habitat from the elevated air temperatures. Indeed, at Llyn Bodlyn, hypolimnetic temperature decreased in warmer summers. These changes in thermocline properties and their buffering effect against climate warming have been detected in other deep, stratified lakes (Richardson *et al.*, 2017), and infers cold-adapted fish may experience little change in average hypolimnetic temperature from summer warming. However, the formation of steeper thermoclines is expected to increase deep-water hypoxia (Jane *et al.*, 2021), an effect that was indicated at two of the lakes studied here (Table 5). Spring temperatures, which were stable over the time-series, were more strongly correlated with hypolimnetic temperature than summer temperatures, presumably because they drive early formation of thermoclines and determine deep-water temperature prior to stratification (Snucins and Gunn, 2000). Warm springs were associated with a thinner summer hypolimnion at three of the sampled lakes. In summary, the results indicate future increases in spring temperature will negatively affect cold-adapted fish by producing a warmer and thinner hypolimnion, whereas summer warming may increase vulnerability to deoxygenation through the formation of steeper thermoclines that reduce mixing.

Regression analysis produced limited explanation for temporal patterns in thermal-oxygen habitat at Llyn Padarn. Sample sizes were relatively small for time-series analysis and explanatory variables had missing data in some years (Table 1). Lakes are highly complex systems, especially when stratification is a factor regulating thermal-oxygen habitat. Additional climate variables, in particular solar radiation, wind, and rainfall, which can influence heating and mixing, might have been important factors if investigated (Jennings *et al.*, 2012; O'Reilly *et al.*, 2015). Secchi depth captures the combined effects of all particles that influence water clarity, rather than just DOC (Tyler, 1968), but the temporal resolution of these data was not adequate. Nutrients other than TP (e.g., nitrates) can increase primary production (Hornung, 1999; Paerl *et al.*, 2020). Finally, variation in sampling day may have added noise to the data. Each of these variables may also have been important for explaining variation between lakes in the spatial analysis, as might aspects of basin morphometry other than maximum depth. In particular, lakes with a large substrate surface area relative to volume are more likely to exhibit oxygen depletion (Molot *et al.*, 1992).

Temporal trends in nutrients

Increases in DOC occurred at all four lakes (Table 4), which is a trend that has been observed in numerous mid to high latitude lakes (Worrall *et al.*, 2004; Brothers *et al.*, 2014; Couture *et al.*, 2015; Knoll *et al.*, 2018). This so called 'brownification' may be caused by several mechanisms, including natural de-acidification of terrestrial soils, landscape disturbance (e.g. forestry activity), increases in air temperature, and higher rainfall (Worrall *et al.*, 2004; Stanley

et al., 2012; Solomon *et al.*, 2015). Because DOC is transported into lakes by river networks and land run-off, management of DOC will require catchment wide measures (Stanley *et al.*, 2012). Interventions such as restoration of natural riparian vegetation may be effective; however, compared with TP and nitrates, DOC has received little attention from a management perspective (Stanley *et al.*, 2012).

Controlling eutrophying nutrients is a central objective of the WFD (Carvalho *et al.*, 2019). TP values of Welsh lakes compare favourably to lakes in other regions of the British Isles and do not appear to have increased since the directive was implemented. However, close attention should be directed toward Llyn Cwellyn and Llyn Tegid where the temporal analysis indicated a possible increase in TP and significant downward effect on deep-water oxygen concentrations. TP is a natural and important feature of lake ecosystems, but certainly a proportion of current TP will be derived from anthropogenic sources, and therefore could be reduced by appropriate management of diffuse and point sources. Controlling anthropogenic influenced factors that affect deep-water oxygen for cold-adapted fish, such as TP and DOC, are actions that can be implemented locally to mitigate habitat squeeze caused by climate warming, which is an issue that will only be addressed through international action.

Conclusion

The study analysed a novel data set documenting deep-water oxygen and temperature conditions at a large number of lakes in England, Wales, and Ireland, including a substantial proportion of the lakes with cold-adapted fish populations. Analysis showed that TDO7 is a useful measure of habitat suitability and that the English Lake district is the most likely region to maintain viable populations, with Irish lakes generally being the least suitable. The poorest quality habitat occurred in shallow, mixed lakes located in the warmest regions. Temporal analysis found hypolimnetic temperature has been stable at stratified lakes in Wales, but a significant increase in the strength of thermal stratification has occurred, with potential negative implications for deep-water dissolved oxygen, which declined in three out of four lakes. The role of DOC in regulating thermal-oxygen habitat is often overlooked, with the focus tending to be on eutrophying nutrients such as phosphorus. However, here it was demonstrated DOC is perhaps more significant than TP, and generally had a negative impact on TDO7 and dissolved oxygen in stratified lakes. It is therefore concerning that DOC has increased across the four lakes in Wales with cold-adapted fish populations, and this trend is probably replicated in other lakes in the British Isles (Worrall *et al.*, 2004). Reducing DOC through catchment

measures could be one method for local managers to improve current thermal-oxygen habitat and mitigate the impacts of climate warming.

Chapter 3. Spawning habits and recruitment success of Arctic charr at Llyn Padarn assessed by snorkelling and emergent fry traps

INTRODUCTION

Arctic charr (*Salvelinus alpinus*) are in widespread decline across the species' southern range, with low survival rate of early life-stages (eggs and alevins) suspected to be one contributing factor (Igoe *et al.*, 2003; Maitland *et al.*, 2007; Winfield and Fletcher, 2009; Jeppesen *et al.*, 2012; Miller *et al.*, 2015). In common with other salmonid species, the early life-stages of Arctic charr require highly oxygenated water, meaning females typically lay their eggs in stony substrates clean from fine sediments that could cause anoxia of the interstitial waters (Low *et al.*, 2011; Riley *et al.*, 2019). As such, spawning areas are vulnerable to increased sedimentation that can follow from eutrophication (Miller *et al.*, 2015). Other disturbances that threaten early-life stages include acidification, heavy metal contamination, predation by nonnative species, desiccation due to water abstraction, and washout of eggs and alevins caused by flooding (Crisp, 1990; Maitland *et al.*, 2007; Setzer, Norrgard and Jonsson, 2011). Finally, because water temperatures experienced by southern range populations are near to the critical thermal limit for egg survival (~ 8.5 °C), predicted anthropogenic climate warming may have a significant impact (Kelly *et al.*, 2020).

Despite concerns regarding the reproductive success of temperate Arctic charr populations, little attention has been directed toward studying early life-stage ecology and survivorship, or toward developing sampling methods to assess fry recruitment (Winfield and Fletcher, 2009; Miller *et al.*, 2015). Researchers in Ireland identified snorkelling as a viable, non-destructive method for measuring the abundance of eggs at shallow littoral sites, and successfully applied the technique to link egg density with habitat features such as depth, substrate particle size, and size of interstitial space (Low *et al.*, 2011; Igoe and Ruane, 2012). As an alternative to visual surveys by snorkelling or SCUBA diving, egg abundance can be measured with suction samplers that extract eggs from the gravel (Butterworth, 1980; Stauffer, 1981). However, suction sampling is a destructive method not desirable for threatened populations. Redd counting from the bank, water, or an air platform, which is a method commonly employed to assess spawning activity of salmonids, is unsuitable because Arctic charr rarely dig defined redds (Frost, 1965; Low *et al.*, 2011). Beyond the alevin stage when fry first emerge from the

gravel, proven methods of sampling are not described and information is greatly lacking on survivorship, habitat use, and phenology. In mild locations such as the British Isles, fry emergence in autumn spawning populations (spawning in late November to December) may occur between March and early May, after which it is thought fry soon depart the spawning grounds to feed in the pelagic or profundal zone (Frost, 1965; Klemetsen *et al.*, 1989; Baroudy and Elliott, 1994). Because fry quickly migrate into open water sampling cannot be undertaken by electric fishing.

A method of sampling emergent fry would be a valuable tool for measuring temporal and spatial patterns in recruitment and for evaluating the effectiveness of habitat remediation efforts. If combined with quantitative assessments of egg abundance, sampling of emergent fry could be used to estimate natural survival rates at the egg and alevin stage. A possible method, and one widely used for closely related lake trout (Salvelinus namaycush), are emergent fry traps (Collins, 1975; Stauffer, 1981; Casscles et al., 2016). These exploit a behaviour exhibited by salmonids, including Arctic charr, whereby fry emerging from the gravel must first surface to fill their swim bladder with air before commencing a free swimming life-style (Collins, 1975; Wallace and Aasjord, 1984). The traps function by enclosing an area of substrate under a mesh cone which directs the upward swimming fry into a collection vessel. Traps can be deployed at most depths and in both flowing and still water; an important requirement for Arctic charr which are highly variable in their choice of spawning habitat (Frost, 1965; Klemetsen et al., 2003; Walker, 2007). A further advantage of emergent fry traps is that they sample a known unit area of substrate, making it possible to investigate relationships between numbers of emergent fry and the habitat beneath each trap, and to extrapolate catch across the whole spawning area to estimate total fry production. Potential applications other than population monitoring could include obtaining samples for assessing genetic polymorphism between spawning sites and studies of phenology.

Llyn Padarn Arctic charr, which are one of only three natural populations in Wales, have been severely affected by spawning habitat disturbance and have declined significantly in recent decades (Thomas, Griffiths and Hall, 2010). A population recovery has been underway since an artificial breeding programme commenced in 2011, but high proportions of hatchery origin fish among adults suggests natural recruitment is low (see Chapter 4). A major factor implicated in reduced recruitment success has been the Dinorwig pumped storage power station (here after power station) which, when constructed in the late 1970s and early 80s, prevented Llyn Padarn Arctic charr from accessing the only confirmed spawning site in the adjoining

upstream lake, Llyn Peris. Subsequently the population has spawned in the Afon y Bala, a short stretch of river that was the historic link between Llyn Padarn and the now impounded Llyn Peris (see Chapter 1, Figure 2). However, early life-stage survival at the Afon y Bala is believed to be low due to extreme flow velocities during spate events (Mould and Todd-Burley, 2015). In addition, large volumes of warm generating water stored in Llyn Peris are frequently discharged into the Afon y Bala through bascule gates operated by the power station (Thomas, Griffiths and Hall, 2010; Mould, 2015). Aside from potentially causing wash-out of eggs and alevins, discharges can increase the water temperature in the Afon y Bala by several degrees above ambient (Thomas, Griffiths and Hall, 2010). Given the southerly location of Llyn Padarn, these temperature spikes lasting several hours have the potential to cause significant egg mortality, especially if they coincide with periods of mild winter weather (Thomas, Griffiths and Hall, 2010). To improve the spawning habitat, a scheme involving substantial reengineering of the river channel was implemented in autumn 2021. However, any future evaluation of this intervention will not be possible without methods for measuring spawning success.

A second factor that may have affected recruitment at Llyn Padarn is eutrophication causing sedimentation of spawning sites in the lake if such sites exist. Spawning in rivers is considered rare in Arctic charr, with most populations utilising shallow littoral habitats (Frost, 1965; Milner, 1985; Klemetsen *et al.*, 2003; Walker, 2007; Low *et al.*, 2011). However, at Llyn Padarn, anecdotal reports of spawning outside of the Afon y Bala, and historically Llyn Peris, have never been corroborated by scientific surveys (Butterworth, 1980; Thomas and Holt, 2011). Identifying additional spawning sites would be an important conservation development, since it would reduce estimates of the population's vulnerability to disturbances affecting the Afon y Bala. Furthermore, density-dependent survival of salmonid early-life stages is strongly correlated with the size of suitable spawning habitat, meaning population carrying capacity should increase with the number of breeding sites (Pulg, Vollset and Lennox, 2019).

The work presented here describes a two-year investigation at Llyn Padarn of Arctic charr fry production using emergent traps. The objectives of the study were to (1) demonstrate that the Arctic charr are captured in emergent traps; (2) obtain information on the timing of Arctic charr fry emergence; (3) investigate relationships between spawning habitat characteristics and the number of emergent fry; and (4) measure variation in fry production between spawning sites and between years. The design of the trap design featured a novel detachable collection vessel that could be retrieved without needing to remove the main body of the trap from the water.

This feature allowed rapid and frequent checking of traps by a single worker to generate finescale temporal information on fry emergence and, of particular importance for objective 3, ensured the exact same area of substrate was sampled by each trap throughout the survey. In addition to fry trapping, visual and camera aided snorkel surveys were undertaken to assess the spatial distribution and density of eggs at the Afon y Bala and identify if spawning occurred at putative sites in the lake.

METHODS

Location of Arctic charr spawning

Surveys of egg distribution and density were undertaken by snorkelling in the winter of 2019 - 2020 and December 2020. Two methods were employed. The first was a general swim-over of putative and known spawning sites to assess the presence-absence and approximate distribution of eggs. The approach involved methodically swimming over the site with occasional dives down to the substrate. Dives often included gentle disturbance of the substrate to check for any buried eggs. When eggs were found the snorkeler communicated to a bank support team who marked the approximate location on an aerial image of the site. The second method was a stratified camera survey which allowed a more precise spatial assessment of egg distribution and gave a semi-quantitative estimate of egg density. The method was only successfully employed at the Afon y Bala. The snorkeler swam the length of a weighted transect line set perpendicular to the bank and took photos of the substrate at 1 m intervals that were marked with cable ties threaded through the line. After completing one transect the line was moved 2.5 m downstream. The camera rig consisted of a Fuji Finepix XP130 digital camera mounted at a height of 48 cm above a weighted square frame. A rope attached to the frame allowed the snorkeler to lower the rig onto the substrate. The camera was programmed to take an image every 10 seconds and produced an image size of 0.16 m^2 . The height of the camera meant that some shallow sites close to the bank could not be surveyed. Eggs visible in each image were manually counted in ImageJ (Schneider, Rasband and Eliceiri, 2012). The egg density data for each image was then matched with transect points mapped in a GIS. Details of the methods employed on each sampling day and the location of survey sites are provided in Table 1 and Figure 1. Putative sites were selected from previous surveys by Butterworth (1980) and Thomas and Holt (2011) and according to suggestions by the local fisheries officer. Attempts were made to conduct quantitative stratified snorkel surveys of egg density as described by Low et al., (2011) for lake spawning Arctic charr in Ireland. However, it was

found that diving to the substrate and counting eggs was not feasible in the flowing water at the locations where eggs were found. Habitat characteristics of sites were not recorded at the time of snorkel surveys but were for emergent fry traps surveys described later.

Location	Dates	Method	Notes
Afon y Bala/Afon Hwch *	03/12/19	Swim-over	No eggs found – spawning
5			probably not commenced
	17/12/19	Swim-over	
		camera (pilot)	
	19/12/20	Camera	
	23/01/20	Camera	
	09/12/20	Swim-over	No eggs found – spawning
			probably not commenced
	18/12/20	Swim-over	Strong flows prevented camera
		Camera	survey
Mouth of Afon y Bala	15/01/20	Swim-over	
Penllyn	23/01/20	Swim-over	
	06/02/20	Swim-over	
	18/12/20	Swim-over	Strong flows prevented camera
			surveys
Mouth of Afon Fachwen	23/01/20	Swim-over	-
Mouth of Afon Coed	23/01/20	Swim-over	

 Table 1. Details of egg snorkel surveys undertaken at Llyn Padarn.

* Previously confirmed spawning area



Figure 1. Survey sites at Llyn Padarn (a) and the approximate area covered by swim-over surveys at each of the five locations (red polygons in b to e).

Emergent fry traps design

The emergent fry traps shared the three basic components of other published designs: a circular rim which rests on the substrate and provides ballast, a mesh cone that is attached to the rim, and a collection vessel at the top of the cone to trap the fry (Figure 2). A relatively heavy trap was required as Arctic charr at Llyn Padarn spawn in flowing water. The rim of the trap was therefore constructed from a 2 m length of large diameter corrugated hose (\emptyset 59 mm) that was filled with sand and joined into a circle using a short section of 58 mm diameter wooden dowel. The cone was made from uncoated aluminium insect screen (1.36 mm mesh size) that was cut

into a circle with a triangular sector removed, the sides of which were then stapled together to form the desired conical shape. To create the hole that would direct fry into the collection vessel, a small incision was cut at the apex of the cone and a 96 mm diameter plastic funnel inserted so that the funnel neck (70 mm long, \emptyset 5 mm), through which the fry would swim, protruded from the top of the cone. The funnel neck was cut in half along the vertical axis down to its base as it was felt fry may struggle to swim upward along the full length of such a narrow tube. Leaving the height of the neck in place, rather than just removing it all together, added stability to the fry collection vessel when anchored to the top of the trap. The funnel was secured to the mesh by bolting a second plastic funnel with the neck removed over the outside of the cone top. The completed mesh cone was then attached to the hose rim using cable ties.

The removable fry collection vessel was made from of a 130 mm length of 68 mm diameter drainage pipe. Inserted into the base of the pipe was a machine cut disc of clear acrylic (5 mm thickness) with a hole that fitted a 90 mm length by 38 mm diameter section of pipe. This internal pipe slotted over the aforementioned plastic funnel that directed fry into the vessel. The lid of the capture vessel was made in the same manner as the base. Small holes (Ø 1.5 mm) were drilled into the lid, base, and sides of the collection vessel to ensure adequate oxygen supply. The vessel was attached to the body of the trap by a 4 mm diameter piece of cord that was tied to a cross shaped piece of acrylic that wedged inside the plastic funnel of the mesh cone. The vessel slid down the cord via the internal pipe and a 6 mm diameter hole in the lid to rest on the top of the external plastic funnel where it was anchored firmly using a heavy duty crocodile clip. Before attaching the vessel, a latex balloon was stretched over the opening of the internal pipe and cut near to the base to inhibit fry from returning into the internal pipe where they would be lost when retrieving the vessel from the trap. The completed traps (N =15) were ~ 43 cm high at the top of the collection vessel, weighed ~ 6 kg in air, and covered an area of 0.32 m². Deployment of traps and retrieval of fry collection vessels was undertaken by wading wearing a membrane dry suit and was possible at depths up to chest high. The only routine maintenance required once traps were deployed was to occasionally remove organic sediment and filamentous algae that accumulated on the mesh (Penllyn site only) which was achieved using a brush attached to a long handle.



Figure 2. Emergent fry traps design: (a) the completed trap; (b) the fry collection vessel with lid in place; (c) the internal pipe with latex balloon; (d) the acrylic wedge inside the funnel that secured the chord for attaching the fry collection vessel; (e) the funnel at the top of the mesh cone that directed fry into the collection vessel; and (f) a diagram showing how the fry collection vessel fitted onto the trap.

Emergent fry traps surveys in spring 2020

Traps were positioned in areas where eggs had been observed by snorkel surveys during the period of egg incubation. Spawning was confirmed at the one previously known site in the lakes inflowing river (Afon y Bala) and at new site near the lake outflow (Penllyn). However, in 2020 traps were only deployed at Penllyn as it was strongly suspected that very few eggs

had survived in the Afon y Bala and a pilot study the previous year had shown there was a high likelihood that frequent flooding and the deeper water at this site and would cause damage to traps and reduce the number of days when traps could be reached by wading. It was decided that deploying all 15 traps at Penllyn would provide a greater opportunity to achieve the objectives of (1) demonstrating that the traps functioned; (2) assessing relationships between fry production and habitat characteristics; and (3) obtaining phenological information on fry emergence. Traps were deployed in four transects running parallel to the direction of water flow (Figure 3). Traps on the same transect were tethered together and anchored to a concrete block to ensure they would not move in floods. The distance between traps and between transects was approximately 1-1.5 m.

The survey began on the 28th of February, when 8 traps were set, followed by a further 7 traps on the 2nd of March. Fry collection vessels were initially examined at 3 - 4 day intervals, decreasing to ~2 days once the first fry were captured ($\mu = 2.08$ days, range 1 - 3 days). The survey continued until the 27th of March when sampling was ended prematurely by the Covid-19 outbreak. Fry in each collection vessel were enumerated and catch per unit effort (CPUE) calculated as the number of fry in a trap divided by nights fished. Nights were used as it is understood that salmonid fry swim-up under the safety of darkness (Brüning, Hölker and Wolter, 2011) and therefore variation in the number of daylight hours fished (which was generally small) was not expected to affect capture numbers. Histograms of CPUE were strongly left skewed, and therefore the geometric mean and 95% confidence intervals were used for summary statistics. To deal with zero values when calculating the geometric mean a value of one was added to all data points and then subtracted from the derived mean (de la Cruz and Kreft, 2019).

Emergent fry traps trap surveys in spring 2021

Traps in spring 2021 were deployed on 1st of March and split evenly between the Afon y Bala and Penllyn (7 traps in two transects at each site, Figure 3). Traps in the Afon y Bala were tethered to a single stake on the bank for security against flooding. Transects were positioned in areas of high egg density that had been determined qualitatively by snorkelling the previous December. Again, the intention was to check the contents of the traps every two days after the date of first capture, but flooding meant that longer periods elapsed on several occasions (Penllyn: $\mu = 2.37$ days, range = 2 – 4 days; Afon y Bala: $\mu = 2.64$ days, range = 2 – 5 days). Access issues caused by flooding were greater at the Afon y Bala due to the deeper water at
the site. In addition, traps at the Afon y Bala had to be lifted and cleaned on two occasions when buried under gravel deposited by flooding. Traps at the Afon y Bala could not be set between March 12th and 16th (i.e., collection vessels were not attached). Data for this period were excluded when comparing fry production of the two sites. Based on the results of 2020 surveys, it was planned that the survey would continue until the end of March, and thereafter until no fry were captured for one week.

Table 2. Details of emergent fry trap deployment

Year	Afon y Bala	Penllyn
2019	Pilot	-
2020	15 traps	-
2021	7 traps	7 traps



Figure 3. Llyn Padarn (a) and the position of swim-up fry traps at Penllyn 2020 (b) and the Afon y Bala 2021 (c). The 7 traps deployed at Penllyn in 2021 were located in the area approximate to traps 5 to 15.

Habitat characteristics at trap locations

Water flow velocity at 5 cm above the substrate was measured adjacent to each trap using a Geopacks flow meter. Water depth was read from a tape measure fixed to a pole. A TinyTag data logger secured inside a hollow breeze block at the Afon y Bala site recorded water temperature at 1-hour intervals starting from mid-December 2020 (approximate spawning time) until the survey end. Temperature at < 2 m depth recorded by an automated monitoring buoy at the centre of the lake was used to represent water temperatures at Penllyn. Temperatures for the lake and Afon y Bala were taken from midday readings because daily data from the buoy were not always at standardised time intervals. Substrate particle size was determined by analysing a photograph of the substrate beneath each trap taken using the same camera equipment as surveys of egg density. A uniform grid of 25 stratified points was

generated for each image and the length of the longest visible dimension of the particle beneath each point then measured (executed in PhotoQuad (Trygonis and Sini, 2012)). The geometric mean was used to summarise the average particle size in each image. Points that covered macroalgae were recorded as such and if a particle was covered by > 1 point only one measurement of particle size was taken. Any points where a substrate particle was not distinguishable (e.g., covered by algae, too small, out of focus) were recorded as no data. At the Afon y Bala the substrate particle size appeared much finer at the end of swim-up surveys in 2021 than had been observed by snorkelling in previous spawning seasons. To assess this, particle size was analysed for 10 images that had been taken at approximately the same location as the traps during camera survey of egg density in December 2019. Due to the cessation of university research activity because of the Covid-19 outbreak, measurements of physical characteristics at trap locations that were planned for the end of fry sampling were delayed until August 2020. In 2021, depth and velocity were measured on the 2nd of April and substrate images taken when sampling ended on the 6th. When the depth of traps was measured the water level of the lake was read from the permanent datum board to standardise measurements for Penllyn in 2020 and 2021. Because of the low numbers of fry captured in 2021 and fewer traps being deployed at each site, relationships between CPUE and habitat characteristics at trap locations were not analysed for this year. All analysis were undertaken using R (R Core Team, 2018).

Confirming species identity of fry

Because the Penllyn site was newly discovered and Atlantic salmon (*Salmo salar*) and trout (*Salmo trutta*) are known to spawn very nearby, one fry captured on each sampling occasion at Penllyn in 2020 was euthanized using clove oil and preserved in ethanol for species confirmation by DNA barcoding. The QIAGEN DNeasy blood tissue kit was used for DNA extractions and PCR conditions and primers (COI-3 primer set) selected from Ivanova *et al.*, (2007). A BLAST search was undertaken to match the sequencing data to reference libraries for salmonid species. A further 2 fry on each day were euthanised for population genetic analysis (Chapter 4). Fry not returned to the laboratory were released at suitable habitat ~100 m from the sampling sites.

RESULTS

Snorkel surveys at the Afon y Bala and Afon Hwch

Swim-over surveys followed by camera surveys in December 2019 identified that eggs were concentrated at the confluence of the Afon y Bala and Afon Hwch (egg density per 0.16 m² camera image: $\mu = 0.86$, SD = 2.52, max = 18; Figure 4). A polygon drawn around the perimeter of camera images where eggs were present measured the spawning area to be 433 m². Swim-over surveys also found eggs deposited on ledges of slate close to the inner bank a short distance up stream of the camera survey area. In a repeat survey of transects 9-19 (see Figure 4) on January 23rd 2020, no eggs were present in images and only one egg (dead) was seen by the snorkeler when making dives to the substrate. The swim-over surveys in December 2020 found eggs in the same location as in 2019 but camera surveys were not possible due to high flow on the sampling day. Unlike surveys in 2019, a few scattered eggs were found in the Afon Hwch a short distance downstream of the road bridge (Figure 10).



Figure 4. The distribution and density of Arctic charr eggs at the Afon y Bala surveyed by camera on December 19^{th} 2019 (a) and the approximated region where Arctic charr eggs were found at Penllyn during a swim-over survey on February 6th 2020 (b). Density is the number of eggs per 0.16 m² camera image.

Snorkel surveys of Putative sites

A small number of eggs were found during a brief swim-over at Penllyn on the January 23rd 2020. A more extensive follow-up survey on the 6th of February revealed an abundance of eggs

that were deposited in the interstices of the cobble substrate (Figure 5). Eggs were found across an area of ~ 267 m² (Figure 5). Swim-over and camera surveys were attempted in the following spawning season but fast water flow on the day made swimming extremely challenging. However, eggs were observed in similar areas as the previous year. No eggs were found at the other putative sites.



Figure 5. Images of Arctic charr eggs taken by the snorkeler at Penllyn on February 6th 2020 (a) and during a pilot camera survey at the Afon y Bala on December 17th 2019 (b).





Figure 6. Microscope image of an ethanol preserved fry captured at Penllyn in March 2020 confirmed as Arctic charr by DNA barcoding.

A total of 260 fry were captured at the Penllyn site in 2020. All fry were alive and lively when emptied from the collection vessels. DNA barcoding confirmed the sampled fry (N = 11) as Arctic charr. Temporal trends in fry CPUE are shown in Figure 7. The first fry was caught on the second sampling day (5th of March). However, it should be noted that on the first sampling day (2nd of March) only 8 traps had been deployed and these traps generally caught the fewest

fry over the survey duration (19% of the total CPUE). The geometric mean of CPUE rose steeply from the 7th of March to its high point on the 12^{th} (= 0.63). Beyond the 12^{th} , the geometric mean remained stable up to the end of the sampling period (range 0.43 - 0.55) but the confidence intervals widened owing to a greater number of fry being captured across fewer traps. To illustrate, between the 9th to 19th of March the total catch was 122 fry in 14 traps, compared with 138 fry distributed across just 9 traps between the 19th and 27th. The geometric mean of CPUE over the entire period of fry emergence was 0.41 (95% CI = 0.16 - 0.71).



Figure 7. Temporal trends in the geometric mean of CPUE (solid line and diamonds) across 15 emergent fry traps deployed at the Penllyn spawning site in March 2020. CPUE is the number of fry in a trap per night fished. Blue shaded areas show geometric 95% confidence intervals.

Habitat characteristics

CPUE varied significantly between traps (Kruskal Wallace test, p < 0.0001). In general, the traps closest to the lake outflow produced the most fry, and in particular, traps 11, 12, and 15 (see Figure 3), which respectively accounted for 14%, 28% and 21% of total CPUE. Analysis

of relationships between number of fry captured and the habitat characteristics at each trap location identified a significant negative correlation with substrate particle size, depth, and % occurrence of macroalgae (depth: rs = -0.94, p < 0.001; substrate particle size: rs = -0.83, p < 0.001; macroalgae: rs = -0.65, p < 0.01). A scatter plot of fry catch against flow low velocity did not show a monotonic relationship. Summary statistics for habitat characteristics at trap locations are provided in Table 1. Substrate particle size was significantly smaller at the Afon y Bala compared with Penllyn and when comparing measurements at the Afon y Bala in 2021 and 2019 (Figure 8 and Table 3). Macroalgae cover was greatest at the Penllyn site (Table 3). Figure 9 shows that water temperatures at the Afon y Bala during the egg incubation, alevin, and swim-up period were on average cooler but more variable compared to the lake surface. Lake surface temperatures in 2019 -2020 were mostly cooler than in 2020 – 2021 with the notable exception of December (during and shortly after spawning) when temperatures were ~ 1.5 °C warmer.

Table 3. Habitat characteristics measured at trap locations. Values are the median (or geometric mean ψ) with 95% CI and the range in parenthesis.ParameterPenllyn 2020 (N = 15)Penllyn 2021 (N = 7)Afon y Bala 2021 (N = 7)

Parameter	Penllyn 2020 (N = 15)	Penllyn 2021 (N = 7)	Afon y Bala 2021 (N = 7)
Substrate size (mm) ψ	53.8 , 32.9 – 94.3 (31.7 – 104.9)	60.3, 31.5 – 71.7 (27.9 – 72.9)	9.8, 5.7 – 15.7 (5.4 – 16.1) † 29.1, 23.4 – 35 (22.4 – 35)
Depth (cm)	69, 55.3 – 81.6 (55 – 82)	69, 57.3 – 79.7 (57 – 80)	92, 74.8 – 99.8 (73 – 100)
Velocity (m/s)	*0.096, 0.04 – 0.41 (0.04 – 0.41)	0.12, 0.05 - 0.16 (0.05 - 0.17)	0.06, 0.02 - 0.37 (0.02 - 0.42)
Occurrence of macro algae (%)	0, 0 - 48.8 (0 - 60)	0, 0 – 19.4 (0 – 20)	(0-0)

[†] Substrate particle size measured in December 2019. * N = 14.

Emergent fry traps 2021

CPUE at Penllyn for March 2021 (geometric mean = 0.06, 95% CI = 0 - 0.13) was significantly lower than in 2020 (Wilcoxon test, p < 0.05). Moreover, of the 16 fry captured, 10 were found dead in the collection vessels and one live individual showed symptoms of swim-up syndrome (poor buoyancy control and swimming capability (Fitzsimons, 1995; Wolgamood *et al.*, 2005)). The highest CPUE value occurred on three sampling days between the 9th and 18th of March (geometric mean = 0.12), with the first and last fry being captured on March 4th and 29th respectively. Only four of the seven traps captured fry, these being located nearest to the lake outflow. The CPUE at the Afon y Bala, where only 1 fry was captured (on 31st March), was not significantly lower than at Penllyn (Afon y Bala: geometric mean = 0, 95% CI = 0 - 0.01; Penllyn excluding dates when Afon y Bala traps were not set: geometric mean = 0.05, 95% CI = 0 - 0.11; Wilcoxon test, p = 0.073). Sampling at both sites was ended on the 6th of April.



Figure 8. Images of the substrate at (a) Penllyn beneath the only trap not to capture fry in 2020; (b) beneath the trap capturing the most fry at Penllyn in 2020; (c) beneath a trap in the Afon y Bala 2021; and (d) at 2021 trap locations in the Afon y Bala taken during egg snorkel surveys in December 2019. Sediment particle size was significantly smaller at the Afon y Bala in 2021 (c) compared with 2019 (d). Each image is the same scale.

DISCUSSION

Reproductive success is critical to understanding the population dynamics of salmonids (Smialek, Pander and Geist, 2021), yet studies of Arctic charr spawning habitat and the survival of eggs and alevins have been rare compared to many other salmonid species. The results presented here add important new information to the sparse literature, including the timing and duration of fry emergence, the characteristics of spawning habitats, and data confirming substantial variation in fry production between years. The suitability of swim-up traps for sampling Arctic charr emergent fry was demonstrated for the first time and could be

a useful technique for population assessment and monitoring, as well as a variety of research topics (e.g., phenology).

The study provides the first confirmation that Arctic charr at Llyn Padarn spawn at more than one location. The newly discovered site at Penllyn had been previously surveyed by Butterworth (1980) and Thomas and Holt (2011). The survey in 2020 suggested the density of emergent fry was high, but without comparable data from other Arctic charr lakes it is difficult to assess the productivity of the site. Comparisons with studies of closely related lake trout show that capture rates at Penllyn in 2020 were at the upper end of the reported range but toward the lower end in 2021 (Table 4). However, differences in sampling strategy, trap design, the nature of habitats sampled, and species vulnerability to traps, may limit comparability between studies.

Table 4. Catches of fry in emergent fry traps reported in studies of lake trout compared with results in the present study of Arctic charr at Llyn Padarn.

Study	CPUE	Reference
Penllyn, March 2020	2.16	This study
Penllyn, March 2021	0.23	This study
Afon y Bala, March 2021	0.01	This study
Grande Isle, Lake Champlain, NY	2.65	Marsden, Chotkowski and Ellrott (2002)
Whallon Bay, Lake Champlain, NY	0.02	Marsden, Chotkowski and Ellrott (2002)
Bissel point, Ostego Lake, NY, 2016	1.17	Winter, Winter and Foster (no date)
Bissel point, Ostego Lake, NY, 2013	0.07	Sawick and Foster (2014)
Bissel point, Ostego Lake, NY, 2003	1.4	Tibbits (2007)
Bissel point, Ostego Lake, NY, 2014	1.65	Lucykanish and Foster (2015)
Bissel point, Ostego Lake, NY, 2015	1.28	Casscles et al. (2016)

CPUE here is the number of fry per trap-day per m^2 .

Assuming that traps capture all the fry emerging from the area of substrate directly underneath then total productivity of a spawning site can be calculated by multiplying the site area by the number of fry captured in the area covered by the traps. Accordingly, using the geometric mean for CPUE in 2020 (0.41, 95% CI =0.16 – 0.71) and the estimated size of the spawning area (267 m²), total fry production at Penllyn for the 25 days between the 2nd and 27th of March 2020 was 8552 (95% CI = 3337 – 14810). The estimate derived from average CPUE in March 2021 (geometric mean = 0.06, 95% CI = 0 - 0.13) is 1401 fry (95% CI = 0 - 3037). However,

almost certainly the catch efficiency of traps was < 100%, as shown in a laboratory study by Collins (1975), who reported values ranging from 28.7% to 69.9% capture efficiency. Factors shown to affect catch efficiency included the species of salmonid, the slope angle of the mesh cone, and how tightly the trap fitted the substrate. The trap tested in the laboratory by Collins (1975) was rigid and therefore may not have fitted the substrate as closely as the flexible trap used here.

From the information available it cannot be determined why lower numbers of fry were captured at Penllyn in 2021 compared with 2020. Likely explanations are (1) differences in survivorship at the egg and alevin stage; and (2) variation in the number of spawning adults, and thus number of eggs deposited. Regarding explanation one, warmer temperatures at the incubation stage are a primary cause of elevated egg mortality (Mari et al., 2016; Kelly et al., 2020), but temperatures prior to 2021 swim-up were mostly lower than in 2020 (Figure 9). Warmer temperatures in summer have been strongly correlated with lower egg viability in aquaculture (Jobling, Tveiten and Hatlen, 1998; Jeuthe, Brännäs and Nilsson, 2015). Poor health of fry in 2021 was indicated by the high proportion that were found dead in the traps and by one fry exhibiting symptoms of swim-up syndrome, a condition which in other Salvelinus spp. has been linked with thiamine deficiency in the parents (Fitzsimons, 1995; Fisher et al., 1996; Wolgamood et al., 2005). Whilst the average and maximum time between checking traps was greater in 2021 than 2020, thus potentially increasing the risk of fry mortality, dead fry were found even when traps were checked after just 2 days. Studies of lake trout referenced in Table 4 often checked traps on only a weekly basis and did not report any mortality. Regarding explanation two, attempts to quantify egg density using snorkelling methods described by Low et al. (2011) were unsuccessful due to high water flow velocity at the site. The adult population size in 2019 and 2020 is unknown because annual NRW hydroacoustic surveys were not undertaken in these years (equipment failure in 2019 and Covid-19 restrictions in 2020). If the Penllyn site is indeed a new breeding site for Arctic charr, rather than just new to science, then the number of spawners might change substantially between years until a stable population with a natal instinct to return becomes established.



Figure 9. Midday temperature of water measured at the Afon y Bala spawning site and at the lake surface (proxy for Penllyn spawning site) between the spawning and swim-up in years when emergent traps were deployed.

The location of highest egg density at the Afon y Bala in December 2019 (Figure 4) matches well with spawning areas mapped by Milner (1984). The absence of eggs in January 2020 and low numbers of fry captured in emergent traps in 2021 are indicators that high rates of egg loss occurred in both years. Given the known hydrodynamic issues at the site and occurrence of multiple flood events in both winters, it is likely that eggs were either washed out or buried beneath newly deposited substrate (and therefore not visible in camera images). The hypothesis of burial is supported by the finding that substrate particle size became significantly finer over the duration of the study. Burial can increase mortality by reducing oxygen concentrations in interstitial waters; limiting the removal of toxic metabolic waste products from the substrate; or by acting as a physical barrier for fry attempting to emerge (Crisp, 1988; Mari *et al.*, 2016). Because the emergent fry traps were not deployed in the power station discharge zone, associated high flow velocity and thermal pollution should not have affected survivorship. However, spawning areas found downstream of the headland separating the Afon Hwch from the channel below the bascule gates are exposed to discharge water (Figure 4).

The inability to detect eggs deposited deeper in the substrate is a limitation of using images to measure egg density. However, the method shows promise for accurately identifying spawning areas at fine spatial scales and does provide a semi-quantitative estimate of density. For these purposes it is recommended camera surveys are undertaken immediately after spawning when eggs are most likely to be on the substrate surface. Surveys were quick and relatively straightforward to complete, taking 1.5 hrs to survey 19 transects of 16 m in length. Here, the method was applied at shallow locations by a snorkeler but could be adapted to deeper sites and using a boat. It is not possible from images to identify the species of egg, and therefore camera surveys may only be reliable at locations where other salmonids do not spawn. Brown trout, which is the most common salmonid to coexist with Arctic charr, tend to bury their eggs deeper and utilise different spawning habitat (typically small, shallow streams with a finer substrate) (Frost, 1965; Klemetsen et al., 2003; Low et al., 2011). Trout and Atlantic salmon spawn in the headwater tributaries of the Afon y Bala (pers. comms. Walter Hanks, NRW), meaning it is possible washed out eggs of these species could be deposited on the spawning area of Arctic charr. It would be expected the density of such eggs would be low. It also likely some Arctic charr eggs in images will have settled after drifting, rather than being deposited at the location by a spawning female. Clustered eggs, as shown in Figure 5, are a good indicator of intentional laying.

The discovery in December 2020 of eggs below the road bridge crossing the Afon Hwch suggests that spawning might have occurred here (Figure 10). Because eggs were few and scattered, it did not appear that they were intentionally laid at this location; more likely they were washed out from further upstream below or beyond the road bridge. Surveying in these areas was not possible due to higher river velocity and limited access for the bank support team but a brief swim directly under the bridge in 2019 did reveal suitable spawning substrate. Approximately 50 m upstream of the bridge is the Afon Peris diversion tunnel and a weir on the Afon Hwch considered impassable for Arctic charr. It has been suggested that spawning may occur in the gravels that accumulate in the tunnel (pers. comms. Walter Hanks, NRW).



Figure 10. Location of eggs found a short distance downstream of the Afon Peris diversion tunnel.

Regarding the putative sites where eggs were not found, surveys at the mouth of the Afon y Bala were challenged by the depth of the site and the fast flows experienced on the survey date. As such, there is a reasonable possibility that eggs could have been missed. However, the nature of the substrate, which consisted mostly of unstable gravel washed out from the river and a smothering of fine sediment seemingly emanating from the sewage discharge upstream, suggests the site has low suitability for spawning. The Afon Fachwen mouth is a small site that shelves steeply into deep water. Because of its limited size there is high confidence that eggs would have been found here if within the depth range of snorkelling (~ 2 m). Small patches of suitable substrate were present but may be exposed when lake levels are low. The Afon Coed appears to offer a relatively large area of spawning substrate that is similar to that found at Penllyn. The site is shallow ($\lesssim 1$ m) and was thoroughly searched, meaning there is high confidence that eggs were not present here. Other potential sites not visited include further downstream of the Afon y Bala between the rail bridge and sewage outflow, the lake bank between the Afon y Bala and Afon Goch, and the lower reaches of other inflowing streams. Additional suitable habitat appeared to be present to the north of the area searched at Penllyn (Figure 4) but the water here was too shallow for the emergent fry traps or for snorkelling without risking significant disturbance of any eggs.

Arctic charr at Llyn Padarn typically spawn over several weeks around the 2nd and 3rd week of December (Chapter 4) meaning peak emergence in 2020 (14th March) occurred 89 days after

spawning (assuming peak spawning on the 15th of December). This was earlier than expected given that the time of 50% hatching for autumn spawning populations at climatically similar Lake Windermere has been measured as 78 days (\pm 5.11) to 86 days (\pm 10.27) (Baroudy and Elliott, 1994), leaving just 3 to 11 days for alevins to reach the swim-up stage. However, in an experimental study of egg survival in the winter of the 2019 – 2020, 50% hatching at the Afon y Bala occurred considerably earlier at ~69 days after fertilisation (Appendix 2). The most likely explanation for early hatching is the temperature of the water, with the winter prior to March 2020 being mild (Appendix 2). However, peak emergence time of fry was similar in 2021 despite winter temperatures being cooler. Because 2020 surveys were curtailed by the Covid-19 pandemic the duration of the swim-up period is unknown, but high numbers of fry captured on the last sampling days suggests emergence was not yet ending. In 2021 no fry were caught for 1 week beyond the 31st of March indicating this date marked the end of the emergence period. Any future monitoring should be undertaken over the month of March.

The size of substrate particles at the Penllyn spawning site (Table 3) was very similar to measurements by Low *et al.*, (2011) at littoral sites in Ireland (geometric mean 5.9 cm, range 3.0 - 7.9 cm). Correlation analysis showed that substrates at the smaller end of the observed range produced higher numbers of fry. Higher fry production could indicate that breeding Arctic charr select this type of substrate or the survival of eggs and alevins was higher. Traps might be are more efficient on smaller substrates because they fit more tightly (Collins, 1975), although this is unlikely to account for the very strong relationship that was detected. Also in agreement with Low *et al.*, (2011), no evidence of redd digging was observed. Eggs were found among large interstitial spaces or on the substrate surface and rarely in large aggregations shown in Figure 5.

Chapter 4. The population dynamics of Llyn Padarn Arctic charr and the efficacy of hatchery stocking using native wild brood stock

INTRODUCTION

Supplementation of wild populations with individuals reared in captivity is a common but controversial management policy (Araki and Schmid, 2010; Milot *et al.*, 2013). The concerns regarding supplementation include the risks of inbreeding depression and reduced genetic diversity (Brown, Woolliams and McAndrew, 2005); loss of locally-adapted phenotypes (Besnier *et al.*, 2015; Savary *et al.*, 2017); suppression of wild recruitment due to competition with captive-reared individuals (Kostow, 2009); low survival rates and reproductive success of captive-reared individuals in the wild (Araki, Cooper and Blouin, 2007; Pollock *et al.*, 2007; White *et al.*, 2018; Lehnert *et al.*, 2020); and lastly, the substantial financial costs of captive breeding programmes (Salminen, Alapassi and Ikonen, 2007). However, it is recognised that supplementation may be necessary if extinction is imminent and the cause of population decline is unknown or cannot be fixed in the short term (Kissel *et al.*, 2014). In such cases, identifying and implementing best practice will be essential to achieve satisfactory outcomes (Cowx, 1994; Savary *et al.*, 2017).

Supplementation of salmonid populations is practiced frequently, either to enhance the productivity of fisheries or to rescue imperilled populations (Kostow, 2009; Araki and Schmid, 2010). The process involves stripping eggs from females that are fertilised with the semen collected from males. The fertilised eggs are either reared in an aquaculture facility (hereafter, hatchery) or planted in artificial nest boxes at spawning grounds (Cowx, 1994). Eggs reared in the hatchery can be stocked shortly after hatching or at a later age, typically at approximately 1 year old to bypasses the period of the life-cycle when survival can be strongly density-dependent (Cowx, 1994). Survival rates of eggs and fry in hatcheries are typically far higher than in the wild (Araki *et al.*, 2008), meaning even a small scale stocking programme may dramatically increase the number of juvenile fish in the target population. However, the increase in juveniles will only have a positive impact on long-term population viability, which is the objective of conservation, if the stocked fish survive to reproduce and do not negatively impact the fitness of wild fish and future generations (Fleming and Petersson, 2001; Araki *et al.*, 2008).

A critical aspect of stocking programmes is the origin of the brood stock (Araki et al., 2008; Milot et al., 2013). Often times, stocked fish have been propagated using captive brood stock founded from native fish or fish from a foreign population (Cooper, Miller and Kapuscinski, 2010; Savary et al., 2017). Where captive brood stocks have been utilised, programmes often fail to meet their management objectives. In a comprehensive review by Araki and Schmid (2010), 23 out of 70 case studies where supplementation used captive brood stock found negative effects of hatchery rearing on the fitness of stocked fish, and 28 studies showed reduced genetic variation in hatchery populations. None of these studies suggested a positive genetic effect on the fitness of hatchery-reared individuals after release and there was limited evidence supporting long-term improvements in wild population size. Loss of genetic diversity in hatchery brood stocks develops over generations due to the increased occurrence of inbreeding (Brown, Woolliams and McAndrew, 2005). Populations with reduced genetic diversity are known to be more vulnerable to stochastic changes and less able to adapt and survive (Hughes et al., 2008). The alternative to a captive brood stock is to collect eggs from wild native adults, hereafter, supportive breeding (Gow et al., 2011; Savary et al., 2017). The approach should maintain locally adapted phenotypes and, by utilising a different brood stock in each year, reduce the risk of inbreeding and declines in genetic diversity (Selly, Hickey and Stevens, 2014). Case studies of supportive breeding are fewer than for captive brood stock, but have generally concluded a positive effect, or no negative effect, on the genetic diversity of the stocked and wild population (Gow et al., 2011; Savary et al., 2017). However, some of the problems associated with a captive brood stock may still arise when using wild brood stock. First, stocked fish may have lower genetic diversity than the wild population if the number of parents is small or there is a parental bias, meaning adherence to correct breeding protocols is essential (Machado-Schiaffino, Dopico and Garcia-Vazquez, 2007; Selly, Hickey and Stevens, 2014). Second, hatchery and wild environments differ in many aspects including fish density, spatial and temporal heterogeneity of physical environment, intra- and interspecific interactions, and food availability, and consequently, conditions in the hatchery do not necessarily select the fittest genotypes for the wild (Milot et al., 2013). Indeed, low survival and reproductive rates of stocked fish may explain the limited impact of supportive breeding on the genetics of the wild population reported in some studies (Gow et al., 2011). Hatchery conditions can also induce plastic phenotypic responses that may result in environmental carryover effects on life-history or behavioural traits which are not beneficial once released in the wild (Milot et al., 2013). Alternatively, stocking of juveniles may supress wild recruitment because of advantages gained from hatchery conditions. In particular, greater food availability

in the hatchery means stocked juveniles are often considerably larger than their wild counterparts, and this may offer a considerable competitive advantage, even if underlying adaptive fitness to the environment is lower (Kostow, 2009). Finally, non-random sampling or artificial breeding can cause unintentional selection on particular traits. The disruption of natural mate choice and hence of sexual selection may have important genetic implications for the next generation (Fleming and Petersson, 2001; Anderson *et al.*, 2013; Perry *et al.*, 2019). In summary, heritable and phenotypic traits selected by artificial breeding or hatchery conditions that are disadvantageous in nature may result in low fitness of stocked fish and have long-lasting evolutionary impacts on the fitness of wild-stocked populations through hybridisation (Milot *et al.*, 2013).

Arctic charr (Salvelinus alpinus) at Llyn Padarn, which are one of only three natural populations in Wales, declined dramatically over the latter part of the 20th and early 21st century (Thomas, Griffiths and Hall, 2010; Beck, 2014). Likely causes of the decline were eutrophication causing deoxygenation and disturbance to spawning habitat after the construction of a hydroelectric power station (Thomas, Griffiths and Hall, 2010). Given the difficulty of immediately fixing the problems of deoxygenation and spawning ground disturbance, it was decided in 2009 that a breeding programme should commence to (a) establish a back-up population at another unpopulated lake (Llyn Crafnant) and (b) support the Llyn Padarn population until the lake habitat could be returned to a more favourable status. Eggs and semen have been collected from fish captured at the Afon y Bala spawning grounds and wet fertilisation undertaken in the field. The standard approach has been to mix the eggs of multiple females in a bowl and then add seamen from approximately the same number of males. Between 2013 and 2015 the number of fish captured and used for crossing was very low, and by 2019 it was necessary to propagate wild caught hatchery origin fish because few wild origin fish were captured (Table 1). These two factors - small brood stock and the use of hatchery fish for propagation, increase the risk of inbreeding and declines in genetic diversity (Machado-Schiaffino, Dopico and Garcia-Vazquez, 2007; Selly, Hickey and Stevens, 2014). The fertilised eggs are reared in a hatchery and mostly stocked as parr (~ 8-13 cm in length) the next autumn (pers. comms. Walter Hanks, NRW, 2019). As of 2021, when a long planned spawning habitat restoration project was completed, NRW have decided to pause the programme (pers. comms. Huw Jones, NRW, 2021).

At the beginning of the supportive breeding programme the DNA of one cohort of hatchery fry was analysed and showed that crossing had maintained favourable genetics (Beck, 2014).

Nearly a decade on, this study aimed to evaluate if the programme has improved the conservation status of Llyn Padarn Arctic charr. Several metrics of population health were investigated. First, longitudinal monitoring data of abundance were analysed to identify if census size has increased since stocking commenced and if population dynamics have been driven by stocking or natural recruitment. Second, the body condition and lengths of wild origin and hatchery origin adults were compared to assess general fitness to the environment. Finally, microsatellite markers were used to assess the effective population size and genetic diversity of various wild and hatchery origin cohorts at time points before the stocking programme commenced and approximately two generations after. An additional objective was to examine if gene frequencies differed between the two spawning sites at Llyn Padarn, as has been observed at other lakes with salmonid populations (Child, 1984; Giles *et al.*, 2004; Ferguson and Taggart, 2008). The study provides valuable information for managers contemplating supportive breeding programmes for threatened populations of freshwater fish.

Year	Females striped	Males striped	Number of eggs	Number of fish
			collected	stocked
2009	23	23	11340	
2010	11	10	11300	600†
2011	7	3	3000	1000, 3200†*
2012	23	14	14800	1150*, 1150†*
2013	4	5	1370	5700
2014	4	1	3000	1800
2015	9	9	7500	2200
2016	25	25	22200	4600
2017	NR	NR	7000	14000
2018	NR	NR	NR	2839
2019	17ψ	13ψ	NR	9254

Table 1. NRW records of stocking activities at Llyn Padarn.

 Ψ Hatchery origin fish used for crossing. *Some fish stocked earlier in the year as fry, rather than parr in autumn. † Fish stocked at Llyn Crafnant. NR = not recorded.

METHODS

Trends in abundance and recruitment of stocked fish

NRW have employed hydro-acoustic surveys and fyke netting to monitor the abundance of Arctic charr at Llyn Padarn. Fyke netting surveys of breeding age fish have been undertaken at the Afon y Bala spawning site since 1997 and are summarised in Table 2. Early surveys were irregular with several missing years in the data series between 1997 and 2009. Beyond 2009, surveys included stripping eggs for the hatchery and occurred in each year, although population data were not recorded in 2017. The general protocol has been to set double-ended fyke nets in a zig zag pattern spanning the width of the river channel which are then left to fish overnight. However, the number of nets deployed and days fished has not been consistent. In this analysis, each year's catch was standardised by calculating the number of fish captured per night divided by the number of nets that were set (hereafter referred to as the catch per unit effort, or CPUE). In 2012 nets were lifted after two nights, and therefore the CPUE was divided by two. Because surveys in each year began on different dates and potentially before spawning had commenced, data were excluded for days before the first Arctic charr was captured in the sampling year. Netting data collected in January 2011 were also excluded, and no fish were captured. In all other years fish were captured on the last survey day, indicating that surveys had finished before the end of spawning. Since 2011 when stocking commenced the number of hatchery origin fish (adipose fin removed when stocked) captured in fyke nets has been recorded. CPUE data and the percentage of stocked origin fish are presented as the median with 95% confidence intervals. These summary statistics could not be calculated for 2014 when data were not reported on a per day basis. In this case, the total fish count for the year was divided by the total number of night's fished and total number of nets set.

Year	Nights fished	Date range	Number of nets	Length	Weight
1997	1	15 th DEC	8	\checkmark	\checkmark
1999	2	10^{th} and 15^{th} DEC	8 and 15, respectively	\checkmark	×
2003	4	$8^{th}-12^{th}\;DEC$	6	\checkmark	×
2009	4	$12^{th}-18^{th} \; DEC$	12	\checkmark	\checkmark
2010	10	$3^{rd}-16^{th}\ DEC$	16	\checkmark	\checkmark
2011	6	$8^{th}-22^{nd}\ DEC$	16	\checkmark	\checkmark
2012	7*	$5^{th}-19^{th} \; DEC$	14	\checkmark	\checkmark
2013	2	5 th and 10 th DEC	14	\checkmark	\checkmark
2014	3	NR	14	×	×
2015	2	15^{th} and 16^{th} DEC	14	\checkmark	\checkmark
2016	4	$6^{th}-15^{th}\;DEC$	14	\checkmark	\checkmark
2018 ψ	1	3 rd DEC	12	\checkmark	\checkmark
2019 †	3	$4^{th}-10^{th} \; DEC$	2	\checkmark	\checkmark

Table 2. Summary of fyke net surveys undertaken by NRW at the Afon y Bala.

 $NR = not recorded. * Nets fished for 48 hrs. † Proportion of stocked fish, weights, and lengths not recorded on 10th December due to extreme weather. <math>\Psi$ Nets were set on a second night but the catch was not enumerated.

Night-time vertical hydro-acoustic surveys have been undertaken each August from 2008 to 2018 (HTI Model 241, split-beam, 200 kHz). The survey boat follows a zig-zag pattern of 10 transects crossing the lakes' pelagic zone, with each transect being surveyed twice (once on the way up the lake and once on the way down). Post processing of the echoes was performed by NRW. These raw echo data were analysed by the author (A. Smith) using NRW's Mobile Survey Analysis Tool (V5.1) to calculate per transect fish densities for three standard length classes: small juveniles (4 - 10 cm), large juveniles (10 - 25 cm), and adults (> 25 cm) (Bean, 2003). Echo targets above 38 cm were excluded since this is the largest recorded length of Llyn Padarn Arctic charr captured in fyke nets. Target strengths of echoes (TS) were converted to lengths using the formula $TS = (20 \log_{10} L) - 67.4$, where L is the fish length in cm (Foote, 1987; Bean, 2003). In addition, density was calculated for large juveniles and adult classes combined in order that density could be assessed against recommended thresholds for good conservation status. According to Bean (2003), the density of fish > 10 cm in length should be ~520 fish ha⁻¹ for mesotrophic lakes and ~37 fish ha⁻¹ and for oligotrophic lakes. As a further means of assessing population status, fish densities were compared with data collected in 2016 and 2017 at Llyn Cwellyn where the Arctic charr population is thought to be healthy and not subject to significant anthropogenic pressures. Protocols for Llyn Cwellyn were identical to Llyn Padarn except only 8 transect were surveyed. Phosphate levels at Llyn Cwellyn and Llyn Padarn (both 7 μ g/l, Chapter 2) are marginally above the middle value of oligotrophic classification (< 12 μ g/l) according Carlson (2007). As recommended by Baroudy & Elliott (1993), fish density was summarised using the geometric mean and 95 % confidence intervals. In early surveys at Llyn Padarn several transects recorded zero fish for some length classes. To deal with zero values when calculating the geometric mean a value of one was added to all data points and then subtracted from the derived mean (de la Cruz and Kreft, 2019). Because the hydro-acoustic surveys focused on the pelagic zone that is infrequently inhabited by other species, it was assumed that all echoes are Arctic charr (Elliott *et al.*, 1995). This assumption is examined in the discussion.

Pettit's non-parametric test for change-point detection was used to assess if and when trends in the density of fish > 10 cm in length changed after stocking commenced (Pohlert, 2020). Pettit's test requires a complete time series, and therefore could only be applied to fyke data between 2009 and 2016. To evaluate if stocking had driven population dynamics, Spearman's rank correlation was performed between the density of large juvenile fish (10 – 25 cm) and the number of parr stocked the previous year. Parr are stocked in autumn at approximately 8 - 13 cm in length, and therefore are highly unlikely to have attained a length > 25 cm by the following summer (Butterworth, 1980).

Body condition

Relative body condition (Kn = predicted weight/observed weight) was calculated using linear regression of natural log transformed fork lengths and weights of fish captured in fyke nets (Figure 1). The regression approach to calculating body condition is preferable to the more common Fulton's condition factor (K = 100 x weight/length³) since it accounts for the non-allometric growth of fish which means that K cannot be compared between fish of different lengths (Cren, 1951; Jones, Petrell and Pauly, 1999). Regressions were implemented separately for gravid males and gravid females sampled in years when both stocked and wild origin fish were captured in fyke nets. The derived condition factors for sexes were then combined for the comparative analysis between stocked and wild fish. Body condition was not calculated for spent fish because of small sample sizes. Sexes were combined for stocked-wild comparisons of fork length because no significant difference was found between males and females (t-test, t = -1.051, df = 732, p = 0.293).



Figure 1. Length-weight relationships for gravid females (left) and gravid males (right). Ln weight of gravid females = -9.278 + 2.613 ln fork length, $r^2 = 0.73$, p < 0.001; ln weight of gravid males = -8.814 + 2.53 ln fork length, $r^2 = 0.77$, p < 0.001.

Population genetics

Details of tissue samples used for DNA extraction are given in Table 3. Cohorts included fish sampled at various time points before and after the stocking programme, and fish of hatchery, stocked, and wild origin. Here, "hatchery" refers to fry returned to the hatchery and sampled before being stocking, "stocked" refers to adult fish of hatchery origin captured at the lake, and "wild" refers to fish that had hatched naturally at the lake. DNA extraction and PCR for samples collected in 2008, 2011, and 2013 were undertaken by previous Bangor University MRes student, S. Beck (Beck, 2014). S. Beck used the hexadecyltrimethylammonium bromide (CTAB) method for DNA extraction and reported that 2008 samples yielded low concentrations of variable quality DNA due to incorrect storage of the tissue samples (ethanol had evaporated). The DNA for samples from 2018 onward was extracted by the current researcher (A. Smith) using the manufactures protocol for the Qiagen DNeasy Blood & Tissue Kit. Spectrophotometer and fluorimeter analysis showed high DNA purity and concentration across all samples by A. Smith.

Year	Sampling location	Capture method	Researcher	Tissue type
2008*	Lake	Gill net	S. Beck	Adipose fin
2011	Hatchery		S. Beck	Adipose fin
2013	Afon y Bala spawning site	Fyke net	S. Beck	Adipose fin
2018	Afon y Bala spawning site	Fyke net	A. Smith	Caudal fin
2019	Afon y Bala spawning site	Fyke net	A. Smith	Caudal fin
2019	Hatchery		A. Smith	Fry tail
2020	Penllyn spawning site	Fry trap	A. Smith	Fry tail

Table 3. Details of tissue samples used for DNA extraction. All samples were stored in ethanol (70 - 95 %) after collection.

* 2 of 21 fish were captured in 2007.

Microsatellite multiplexes and PCR conditions were developed by S. Beck (see Beck (2014) for details of multiplex development). Reactions for multiplex 1 (4 loci) were performed in 5µl PCR reactions using Qiagen Type-it Microsatellite PCR Kit with primer concentrations stated in Appendix 3, 1µl of DNA, 50mg/ml of Bovine Serum Albumin (BSA), and PCR grade H₂O to make up the final volume of 5µl. PCR conditions were 10 minutes at 95 °C to activate the hot start polymerase, followed by 34 cycles of 30 s at 94 °C, 90 s at 57 °C, and 60 s at 72 °C, followed by 31 cycles of 30 s at 94 °C, 90 s at 50 °C, and 60 s at 72 °C, and finalised with a 30 min extension phase at 60 °C. Reactions for multiplex 2 (3 loci) were identical except for the addition of 1 µl of Q-solution due to the poor amplification of primers when combined within the multiplex. For PCRs undertaken by A. Smith, reaction size was increased to 18.25 µl total volume and BSA added at 20 mg/ml. The number of cycles after hot start was reduced from 34 to 28 due to better quality DNA which was standardised across samples to a concentration of $\sim 10 - 20$ ng/µl. Primers (sourced from Eurofins) were ordered HPSF and HPLC purified by S. Beck and A. Smith, respectively (HPSF service no longer offered in 2020). Most primer dyes used by S. Beck had been discontinued (NED, VIC and PET) so were replaced with the Eurofins recommended alternatives (NED = ATTO 550, VIC = YAKE, PET = ATTO 565). Capillary sequencing of PCR products was undertaken by S. Beck (2008 – 2013 samples) and by Eurofins (2018 – 2020 samples) using the Applied Biosystems ABI 3130XL genetic analyser with either LIZ-600 (S. Beck samples) or LIZ-500 (A. Smith samples) dye size standards. To check for possible cross contamination between samples, each PCR plate sent for sequencing by A. Smith included a minimum of one negative control.

Genotype scoring for all sequence data (2008 – 2020) was undertaken by a single researcher (A. Smith) using the R package 'Fragman' (Covarrubias-Pazaran *et al.*, 2016; R Core Team, 2018). The pipeline for scoring microsatellite alleles using the Fragman package utilises four functions: (1) the storing inds function to read the raw FSA file data with Fourier smoothing and correction for cross channel noise and saturated peaks, (2) the ladder.info.attach function which matches the size standard to peaks detected in the ladder channel, (3) the overview2 function for creating allele panels; and (4) the score easy function which automatically scores the alleles of individuals. These automated allele calls were verified by eye and adjusted where necessary. Unfortunately, for reasons described in Appendix 4, alleles of four markers could not be scored reliably and were consequently excluded from the analysis.

The three markers that could be scored (SsaD48, SalF56, and SalP61) were assessed for genotyping errors (scoring error due to 'stutter' bands, null alleles, and allelic dropout) using Microchecker (Van Oosterhout *et al.*, 2004). Microchecker calculates the expected and observed number of homozygotes (based on Hardy-Weinberg expectations) at each locus to determine whether there is significant homozygote excess, indicative of null alleles. Scoring error due to 'stutter' bands is detected when there is a deficit of heterozygotes with alleles differing in size by one nucleotide repeat. Finally, large allelic dropout is identified by an excess of homozygotes that are biased towards the extreme of the allele size distribution (Van Oosterhout *et al.*, 2004).

Measures of genetic diversity and inbreeding were calculated per locus and across loci (global) for the following cohorts: 2008 wild origin adults, 2011 hatchery fry, 2013 wild origin adults, 2018 wild origin adults, 2019 wild origin adults, 2019 stocked origin adults, 2019 hatchery fry, and 2020 wild origin fry captured at the newly discovered spawning site (Penllyn). Because the first hatchery fished were stocked in 2011 and Llyn Padarn Arctic charr mature at approximately 3 to 4 years old (Butterworth, 1980), it is possible that 2018 to 2020 samples included stocked-wild hybrids. Parameters investigated included allelic richness with rarefaction (A_R), observed heterozygosity (H_o), expected heterozygosity or gene diversity (H_e), and F_{is}, which measures the deficit of heterozygosity caused by inbreeding (F_{is} > 0 indicating inbreeding). The hypotheses that genetic diversity and inbreeding were significantly different between sample groups was tested using the Kruskal Wallace test and post-hoc Wilcoxon test with Bonferroni correction for pairwise comparisons. Genetic structure between sample groups was assessed using pairwise F_{st} values (0 indicating high levels of gene flow and 1 indicating no gene flow). The hypotheses that F_{is} and F_{st} were significantly different

from 0 was tested by calculating the bootstrapped 95% confidence intervals. The above genetic analysis were all performed using the package Heirfstat (Jerome *et al.*, 2020). Tests for per locus linkage disequilibrium and deviation from Hardy-Weinberg expectations were undertaken using the LD2 and hw.test from the 'pegas' package, respectively (Winter, 2021). P-values for linkage disequilibrium and Hardy-Weinberg expectations were adjusted for the number of loci using the Holm-Bonferroni method (Holm, 1979). Effective population size (N_e) of the parental population was estimated for each sampling year using the linkage disequilibrium method executed in NeEstimator V2 (Do *et al.*, 2014). Rare alleles with frequencies below 0.05 were removed to calculate N_e (Do *et al.*, 2014).

RESULTS

Fyke net and hydro-acoustic data

Analysis of the fyke net and hydro-acoustic survey data showed the abundance of adult Arctic charr at Llyn Padarn increased in the period after the stocking programme commenced (Figure 2 and Figure 3). Pettit's test applied to the hydro-acoustic data indicated a significant change to the trend in fish density occurred in 2013, two years after the first stocking event (U = 30, p < 0.05). The change point for the fyke netting data between 2009 and 2016 occurred in 2014 but was not significant (U = 12, p = 0.44). Fyke netting data showed a steep decline in population size between 1997 and 2011. By the end of the time series (2019), the 95 % confidence intervals for CPUE suggested abundance returned to levels similar to, or perhaps exceeding, abundance at previous high points in 1997 and 2003 (Figure 2). However, whilst population size at Llyn Padarn has increased, hydro-acoustic estimates showed the density of fish 10 to 38 cm in length was significantly lower than at Llyn Cwellyn in the two years when both lakes were surveyed (Figure 3). Lower bound estimates at Llyn Cwellyn for 10 to 38 cm fish density were above the 37 fish ha⁻¹ threshold for good conservation status. The median estimate for Llyn Padarn exceeded the 37 fish ha⁻¹ threshold by 2018 (64.7 fish ha⁻¹) but the lower bound estimate remained unfavourable (31.2 fish ha⁻¹). Trends in the density of fish <10 cm in length, which may be indicative of natural recruitment, showed a steep decline at Llyn Padarn between 2008 and 2013, which was followed by a gradual recovery in the subsequent years (Figure 3).

First recruitment of stocked fish to the breeding population occurred in 2012, just 1 year after the first stocking event, when two fin clipped females of 24 and 26 cm in length were captured in fyke nets. No stocked fish were captured in 2013. By 2014, stocked fish represented 50% of

the population, and by the end of the time series this number had increased to 71 % (Figure 1). Spearman's rank correlation found a strong and significant positive correlation between the number of part stocked in autumn and the density of juvenile fish (10 - 25 cm in length) in the following summer hydro-acoustic survey (N = 7, *rs* = 0.89, *p* < 0.05).

Fork lengths and relative condition factors for stocked and wild fish are shown in Figure 4. Ttests found the fork length and condition of stocked origin fish were not significantly different from wild origin fish (fork length, t = -0.106, df = 435, p = 0.91; body condition, t = -0.84, df = 159, p = 0.39).



Figure 2. Temporal trends in the number of breeding Arctic charr captured in fyke nets (left) and the percentage of fish that were of stocked origin (right). CPUE is the number of fish captured in one fishing night divided by the number of nets fished. The orange line of the left-hand plot shows CPUE for wild and stocked fish combined and the dashed blue line shows CPUE for wild fish after stocking commenced (autumn 2011, dashed vertical line). Points are the median and bars show 95% confidence intervals.



Figure 3. Temporal trends in fish density by length class at Llyn Padarn and Llyn Cwellyn assessed by hydro-acoustic surveys. The dashed vertical line marks the date of the first stocking event (autumn 2011). The solid lines show the geometric mean, and the shaded areas show geometric 95% confidence intervals.



Figure 4. Fork length (left) and relative condition factor (right) of gravid wild and stocked origin Arctic charr captured in fyke nets at the Afon y Bala spawning site between 2012 and 2019. Sample size for fork length was 262 wild 193 stocked and for condition factor 214 wild 83 stocked.

Population genetics

Microchecker did not indicate scoring errors due to null alleles, allelic dropout, or stutter bands for the three loci. All loci were polymorphic within each of the 9 samples analysed. The number of alleles per locus ranged from 7-9 (SalF56), 4-6 (Salp61), and 9-15 (SsaD48). Significant deviation from Hardy-Weinberg expectations was detected at the SSaD48 and SalP61 loci for the wild cohort sampled before stocking in 2008. For subsequent cohorts loci conformed to Hardy-Weinberg expectations except SalP61 for the 2018 and 2019 wild cohorts (SalP61) and for 2019 hatchery fry (SSaD48 and SalP61). Linkage disequilibrium analysis identified a significant non-random association between loci for wild cohorts in both years prior to stocking (2008 and 2013).

Statistics for genetic diversity are displayed in Table 4. The global F_{is} values of all cohorts other than hatchery fry from 2019 were negative (range = -0.22 to -0.04), suggesting a heterozygote excess, rather than a deficit, that would have been indicative of inbreeding (Table 4). Global F_{is} was significantly below 0 for four of the sample groups (wild 2008, stocked 2018, stocked 2019, and Penllyn 2020). Global F_{is} for hatchery fry in 2019 was 0.04 but did not differ significantly from 0. Kruskal Wallis tests showed there was no significant difference between

sampling groups in F_{is} or other metrics of genetic diversity (F_{is} , p = 0.56; A_R , p = 0.94; H_o , p = 0.69; H_e , p = 0.96).

Year	Origin	Sampling	Locus	N	AR	Ho	He	Fis
	- 0	location				Ŭ	ĩ	
2008	W	LK	SalF56	21	5.18	1.0	0.7	-0.42
			SalP61*	20	4.89	0.8	0.72	-0.1
			SsaD48*	20	8.62	1.0	0.85	-0.15
			Global	21	5.84	0.93	0.75	-0.23*
2011	Н	Hatchery	SalF56	16	5.54	0.93	0.73	-0.27
			SalP61	16	5.58	0.62	0.78	0.2
			SsaD48	17	10	0.88	0.85	-0.03
			Global	16	6.53	0.81	0.79	-0.02
2013	W	AYB	SalF56	16	5.88	0.87	0.69	-0.25
			SalP61	16	6.58	0.81	0.83	0.03
			SsaD48	16	8.91	0.75	0.78	0.04
			Global	16	6.91	0.81	0.77	-0.04
2018	W	AYB	SalF56	16	5.45	0.94	0.75	-0.25
			SalP61*	14	3.96	0.5	0.73	0.32
			SsaD48	17	10.58	0.88	0.77	-0.14
			Global	18	5.67	0.77	0.75	-0.02
2018	S	AYB	SalF56	12	5.84	0.85	0.72	-0.18
			SalP61	12	3	0.75	0.66	-0.12
			SsaD48	14	8.09	0.92	0.85	-0.19
			Global	14	4.77	0.84	0.74	-0.13*
2019	W	AYB	SalF56	11	5.97	0.86	0.84	0
			SalP61*	11	4	0.54	0.62	0.12
			SsaD48	13	9.76	0.92	0.77	-0.19
			Global	13	5.77	0.77	0.76	-0.03
2019	S	AYB	SalF56	27	5.87	0.82	0.77	-0.05
			SalP61	25	4.39	0.8	0.72	-0.1
			SsaD48	28	9.14	0.89	0.83	-0.07
			Global	28	5.91	0.83	0.77	-0.07*
2019	Н	Hatchery	SalF56	23	5.43	0.82	0.78	-0.04
			SalP61*	18	3.6	0.55	0.64	0.14
			SsaD48*	23	8.05	0.69	0.73	0.05
			Global	18	5.12	0.69	0.72	0.04
2020	W	PL	SalF56	35	5.53	0.94	0.77	-0.22
			SalP61	35	4.63	0.77	0.72	-0.06
			SsaD48	35	9.7	0.88	0.86	-0.02
			Global	35	6	0.86	0.78	-0.18*

Table 4. Summary statistics for allelic richness with rarefaction (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F_{is}). N = number of individuals successfully genotyped.

Locus * = significant deviation from Hardy-Weinberg equilibrium. F_{is} *

significantly different from 0. W = wild origin fish; H = hatchery origin wild caught adults. YPB = Afon y Bala spawning site; PL = Penllyn spawning site; LK = lake.

Confidence intervals for pairwise f_{st} values indicated large temporal variation in genetic structure between the two cohorts sampled before stocking (2008 and 2013) and between cohorts sampled before and after stocking (Figure 5). Comparisons of F_{st} between the new spawning site (Penllyn 2020 wild caught fry) and recent samples from the Afon y Bala spawning site (2018 and 2019 cohorts) did not show a significant difference in gene frequencies (F_{st} confidence intervals spanned 0), except for stocked adults sampled in 2019 ($F_{st} = 0.01 - 0.02$). Significant pairwise F_{st} scores were not found between stocked origin and wild origin adults captured at the Afon y Bala in the same sampling year.



Figure 5. 95% confidence intervals for pairwise F_{st} values measuring differences in genetic structure between cohorts. Axis labels: numbers are the sampling year; AYB = Afon y Bala spawning site; LK = lake; PL = Penllyn spawning site; W = wild origin fish; S = stocked origin fish; H = hatchery fry. Darker colouration signifies higher F_{st} values.

Estimates of effective population size (N_e, Table 5) were highest for the two most recent cohorts sampled at the Afon y Bala (2018 and 2019). Upper estimates of N_e were often infinity, and therefore not useful, but the lower bound estimate indicated an increasing trend in N_e for fish sampled from the wild between 2008 and 2019. Confidence intervals for the stocked-wild population in 2018 and 2019 were higher and not overlapping with estimates for 2008 (before stocking). However, the central estimate of N_e in 2013, which was the other cohort sampled before stocked fish or possible stocked-wild hybrids recruited to the adult population, fell within the confidence intervals for N_e in 2018 and 2019. The effective population size of hatchery fry was indicated to be lower in 2019 compared with 2011.

population size (14e) by year and sampling location.					
Year	Origin	Location	Ν	Ne (95% CI)	
2008	W	LK	21	3 (1.7 – 10.3)	
2011	Н	Hatchery	17	$20.8 (4.1 - \infty)$	
2013	W	AYB	16	19.5 $(2.9 - \infty)$	
2018	S and W	AYB	32	132.7 (10.6 - ∞)	
2019	S and W	AYB	41	$107 (16.4 - \infty)$	
2019	Н	Hatchery	23	6.1 (1.9 – 29.6)	
2020	W	PL	35	19.5 (6.6 - 98.8)	

Table 5. Linkage disequilibrium estimates of effective population size (N_e) by year and sampling location.

N = sample size; W = fish of wild origin; S = fish of stocked origin; H = hatchery fry; AYB = fish caught at the Afon y Bala spawning site; PL = wild fry caught at the Penllyn spawning site; LK= fish caught in the lake.

DISCUSSION

The results show that the census size of Arctic charr increased between 2011 (start of stocking) and 2019. Three pieces of evidence support the hypotheses that the supportive breeding programme was responsible for the increase. First, the significant change point to the trend in fish density measured by hydro-acoustics occurred in 2013, after stocking commenced; second, fish of stocked origin represented the majority of spawning adults captured in fyke nets during the period of population increase, and third, there was a very strong correlation between the number of juvenile fish stocked each year and the density of juvenile fish measured by hydro-acoustics. Regarding point three, it would be expected that this correlation would be weak or not present if natural recruitment was driving population dynamics. However, there was some indication of a natural population recovery. Estimates of effective size increased before

stocking between 2008 and 2013, although a much larger increase in N_e occurred between 2013 and after stocking (2018 – 2019). There was however considerable uncertainty in estimates of N_e . Natural population processes were probably responsible for increases in the CPUE of wild origin fish that occurred in 2015 and 2016 (Figure 3), as it is highly unlikely any offspring of stocked fish had reached breeding age by these two dates: the earliest age at maturity of Arctic charr at Llyn Padarn is understood to be 3 (Butterworth, 1980) and strong recruitment of stocked fish to the breeding population did not occur until 2014 (Figure 3). Therefore, it seems reasonable to conclude that a natural recovery may have occurred without supportive breeding but that any such recovery would have been substantially smaller than observed. This being said, it is not known to what extent supplementation may have supressed a natural recovery through competitive interactions between wild and stocked juveniles (Kostow, 2009). Furthermore, removing eggs for the hatchery inevitably reduces the potential for wild recruitment and netting activities may cause stress to adults and interfere with natural spawning, including disturbance of redds.

Monitoring methods used by NRW have several limitations which require consideration. Perhaps most important is the question of what proportion of echoes detected in hydro-acoustic surveys are Arctic charr. Other species at Llyn Padarn that are within the targeted size range include brown trout (Salmo trutta), minnow (Phoxinus phoxinus), stickleback (Gasterosteus aculeatus), eel (Anguilla anguilla), Atlantic salmon (Salmo salar), and perch (Perca *fluviatilis*). Ideally, direct sampling methods to measure community composition, such as gill netting, should be undertaken alongside hydro-acoustic surveys in order that echoes can be apportioned accordingly (Bean, 2003). However, because gill netting is destructive, such an approach has not been adopted. It is generally the case that Arctic charr are the dominant species in the pelagic zone (Elliott et al., 1995; Bean, Winfield and Fletcher, 1996), but sampling at Llyn Padarn in the 1970s indicated brown trout may represent approximately 50 % of the fish community (Butterworth, 1980). However, provided that the relative abundance of species did not change substantially between years then hydro acoustics should provide a good indication of temporal trends. The very strong correlation between the number of juvenile Arctic charr stocked each year and the density of juvenile fish measured by hydro-acoustics suggests that data for larger length classes (10- 38 cm) strongly reflect Arctic charr population dynamics. It would be expected that this correlation would be weak or not present if the population dynamics of other species are having a large influence on fish density in the lakes pelagic zone. Given that confidence intervals of density estimates at the end of the time series overlapped the lower threshold for good conservation status and the likelihood that some proportion of echoes were not Arctic charr, the population status is indicated to be unfavourable.

Regarding fyke netting, fishing effort was a key element of potential bias in survey protocols between years, and this was accounted for by adjusting the catch according to the number of nets deployed (CPUE) and by treating nights fished as replicates to show uncertainty in CPUE estimates. The approach for calculating CPUE assumes a linear relationship between the number of nets and the catch, which is not necessarily the case (Bordalo-Machado, 2006). For example, a net downstream will likely capture more fish than nets added upstream (Jellyman and Graynoth, 2005). Similarly, the temporal intensity of spawning migration will broadly follow a bell shaped curve (Frost, 1965), meaning sampling on a day toward the end or beginning of the breeding season will not be equivalent to sampling nearer to the time of peak spawning. Spawning may occur over 4 weeks or more and the timing of spawning is likely to have varied between years dependent on temperature and flow conditions in the river (Frost, 1965; Austin, Essington and Quinn, 2019). The effect on CPUE of inter-annual variation in spawning activity could be large because the surveys rarely continued for more than one week (Table 2). Given these methodological issues, over emphasising inferences from short term temporal changes in CPUE is unwise, but the general trend showing a decline at the start of the time series and then subsequent recovery after stocking is probably a true reflection of population dynamics. Finally, it is important to note that fyke netting has not monitored the newly discovered spawning site at Penllyn. If the site has only recently been utilised by Arctic charr then this might not be too significant in terms of population monitoring, but otherwise, trends measured at the Afon y Bala over the last two decades may not be representative of overall trends in the lake. It is possible that the new spawning site at Penllyn is utilised mostly by stocked fish which may not have a natal instinct to spawn at the Afon y Bala, meaning ratios of stocked to wild fish at the lake could be higher than reported here.

The success of a conservation-focused supportive breeding programme depends on the extent to which stocked fish contribute to population growth by reproducing in the wild (Fleming and Petersson, 2001; Araki *et al.*, 2008). Clearly, fyke netting surveys have demonstrated stocked fish are reaching breeding age and locating native spawning grounds. Similarities in body length indicate stocked fish are surviving to the same age as their wild counterparts, and therefore have a similar reproductive lifespan. However, it is possible stocked juveniles are larger than wild juveniles due to greater food availability in the hatchery, and that this size

difference is retained, or increases, throughout life. As such, length may not be a suitable comparative proxy for age. Juveniles stocked at 8 months are approximately 8 to 13 cm, which is larger than one year old fish measured in the 1970s by Butterworth (1980), which were an average 7 cm in length (SD = 0.83 cm). These differences may not be as significant for the contemporary population: recently sampled wild adults are considerably larger than in the 1970s (McCarthy, 2007) and this may also be the case for wild juveniles. A more precise assessment of survival and reproductive lifespan would require otolith ageing or mark-recapture studies.

Body condition measures an individual's stored energy or nutritional reserves, giving an indication of fitness, well-being, and ability to exploit the environment (Jakob et al., 1996; Stevenson and Woods, 2006; Gibbs and Chiucchi, 2012; Brosset et al., 2015). Individuals with larger nutritional reserves may have a higher probability of survival and reproductive success (Stevenson and Woods, 2006; Robinson et al., 2008; Brosset et al., 2015). High parasite load is a common cause of low body condition (Santoro *et al.*, 2013). The conclusion of the analysis is that stocked fish have a similar body condition to their wild counterparts, indicating they are equally adapted to exploit the environment of the lake to obtain body mass. Furthermore, because condition was calculated for gravid fish, the results suggest stocked fish are equally fecund, although it would have been preferable to also calculate body condition for spent fish to differentiate somatic from reproductive mass. However, whilst fecundity may be similar for stocked and wild fish, this does not necessarily translate in to an equal reproductive output, which will depend also on factors such as spawning behaviours and sexual selection which can act to reduce the breeding success of stocked fish (Fleming et al., 1996; Fukui et al., 2018). In terms of evaluating whether the supplemental breeding programme has increased population viability, the absence of information on the breeding success of stocked fish is a major limitation.

Assuming the stocked fish are reproducing at the lake, a critical question is how breeding between stocked fish and hybridisation with wild fish may impact the fitness of future generations (Fleming and Petersson, 2001; Araki *et al.*, 2008). In numerous studies it has been shown that artificial breeding typically increases the frequency of inbreeding and produces individuals with lower genetic diversity relative to wild fish, even when wild-native brood stock are used (Machado-Schiaffino, Dopico and Garcia-Vazquez, 2007; Araki and Schmid, 2010; Selly, Hickey and Stevens, 2014). Populations with reduced genetic diversity are known to be more vulnerable to stochastic changes and less able to adapt and survive (Hughes *et al.*,

2008). Inbreeding increases the probability that harmful mutations accumulate in the population, resulting in generational declines in fitness, and ultimately, extinction (Gibbs and Chiucchi, 2012; Bosse *et al.*, 2019). The genetic analysis of Llyn Padarn Arctic charr indicate these deleterious effects have thus far been avoided. Inbreeding coefficients and measures of genetic diversity were not significantly different between wild cohorts before and after stocking or between wild and stocked origin adults (Table 4). The later cohorts of wild fish (2018 and 2019) are likely to have included first generation hybrids of stocked and wild origin fish, but this cannot be said with certainty. There was some indication that recent utilisation of stocked origin adults for propagation produced a hatchery cohort with a small effective size, lower genetic diversity, and higher inbreeding coefficient (hatchery fry 2019). These difference were not statistically significant (perhaps due to the small number of loci analysed), and genetic diversity indexes and the inbreeding coefficient for the 2019 hatchery cohort were within the range measured across 6 Arctic charr populations in Wales (Beck, 2014). Nethertheless, it would be prudent to avoid such practices if the breeding programme is to be restarted. Given the recent high percentage of stocked fish in the lake, this could be difficult to achieve.

It was notable that large differences in genetic structure (F_{st}) were detected between samples from different years (Figure 4), which may be the result of genetic drift (Child, 1984; Broquet, Viard and Yearsley, 2013). The magnitude of temporal differences in gene frequencies was toward the middle and lower end of F_{st} values measured among Arctic charr populations in Wales (0.094 – 0.399, Beck (2014)). Genetic drift produces more rapid and extreme shifts in allele frequencies in small populations and is often pronounced following a bottleneck and during subsequent population expansion (Yamamoto *et al.*, 2004; Nussbaum, McInnes and Willard, 2016). Analysis by Beck (2014) suggested a bottleneck occurred at Llyn Padarn around 2008, and the significant association between loci (linkage disequilibrium) detected here for the 2008 cohort, together with deviation from Hardy-Weinberg expectations (Table 4), support this finding (Wang, Caballero and Hill, 1998). Population size (effective and census) increased substantially over a relatively short period in the years subsequent to the likely bottleneck. Similarly large F_{st} values were found between cohorts processed in the laboratory by both researchers, and therefore any known or unknown differences in PCR or sequencing procedures are not a likely explanation for temporal variation in F_{st} .

Genetic structuring between spawning sites has been detected at several lakes with salmonid populations (Child, 1984; Giles *et al.*, 2004; Ferguson and Taggart, 2008; Markevich *et al.*, 2021). Genetic divergence may occur over time due the natal instinct of individuals to breed at

the spawning site of birth, leading to reproductive isolation (Horrall, 1981). Separate spawning sites might be a relic of a past period of geographic isolation or be utilised by populations that colonised the lake on separate occasions in history (Frost, 1965; Schluter, 1996). In the case of Llyn Padarn, the phenomenon of genetic separation was not evident for the two spawning sites located at opposite ends of the lake. In the pairwise comparisons of 2020 samples collected at Penllyn and recent samples collected at the Afon y Bala (2018 - 2019) the lower bound confidence intervals for F_{st} were not greater than 0 except when comparing Penllyn with stocked origin adults in 2019 (95 % confidence interval 0.01 - 0.02, Figure 4). The closest temporal comparison was between Penllyn fry (March 2020) and hatchery fry propagated in December 2019, and in this case the 95 % confidence interval spanned 0 (-0.01 - 0.7). Three explanations are proposed for the absence of genetic divergence between spawning sites: one, there is not a strong instinct to spawn at the location of birth and straying is common (Savary et al., 2017); two, the Penllyn spawning site has only recently been established by straying individuals from the Afon y Bala site or by stocked fish that may not have an instinct to breed at a particular site; or three, the Penllyn site is long established but introgression with stocked fish propagated from breeders at the Afon y Bala has homogenised any genetic structure that might have existed. Significant genetic structure may have been revealed if more loci were investigated, although it has been shown that as few as 2 polymorphic loci can be sufficient (Arthofer *et al.*, 2018).

In conclusion, the supplemental breeding programme can be considered a success according to three criteria: (1) census and effective population size has increased; (2) stocked fish are recruiting to the breeding population and appear to have comparable fitness to their wild counterparts (i.e., comparable body condition); and (3) breeding protocols and likely hybridisation between stocked and wild fish have not impacted genetic diversity. However, with the available information it is not possible to assess the reproductive success of stocked fish, which will ultimately determine the efficacy of the programme in terms of increasing population viability. As of 2018, conservation status remains unfavourable according to population density criteria for hydro-acoustic surveys. Supplementation should not be viewed as a long-term strategy to achieve favourable conservation status due to the potential current and long-term impacts and outcomes that are unknown and will require further study, and because of the large financial cost. The programme was implemented to prevent seemingly imminent extinction, but now that this threat appears to have been adverted, returning lake and spawning habitat to a more favourable condition must be the priority.
Chapter 5. Sensitivity-elasticity analysis of a population projection matrix for Llyn Padarn Arctic charr

INTRODUCTION

Demographic models are commonly used to guide management decisions for wild populations of threatened or endangered species (Earl, 2019; Doak et al., 2021). A popular approach is sensitivity-elasticity analysis of population projection matrices (PPMs) (Benton and Grant, 1999; Manlik, Lacy and Sherwin, 2018; Doak et al., 2021). PPMs provide a transparent mathematical framework for organising lifetime mortality and reproductive schedules, which together determine the rate at which a population is growing or declining (Benton and Grant, 1999). Sensitivity-elasticity analysis quantifies the relative importance of life-stages by calculating the change in the population growth rate (λ) caused by an equal-sized absolute or proportional perturbation to each life-stage vital rate (e.g., fecundity or juvenile survivorship) (Benton and Grant, 1999). All other things being equal, management should focus on protecting or increasing life-stage vital rates that have a large effect on λ (Heppell, Caswell and Crowder, 2000; van de Kerk et al., 2013; Wang et al., 2017). Likewise, sensitivityelasticity analysis is important process in developing models suitable for predicting population dynamics (Caswell, 2009). If a model parameter, for example juvenile survival, has a large impact on λ , then the greatest improvements in model accuracy will be achieved by collecting field measurements of juvenile survival and understanding the environmental and demographic factors regulating temporal variation.

Simple deterministic PPMs can be constructed from a basic static life-table documenting population vital rates at a single time point (i.e., age- or stage-specific growth, survival, and reproductive rates), meaning they are a valuable tool for guiding management of populations that are data-limited (Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Wang *et al.*, 2017). However, because incorrect estimation of PPM parameters may change the outcomes of sensitivity-elasticity analysis, it is important that different permutations of vital rates are investigated to assess confidence in the results of sensitivity-elasticity analysis and associated recommendations for management (Mills, Doak and Wisdom, 1999; Wisdom, Mills and Doak, 2000; Earl, 2019). In the case of Llyn Padarn Arctic charr, life-table information is uncertain as data are either historical or parameters have not been directly measured. Longevity, fecundity, and maturity information were last collected approximately 40 years ago (Butterworth, 1980). In the time since, the body length of mature

fish has increased substantially by ~ 45% (McCarthy, 2007), which could be due to a faster growth rate or a change to life-history traits (e.g., delayed age at maturity) in response to disturbances or environmental changes at the lake (McCarthy, 2007; Johnston and Post, 2009). Determining the age of fish is destructive as only the annuli of otoliths provide an accurate record of age in Arctic charr (Frost and Kipling, 1980; Barbour and Einarsson, 1987). Likewise, assessment of maturity status by inspecting gonad development requires that fish are sacrificed (for example, Butterworth, 1980). Given the precarious status of the population, sacrificing individuals to collect new data on age, growth and maturity could not be justified. Markrecapture methods are a non-destructive alternative for collecting demographic information, but require long-term, multiple years, resource and manpower commitments that are difficult to maintain (Kipling and Le Cren, 1984; Pine *et al.*, 2003; Pollock *et al.*, 2007). Given the currently available life history data, a PPM for Llyn Padarn Arctic charr using historical data is the most viable option, but the analysis should explore if uncertainty in parameters significantly changes the outcomes of sensitivities-elasticities.

Life-stage simulation analysis (LSA) is a framework for assessing uncertainty in sensitivitieselasticities. LSA employs the following analytical steps: (1) vital rates are drawn randomly from a specified probability distribution that reflects uncertainty for the population; (2) large numbers of PPMs are generated through resampling of the probability distribution of each vital rate; and (3) confidence intervals of sensitivities-elasticities are calculated from replicate PPMs to index the probability of potential effects of each vital rate on λ (Wisdom, Mills and Doak, 2000; Biek *et al.*, 2002; Cox *et al.*, 2013). The study presented here adopts this robust permutation approach to assess the relative importance of vital rates for the population growth rate of Llyn Padarn Arctic charr, with the overall objective of identifying priorities for management and further research.

METHODS

Population projection matrix model

The PPM classified fish according to body length, rather than age, so that permutations of the PPM could be parameterised using different growth rates of fish. The model followed a prebirth pulse formula with eight length stages (Kendall *et al.*, 2019), each representing the average length of fish at ages 1 to 8, which is the maximum recorded age of Arctic charr at Llyn Padarn, and in Wales generally (McCarthy, 2007). Length-age data were available from Butterworth (1980), but it was known that the lengths of fish has increased substantially since these data were collected (Chapter 4). To adjust for the increase in fish length, the historical age-length data were calibrated to recent measurements of adult fish length using the equation L_B (L_{M2}/L_{M1}), where L_B is the length of fish at age 1 to 8 sampled by Butterworth (1980), L_{M2} is the mean fork length of breeding fish measured in recent (2008 – 2019) netting surveys (= 280 mm), and L_{M1} is the mean fork length of breeding fish measured by Butterworth (= 210 mm). Thus, it was assumed that the larger body length of the contemporary population is due to a faster growth rate, rather than delayed age at maturity or greater longevity. Increasing fish lengths to reflect the contemporary population was necessary as larger body size infers greater fecundity. The Von Bertalanffy growth (VBG) function from the R package fishmethods (Nelson, 2017) was applied to the adjusted length-at age data to determine lengths for each matrix model stage. The parameter values calculated for VBG function were K = 0.31 (growth completion rate), $L_{\infty} = 362$ mm (asymptotic maximum size), and $t_0 = 0.053$ (theoretical size at birth). Accordingly, the fork lengths for stages 1 to 8 (i.e., age 1 – 8) were 93, 165, 218, 257, 286, 307, 323, and 335 mm. The equation for the pre-birth pulse length structured matrix model (*A*) was as follows

	$F_i M_i S_E S_F$	•••	•••		•••	•••	•••	$F_i M_i S_E S_F$
Eq 1. <i>A</i> =	$S_i T_{i+1}$	S_iT_i	0	0	0	0	0	0
	$S_i T_{i+2}$	·.	•.	÷	÷	:	:	÷
	0	·.	•.	۰.	:	:	:	:
	:	0	•.	•.	•.	:	:	:
	:	:	0	•.	·.	•.	:	:
	:	:	:	0	·.	۰.	•.	:
	L :	:	÷	:	0	$S_i T_{i+2}$	$S_i T_{i+1}$	S_i

where *i* is the length stage, *S* is the probability of surviving one year at length stage *i*, *T* is the probability of growth to a subsequent length stage or remaining at the current stage, *F* is the number of eggs produced by a female at length-stage *i*, *M* is the probability that fish are mature at length-stage *i*, *S*_E is the survival probability of eggs and alevins, and *S*_F is the survival probability of fry from emergence to the first birthday (i.e., length stage 1). As is common for matrix models, only the female portion of the population was modelled as the availability of males is not expected to limit λ (Caswell, 2001). Half of the eggs were assumed to hatch as females and only female-specific data were used for the model parameters. The projection interval of the matrix was one year. Eq 1 is represented graphically in Figure 1.

Parameter estimates

To explore the effect of uncertainty in model parameters on sensitivities, 5000 matrices were generated with different values for stage-specific fecundity, breeding probability, survival, and growth (Table 1). Average fecundity (F) at each length class was calculated by linear regression of length-fecundity data collected by Butterworth (1980). Variations of the fecundity regression were generated by resampling the length-fecundity data. Likewise, variations of the VBG model were generated by resampling the length at age data. The growth probability of remaining at a length stage or transitioning to a subsequent stage was calculated by examining the time taken to grow between stages according to the VBG parameters sampled. To illustrate, for the PPM with sampled VBG parameters predicting slower than average growth (e.g., K =0.29, $l_{\infty} = 362$ mm, $t_0 = -0.033$), the time to grow the 53 mm from stage 2 (165 mm) to stage 3 (218 mm) was 1.04 years, meaning the probability of growth to stage 3 was 0.96. Conversely, with VBG parameters predicting faster than average growth (e.g., K = 0.30, $L_{\infty} = 370$ mm, $t_0 =$ 0.08), the predicted time to grow from stage 2 to stage 3 was 0.97 years, meaning the probability of fish transitioning out of stage 2 after 1 year was 1, with a 0.03 probability of transitioning from stage 2 to stage 4. Finally, if average VBG parameters were sampled for the PPM then growth to the next stage and remaining probabilities were 1 and 0, respectively.

Breeding probabilities (*M*) were estimated by logistic regression of data collected by Butterworth (1980) measuring the frequency of sexually mature females in each age class (matched to length classes). The first breeding was at age 3 (stage 218 mm), which compared favourably with the smallest fish captured in recent fyke net surveys (Chapter 3). Variations of the maturity regression were generated by resampling of the age-maturity data.

Direct estimates of natural mortality could not be made from the available data. The population was assumed to follow a type-III survivorship curve, reaching an asymptotic survival rate at the approximate length at age 2 (165 mm stage), as indicated for brown trout (*Salmo trutta*) in British waters (Elliott, 1993). Asymptotic natural mortality (*Z*) was calculated using Then's *et al.*, (2015) estimator $Z = 4.899t_{max}^{-0.916}$, where t_{max} is the maximum recorded age of the population. The published error terms for each coefficient (0.33 and 0.04) were used to generate variation in estimates of *Z*. The mortality rate of at the first stage was calculated using the equation $Z(L_M/L)$, where L_M is the length at the first stage of asymptotic natural mortality (stage 2) and *L* is the length at stage 1 according to the VBG parameters sampled for the matrix (Lorenzen, 2000; Brodziak *et al.*, 2011). Thus, juvenile mortality varied among matrices by

sampling different VBG parameters and values for *Z*. Faster growth increased juvenile mortality, which is consistent with life-history theory (Chen and Watanabe, 1989; Lorenzen, 2000). Mortality was converted to survival probability (*S*) according to Ricker (1975) as $S = e^{-Z}$.

Only one published study of Arctic charr had measured egg survival under quasi-natural conditions. In the study, Setzer, Norrgard and Jonsson, (2011) protected incubating eggs at Lake Vattern (Sweden) with cages that either (a) excluded predation by fish and an invasive crayfish, (b) allowed only fish predation, and (c) allowed both crayfish and fish predation. Here, a beta distribution using the mean and standard deviation for daily egg survival probability from the fish predation treatment (0.996 and 0.0007, respectively) was resampled to parametrise the matrices. Daily survival was extrapolated to the entire incubation period, which was determined as 75 days based on measurements for the eggs of the autumn spawning Arctic charr population at nearby Lake Windermere incubated at natural water temperatures (Baroudy and Elliott, 1994). Alevin mortality data from the field were not available so the egg survival value sampled for each matrix was simply multiplied by 0.32, which is the survival rate of alevins reported by Baroudy and Elliott (1994). Survival of fry from alevin emergence to age 1 (S_F) was estimated by iteration to find the value necessary for the λ to be at equilibrium (i.e., $\lambda = 1$) (Vaughan and Saila, 1976; Saether and Bakke, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Wang et al., 2017). The iteration process was undertaken for each of the 5000 matrices to generate a distribution for S_F . The value of S_F for each equilibrium matrix was then replaced by resampling from the S_F distribution so that PPMs represented both growing and declining populations and populations where the cause of decline was being driven by different vital rates. Analysis of matrices with different values for λ was important because sensitivities and elasticities may change for declining and growing populations (Mills, Doak and Wisdom, 1999). The parameter value ranges that were sampled for the 5000 matrices are shown in Table 1.



Figure 1. Life-cycle graph for the stage structured matrix model (Eq. 1). Stages (diamonds) are the lengths of fish at age 1 to 8 according to the average VBG parameters. Recruitment to stage 1 is the stage fecundity of female fish multiplied by the product of stage breeding probability, nest survival (egg and alevins), and fry survival. Transitions (blue, green, and red arrows) are a function of survival and growth probabilities.

Deterministic sensitivities-elasticities analysis

The popbio package in R was used to calculate the stable stage distribution, generation time (G_T), population growth rate (λ), sensitivities, and elasticities (Stubben, Milligan and Maintainer, 2016; R Core Team, 2018). The sensitivity value of a particular transition in the matrix is the sensitivity of λ to absolute changes in the transition value. However, because transitions may be expressed in different units (i.e., survival is a probability whereas fecundity is a continuous number), it is difficult to compare the sensitivities of all transitions in a life cycle. Transformation of sensitivities into proportional sensitivities (elasticities) provides an estimate of the proportional change in λ that would result from a minuscule proportional change in the matrix transition. Importantly, many transitions in the life cycle (Figure 1) involve several lower-level parameters (e.g. recruitment of 93 mm fish is a function of fecundity and survival of offspring), which complicates evaluation of the relative contributions of specific lower-level vital rates (Wisdom, Mills and Doak, 2000). Therefore, sensitivities and elasticities were estimated for each vital rate by calculating the partial sensitivity and elasticity from values

for each transition using the chain rule (Caswell, 2001, p. 232). Values for vital rate sensitivities and elasticities were presented for each lower-level vital rate and as summed values representing more general aspects of the life-cycle relevant to management. These stages were fecundity (*F*), which was the sum of all values, egg and alevin survival, or nest survival (*S_N*), survival of fry (*S_F*), survival of juvenile stages (stages 93 and 165 mm) (*S_J*), survival of young adults before 100 % maturity (stages 218 and 255 mm) (*S_{AI}*), and survival of adult fish after 100% maturity (length stages 286 – 333 mm) (*S_A*).

Stage	Length (mm)	Survival	Growth	Maturity	Fecundity
Egg	NA	0.50 - 0.94	NA	0	0
Alevin	NA	0.32	NA	0	0
Fry	NA	0.03 - 0.2	NA	0	0
93 mm	61 – 135	0.2 - 0.35	0.95 - 1.06	0	0
165 mm	128 - 210	0.40 - 0.55	0.97 - 1.04	0	0
218 mm	174 - 251	0.40 - 0.55	0.94 - 1.06	0.18 - 0.27	213 - 243
257 mm	214 - 296	0.40 - 0.55	0.89 - 1.14	0.88 - 0.92	287 - 364
286 mm	224 - 327	0.40 - 0.55	0.81 - 1.23	1	339 - 482
308 mm	277 - 333	0.40 - 0.55	0.75 - 1.48	1	378 - 580
323 mm	289 - 349	0.40 - 0.55	0.68 - 1.97	1	410 - 662
335 mm	315 - 358	0.40 - 0.55	NA	1	434 - 728

Table 1. Life-table information used for generating 5000 population projection matrices for Llyn Padarn Arctic charr. Values are the ranges sampled for the permutation matrices.

Lengths are the value ranges sampled for calculating VBG parameters; survival is the probability of surviving to the next stage; growth is the time taken in years to transition out of the current stage; maturity is the probability of breeding; and fecundity is the number of female ova produced by a single fish. The combined duration of the egg, alevin and fry stages was always 1 year for each permutation of the PPM.

RESULTS

The generation time of PPMs ranged from 4.56 to 5.45 years (MD = 4.92, 95% CI = 4.68 – 5.2) and λ from 0.72 to 1.42, meaning PPMs represented both rapidly growing and declining populations. For equilibrium matrices (i.e., $\lambda = 1$), median survival from alevin swim-up to the 93 mm stage (fry survival) was estimated as 7.7 % (95% CI = 4.2 – 14.7 %) and median survival from the start of the egg incubation period to the 93 mm stage was estimated as 1.9% (95% CI = 1 - 3.6%) %). The stable stage distribution for the 5000 matrices is plotted in Figure 2, which

shows an exponentially decreasing trend, with adults beyond the stage of 50 % maturity (257 mm stage) representing a very small proportion of the population (4.9 %).



Figure 2. Median stable stage distribution and 95% confidence intervals for Llyn Padarn Arctic char.

Sensitivities and elasticities for survival, growth, fecundity and maturity generally decreased over the life cycle (Figure 3). The two exceptions were egg survival, which had a lower sensitivity value than survival of fry and vital rates elasticities for each stage prior to the first mature stage (i.e., egg survival to survival and growth at stage 165 mm) which had equal values. Reproduction elasticities and sensitivities (fecundity and breeding probability) peaked at the second mature stage (257 mm stage) and then gradually declined. For the early mature stages (stage 218 and 257 mm) survival and growth were more important for λ then fecundity and maturity, whereas in the later length stages (286 – 335 mm) the elasticities for each vital rate became similar. Elasticities for growth and survival probabilities contributing to each PPM transition were always equal, whereas for sensitivities the importance of growth for λ was less than survival at the 93 mm stage but greater than survival for all subsequent stages.



Figure 3. Lower-level vital rate elasticities (top) and sensitivities (bottom) calculated from 5000 population projection matrices for Llyn Padarn Arctic charr. Bars are the median and whiskers show the 95% confidence interval. $S_N = \text{egg}$ and alevin survival; $S_F = \text{survival}$ from emergence to first birthday; S = probability of surviving; T = probability of remaining at a stage or growing to a subsequent stage; F = fecundity; and M = the probability of breeding. Subscript numbers refer to length stages 1 - 8 (see Figure 1).

Confidence intervals among sensitivity and elasticity values summed into general life-cycle stages rarely overlapped, indicating the pattern observed in the sensitivity-elasticity analysis would not change at the level of uncertainty incorporated into simulations (Table 2). The elasticity ranking orders of fecundity, nest survival and fry survival did sometimes switch between PPMs but this was only because of very small differences in the actual elasticity value.

Summed elasticities were greatest for juvenile survival and were nearly 50 % higher than the elasticity of young adult survival, which was the next most important stage for λ , and almost four times higher than the summed survivorship elasticity for stages after 100 % maturity, which was the least important life-cycle stage. Sensitivities analysis showed that an equal absolute change in fry survival had a substantially larger effect on λ than other life-stages. However, the true value for fry survival sensitivity was highly uncertain, reflecting large variation in this parameter among PPMs (Table 1). Juvenile survival and nest survival had similar sensitivity values and were the second and third most important life-cycle stages for λ , respectively. Because parameter values for fecundity were always extremely high relative to survival probabilities (Table 1), which are constrained to 1, λ was very insensitive to an absolute change in fecundity.

Table 2. Lower-level vital rate elasticities and sensitivities summed into general life-cycle stages. Values are medians with 95% confidence intervals in parenthesis. The ranking importance of life-cycle stages are presented on a scale of 1 to 6 (highest importance for λ to lowest importance for λ).

Stage vital rate	Elasticity	Elasticity rank	Sensitivity	Sensitivity rank
S_N	0.20 (0.19 – 0.22)	3 (2 – 4)	0.8 (0.62 – 1.03)	3 (3 – 3)
S_F	0.20 (0.19 – 0.22)	3 (2 – 4)	2.61 (1.64 – 4.11)	1 (1 – 1)
S_J	0.41 (0.37 – 0.44)	1 (1 – 1)	1.16 (0.93 – 1.43)	2 (2 – 2)
S _{AD1}	0.28 (0.27 – 0.29)	2 (2 – 2)	0.58 (0.51 - 0.65)	4 (4 – 4)
S_{AD2}	0.11 (0.07 – 0.16)	6 (1 – 1)	0.23 (0.18 - 0.28)	5 (5 – 5)
F	0.20 (0.19 – 0.22)	3 (2 – 4)	0.0005 (0.0004 -	6 (6 – 6)
			0.0007)	

 S_N = egg and alevin survival; S_F = survival of fry from emergency to the first birthday; S_J = survival of juveniles before the first maturity stage (93 and 165 mm stage); S_{AI} = survival of young adults before ~100 % maturity (218 and 257 mm stage); S_{AI} = survival of adults after ~100 % maturity (218 and 257 mm stage); and F = fecundity.

DISCUSSION

The analysis applied a robust life-stage simulation approach to a PPM for Llyn Padarn Arctic charr and demonstrated that the relative importance of life-cycle stages was consistent across a large range of possible parameter values. The general pattern was for survival rate, fecundity, and breeding probability to decline in importance for λ over the life cycle (Figure 3), reflecting the stable stage distribution (Figure 2), whereby the stage with the smallest number of

individuals contributes least to population growth. A notable exception was the sensitivity of fry survival, which was significantly higher than the previous stage, survival in the nest (egg and alevin survival). This aberration reflects the life-history strategy of Arctic charr, and salmonids generally, whereby parental investment in eggs (i.e., migrating to optimal spawning sites and, to some extent, redd digging) means egg and alevin survival is high relative to broadcast spawners, and higher than fry survival (Winemiller and Rose, 1992; Power, Reist and Dempson, 2008). As such, an absolute change in nest survival produces a smaller increase in the parameter value than an absolute change in fry survival, resulting in a lower sensitivity value for nest survival. A further interesting pattern was the change over the lifecycle in the importance of survival relative to reproduction elasticities. In young adults, survival was substantially more important for λ than maturity probability and fecundity, whereas as in the last two adult stages, the two aspects of reproduction became the marginally more important vital rates (Figure 3). The pattern appears to reflect the curve of survivorship and fecundity, whereby the former flattens after maturity whilst fecundity continues to increase due to indeterminate growth (Table 1). Growth sensitivities were higher than survival for 165 mm to 307 mm stages but lower than survival for young juveniles (93 mm) and equal to survival for the penultimate adult stage (Figure 3), suggesting the widely recognised trade-off been growth and survival switches over the life-cycle (Pauly, 1980; Chen and Watanabe, 1989; Stearns, 1992). In the PPMs Arctic charr reached the asymptomatic survival rate at the 165 mm stage, and it was at this point that growth became the more important parameter relative to survival. According to the VBG model, growth slows as it approaches the asymptomatic length (Ricker, 1975, Table 1), meaning the percentage increase in fecundity between stages becomes less as fish get larger (Table 1), reducing the benefit of growing to the next stage toward the end of the life-cycle. Finally, it should be noted, unlike an age-structured PPM, the time of death is not determined in a stage-structured PPM (Caswell, 2001), as used here. Consequently, fish can remain in the final stage of the model for longer than the previous stage, explaining the similar sensitivity-elasticity values for both stages.

The analysis explored a large range of plausible values for vital rates, however, an important parameter that did not vary among PPM permutations was the stage at first maturity (218 mm stage). Comparative analyses across numerous taxa have demonstrated a change in the age or stage of first maturity can have a large impact on sensitivities and elasticities (Cole, 1954; Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006). Recent empirical data for the lengths of breeding fish captured at Llyn Padarn supported the selection

of the 218 mm stage as the length at first reproduction (Chapter 4). Less certain was the growing time required to reach the 218 mm stage and the proportion of females breeding, as these data were collected approximately 40 years previously when Arctic charr at Llyn Padarn were substantially smaller in size. The effect of these uncertainties was explored by varying the VBG parameters and the probability of maturity, which changed the generation time between PPMs by nearly 1 year. Irrespective of variation in generation time the qualitative outcomes of sensitivities and elasticities were stable. The effect on elasticities of larger changes in the age of maturity (and therefore generation time) is investigated further in the following chapter through comparison of Arctic charr populations that are highly divergent in these traits.

Scope of deterministic methods

Deterministic elasticities and sensitivities are not a forecast tool for population dynamics because the dynamics of wild populations are influenced by factors not considered in the linear form of PPM used here which assumed vital rates are constant and that population has a stablestage distribution (Vélez-Espino, Fox and McLaughlin, 2006). Disturbed populations typically exhibit high temporal variability in vital rates and rarely have a stable age distribution (Ackleh *et al.*, 2018). The elasticities and sensitivities of λ may differ from deterministic estimates when populations are recovering from a catastrophic disturbance causing an unbalanced population structure (Ackleh et al., 2018). As is generally the case for salmonids, density-dependent compensation may act on the vital rates of Arctic charr (Grossman and Simon, 2020), causing sensitivities and elasticities to change according to population size and carrying capacity of the environment (Grant and Benton, 2003). However, since conservation is generally concerned with declining populations that are below the carrying capacity, the phenomena of densitydependent compensation is perhaps not important when considering elasticities and sensitivities (Grant and Benton, 2003). Moreover, whilst non-linear PPMs may have quantitatively different sensitivity and elasticities values compared to linear models, the qualitative ranking order of importance used to guide management prioritisation appears invariant for iteroparous species, such as Arctic charr (Silva Matos, Freckleton and Watkinson, 1999; Vonesh and De la Cruz, 2002; Caswell and Takada, 2004). Quantitative difference may however be a consideration when weighing up the wider cost-benefits and feasibility of different management interventions. Non-linear models are not easy to construct; they require not only data on the vital rates, but data on the functional dependence of the stage specific vital rates on density and the environmental carrying capacity (Caswell and Takada, 2004). Such

data are not available for Llyn Padarn Arctic charr, or Arctic charr in the wild generally (Grossman and Simon, 2020).

Implications for management

Sensitivities and elasticities can be applied in evaluating management strategies in conservation and identifying research priorities: all other things being equal, management efforts and research should focus on vital rates with a large effect on the population growth rate (Heppell, Caswell and Crowder, 2000; van de Kerk *et al.*, 2013; Earl, 2019). According to elasticities analysis, which measures the change to λ caused by an equal proportional change in vital rates, the rankings in Table 2 suggest managers at Llyn Padarn should assign highest priority to interventions improving juvenile survivorship, followed by young adults, fecundity, nest and fry survival equal third, and lastly adults > 287 mm in length. In sensitivities analysis, which measures the change in λ caused by an equal absolute change in vital rates, this order was replicated except fry survival was the most important parameter and fecundity the least important (Table 2). However, there are several practical aspects that managers must consider alongside elasticities and sensitivities, and these are examined at length in the general discussion (Chapter 7), with specific reference to Llyn Padarn Arctic charr.

Chapter 6. Variation in the elasticities of Arctic charr lifehistory phenotypes

INTRODUCTION

As the last glacial period ended and the ice sheet retreated poleward (~12,000 to 15,000 ybp), new, vacant, but often only intermittently accessible habitats were formed that offered much ecological opportunity to early colonising species (Schluter, 1996). Because of these factors short evolutionary time scales, oscillating periods of geographic isolation, and low interspecific competition, species in postglacial regions are often characterised by high levels of intraspecific diversity (Schluter, 2000; Stone, Flynn and Cook, 2002; Lecocq et al., 2013; Doenz et al., 2019). Perhaps most impressive is the variability exhibited by postglacial freshwater fish, which can include a bewildering array of phenotypes, both sympatric and allopatric, that have diverged in life-history, functional traits, marine migratory behaviour, and trophic niche (Sandlund et al., 1992; Schluter, 1996; Klemetsen, 2013; Muir et al., 2016; Doenz et al., 2019). This intraspecific variability is considered remarkable among vertebrates (Klemetsen, 2013), and may make an important contribution to the biodiversity, and therefore stability, of depauperate high latitude and upland ecosystems (Adams et al., 2007; Reist et al., 2013). Accordingly, it is argued, that to preserve the biodiversity necessary for the generation of new species, persistence of species following environmental change, and local adaptation, management should focus on units such as phenotype, geographic region, or population, rather than biological species (Waples, 1991; Crandall et al., 2000; Adams and Maitland, 2007; Adams et al., 2007; Bush and Adams, 2007; Mee et al., 2015).

Studies discussing management units for postglacial fish have focused on criteria for delineating units (e.g., Waples, 1991; Bush and Adams, 2007; Mee et al., 2015), whereas the extent to which trait differences within or among these units may result in contrasting population dynamic responses to management interventions or natural disturbances, has not been considered. Investigations of population dynamics across numerous taxa have found that the population growth rate (λ) is typically more sensitive to perturbations in vital rates at certain stages of the life-cycle than others, and that these patterns in the relative importance of life-stages for λ , and the differences in patterns observed between taxa, are an emergent property of life-history traits (Cole, 1954; Saether and Bakke, 2000; Oli and Dobson, 2003; Vélez-Espino, Fox and McLaughlin, 2006). Therefore, given the exceptional intraspecific variability

in life-history of postglacial fish, large differences in the importance of life-stages for λ may exist between populations, with significant implications for management and predicting population dynamics. If the contribution that a life-stage vital rate (e.g., adult survivorship) makes to λ differs between two populations, then contrasting trajectories in λ should be expected in response to a natural disturbance or management intervention affecting that vital rate (Heppell, 1998; Benton and Grant, 1999; Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; van de Kerk *et al.*, 2013). In conservation, a fundamental objective is to increase λ , meaning it is desirable to identify the most and least influential lifecycle stages in order that management and research efforts can be focused accordingly (Heppell, 1998; Benton and Grant, 1999; Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; van de Kerk *et al.*, 2013). Therefore, to optimise management strategies at the sub-species level, variation in life-history and its implications for population dynamics must be considered.

Population projection matrices (PPMs) and associated elasticities analysis are the predominant approach for investigating the importance of life-cycle stages for λ (Manlik, Lacy and Sherwin, 2018; Doak *et al.*, 2021). PPMs provide a transparent mathematical framework for organising lifetime mortality and reproductive schedules, which together determine the rate at which a population grows or declines (Caswell, 2001). Elasticities quantify the importance of lifestages by calculating the change in λ caused by an equal sized and proportional perturbation to each life-stage vital rate (e.g., fecundity or juvenile survivorship) (Caswell, 2001). Assuming λ is the appropriate measure of mean fitness, then elasticities indicate the strength of selection acting on different elements of the life-cycle, and therefore are of interest to evolutionary ecologists (Stearns, 1992; Caswell, 2009). Unlike non-proportional perturbation analysis (sensitivities), the elasticities of vital rates always sum to unity, meaning their relative contribution to λ can be compared among populations with different life histories (Benton and Grant, 1999).

Calculation of elasticities is often challenged because the complete life-history and demographic information needed to construct PPMs are unavailable for many populations, or is uncertain due to the difficulties in measuring some parameters, in particular survivorship (Heppell, Caswell and Crowder, 2000; Saether and Bakke, 2000). Moreover, historical information on a population may become invalid due to subsequent disturbance regimes altering life-history traits (Heppell, Caswell and Crowder, 2000; Johnston and Post, 2009). Accordingly, it is informative to compare a large number of populations in order to identify

general relationships between elasticities and commonly measured life-history traits (e.g., age at maturity, longevity) (Heppell, Caswell and Crowder, 2000; Stahl and Oli, 2006; Vélez-Espino, Fox and McLaughlin, 2006; van de Kerk et al., 2013), or characteristics that are often correlated with life-history, such as phylogeny (Heppell, Caswell and Crowder, 2000; Oli and Dobson, 2003; Vélez-Espino, Fox and McLaughlin, 2006), functional traits (Adler et al., 2014), or environment (Silvertown, Franco and Menges, 1996; Oli and Dobson, 2003; Hanson, 2011). Generalities based on population characteristics could be used to infer elasticities for populations which do not have sufficient information to calculate them directly and would provide a rapid method to categorise numerous populations according to their likely response to natural disturbances or management interventions affecting certain life-stages (Silvertown, Franco and Menges, 1996; Heppell, Caswell and Crowder, 2000; Rose, 2005; Vélez-Espino, Fox and McLaughlin, 2006; van de Kerk et al., 2013; Adler et al., 2014). In the case of environmental parameters, relationships would allow estimates of elasticities for a population without requiring any life-history information. Furthermore, relationships could indicate how elasticity patterns might change if life-history traits adapt in response to a longer term change in the environmental parameter, or which populations have traits and elasticity patterns that suggest they are better or worse adapted to cope with any such perturbation (Beukhof et al., 2019). In this regard, climate temperature, which is predicted to increase substantially over this century due to climate change (IPCC, 2018), is a parameter of particular interest and significance.

Life history theory proposes that natural selection shapes the timing and duration of key events in an organism's life to maximise fitness (Roff 1992; Stearns 1992). The various life history events are linked to fitness components (i.e., life-stage or age specific reproduction survival rates) through trade-offs. Contrasting selection regimes between populations occupying different environments may lead to variation in life-history traits such as age at maturity, fecundity, body size, and longevity. According to the influential Winemiller & Rose model which proposes fish life-histories can be classified on a trilateral continuum, populations from high latitude habitats are expected to display later maturation, larger body size, greater batch fecundity, and lower investment in offspring compared with populations occupying lower latitude habitats (Winemiller and Rose, 1992; Winemiller, 2005). Winemiller & Rose (1992) named this strategy as 'periodic' and proposed that a more periodic strategy would be selected when resource availability was limited or variable but competition and threats from natural enemies were low. The strategy is in several ways analogous to the slow end point of the slowfast life-history continuum commonly used to characterise life-histories in other vertebrate groups (Dobson and Oli, 2007; Tökölyi, Schmidt and Barta, 2014). In effect a periodic strategy represents an iteroparous "bet-hedging tactic" over an inter-annual time scale, whereby it is advantageous to prioritise surviving and increasing biomass to produce a greater number of offspring in a subsequent year over current reproduction because of high temporal variation in offspring survival caused by environmental stochasticity (Winemiller and Rose, 1992; Saether and Bakke, 2000). Accordingly, it follows that the strength of selection on surviving (i.e., importance of survival for mean fitness) should increase with latitude. Since cooler temperatures can also be associated with lower resource availability and density of competitors and natural enemies (Schemske *et al.*, 2009), then we should expect that life-histories become less periodic along a gradient of increasing climate temperature, and selection on survival be relaxed.

In the present study, I assess the extent to which elasticities vary within a species of phenotypically diverse postglacial fish and test the hypotheses that variation can be explained by adaptations in life-history traits associated with climate temperature gradient and marine migratory behaviour (freshwater resident vs anadromous). The Arctic charr (Salmonidae, Salvelinus alpinus) was considered an ideal species for such an investigation for several reasons. First, populations are distributed across a large climate range and include anadromous and freshwater resident forms (Klemetsen et al., 2003). Second, resident Arctic charr have a shared anadromous ancestor and have only recently (~10,000 - 15,000 ybp) colonised the habitats in which they now reside (Brunner et al., 2001), meaning it is likely that differences in life-history traits and elasticities reflect the change from an anadromous to resident lifestyle and adaptations to shifts in selective pressures across environmental gradients (Baker et al., 1998). Third, a large body of literature with the necessary data for a PPM is available as the variable life-history tactics of Arctic charr has generated intense interest among the research community. PPMs were constructed using literature data for 41 populations and elasticities then calculated for four life-stage vital rates: reproduction, juvenile survivorship, adult survivorship, and overall survivorship. Previous comparative analyses of fish and non-fish taxa (e.g., Saether and Bakke, 2000; Oli and Dobson, 2003; Vélez-Espino, Fox and McLaughlin, 2006; Wang et al., 2017) have shown the age at maturity strongly determines the elasticity of reproduction and overall survivorship (decrease and increase with age at maturity, respectively). However, relationships between life-history traits and the combination of reproduction and life-stage survivorship elasticities have not been as clearly resolved due to

the difficulty in analysing these multi-dimensional compositional data with methods that are designed for a single dependent variable. To address this, I employ Dirichlet regression, which in the field of elasticities has previously only been utilised to study relationships with functional traits in plants (Adler *et al.*, 2014). Dirichlet regression is a relatively new analytical tool available to ecologists, and unlike simple linear regression or correlation analysis, it accounts for the sum to unity property of elasticities and allows the visualisation of elasticity relationships with independent variables in three-dimensional space (Maier, 2014). The study represents the most extensive comparative analysis of elasticities at the interspecific level and the first to investigate if elasticities change in a predictable manner according to climate temperature gradient and the anadromous *vs* resident dichotomy that is common in fish.

METHODS

Data compilation

Peer review articles, dissertations, and grey literature were surveyed for studies of Arctic charr populations containing the following information required to construct and parameterise a PPM: (1) adult survival estimates from catch curve analysis or the necessary age frequency data; (2) a length fecundity relationship or raw data; (3) mean lengths of mature age classes; and (4) the proportion of fish in each age class expected to breed that year. These data were found for 25 populations from Europe and 16 from North America, covering a latitudinal range of 49.58 to 69.94 °N (Figure 1, Appendix 5). We only included populations where age had been determined from the annuli of the otolith, rather than scales, as the latter method has been shown to underestimate the age of Arctic charr > 5 years (Frost and Kipling, 1980; Barbour and Einarsson, 1987). If data were presented separately for survey years or available from more than one source then average values were used for PPM parameters and life-history traits. Data presented in figures rather than in tables were extracted digitally using the open source application WebPlotDigitizer (Rohatgi, 2019). Adult fish residing in lakes had been sampled using benthic or pelagic gill nets, whilst sampling of populations from rivers used a variety of methods, including fyke nets, seine nets, migration traps, and angling. Differences in sampling strategy, including sampling gear, time of year, and location, may have influenced estimates of model parameters and life-history traits, but we have here assumed studies are equally representative of the population.



Figure 1. Locations of Arctic charr populations sampled. The red line marks the southern limit of anadromous populations according to Jørgensen and Johnsen (2014). In this study, populations below this line were classified as southern region populations and above the line as northern region populations. Population names for location numbers are given in Appendix 5.

Matrix model

I used the post-breeding form of birth pulse Leslie matrix (age-structured PPM) to represent the seasonal reproduction of Arctic charr. In a post-birth pulse model, the projection interval begins immediately after spawning and the reproduction transition of the matrix is weighted by the probability of adults surviving to the next spawning event (Kendall *et al.*, 2019). In the alternative approach, a pre-birth pulse PPM, the projection interval begins immediately before spawning and the reproduction transition is weighted by the probability of offspring surviving to the next year (Kendall *et al.*, 2019). As is common in demographic studies, only the female portion of the population was modelled as the availability of males is not expected to limit λ (Caswell, 2001). It was assumed that half of the eggs hatched as females and only femalespecific data from the literature search were used accept when only averages across sexes were available. The parameters for our post-birth pulse Leslie matrix (A) were as follows:

(Eq. 1)
$$A = \begin{bmatrix} R_x = F_x B_x P_x & \cdots & R_x = F_x B_x P_x & R_x = F_x B_x P_x \\ P_x & 0 & \cdots & 0 \\ 0 & \ddots & \ddots & & \vdots \\ \vdots & 0 & P_x & \ddots \end{bmatrix}$$

where P_x is the probability of surviving to age x + 1 and R (reproduction) is the number of eggs (*F*) a female produces at age x weighted by P_x and the proportion of fish at age x that reproduce (*B*).

Elasticities analysis

Elasticities were calculated using Caswell's (2001) analytical method which measures the response of λ to a proportional and equal change in each matrix transition. The elasticity matrix is calculated as

(Eq. 2)
$$e_{ij} = (a_{ij}/\lambda)(\partial \lambda / \partial a_{ij})$$

where e_{ij} is the elasticity, and a_{ij} is the (i,j) element of the matrix.

The elasticities of matrix transitions always sum to unity, permitting comparisons of populations with different life-histories (Benton and Grant, 1999). However, comparing the elasticities at each age is only possible if the matrix dimensions of populations are identical. Since this is rarely the case, it is necessary to sum transition elasticities into important life-cycle stages that are shared across populations, which in fish and animals generally, are usually juvenile survivorship, adult survivorship, and reproduction (Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Wang *et al.*, 2017). Thus, the relative contribution of these composite vital rates to λ were calculated according to Heppell, Caswell and Crowder (2000) by summing the elasticities of the matrix transitions (Eq 1) as follows:

(Eq. 3) elasticity of juvenile survivorship $(e_{Sj}) = \sum e_{Px} x < A_M$

(Eq. 4) elasticity of adult survivorship $(e_{Sa}) = \sum e_{Px} x \ge A_M$

(Eq. 5) elasticity of reproduction $(e_R) = \sum e_{Rx}$

where A_M is the age at maturity (defined later). The sum of e_{Sj} and $e_{Sa} = 1 - e_R$, meaning it is not necessary to analyse both overall survivorship elasticity (e_S) and e_R separately: a relationship between e_S and an independent variable (e.g. longevity) will always be the inverse of the relationship with e_R . A second mathematical property of elasticities is that the elasticity of the first PPM transition is always equal to e_R (Heppell, Caswell and Crowder, 2000), meaning e_R also represents the sensitivity of λ to changes in early life-stage survival (i.e. egg to year 1). Elasticities were calculated using the 'elasticity' function from the R package 'popbio' which assumes a stable population age structure and no density dependence of vital rates (Stubben and Milligan, 2007). All analyses described hereafter were also implemented in R (R Core Team, 2018).

Matrix model parameters

The fecundity of age classes (F_x , Eq 1) was estimated from length-fecundity relationships in the source material, or when absent, regression equations derived from raw data using the formula $\ln(F) = a + b \ln(L)$, where F is the number of eggs and L is the body length at age x. In the case of two dwarf populations, the authors did not find a significant relationship between fecundity and length and reported only a mean fecundity value. The inclusion of these populations was deemed acceptable as each grew very little after reaching maturity. Two studies where more than one population had been sampled (Jonsson and Hindar, 1982b; Loewen, 2008) only provided a combined regression equation as the fecundity-length relationship did not vary among the populations. Mean length at age data were extracted from tables and figures or calculated from the parameters provided for the von Bertalanffy growth function (VBGF) $L_x = L_\infty$ ($1 - e^{-K(t+t0)}$), where L_∞ is the asymptotic maximum length, K is the growth completion rate, and t_0 is the theoretical length at birth (Ricker, 1975).

The proportion of females in an age class expected to breed at the next spawning season (B_x , Eq 1) had been determined by researchers according to the stage of gonad development or the size of gonads relative to body size. For the PPMs, age groups younger than the first age when the expected breeding frequency was $\geq 50\%$ (A_M) were classified as juveniles and assigned a B value of zero. The removal of reproduction prior to A_M is necessary for e_{Sj} to be comparable across populations. For two populations the breeding frequency did not equal 50% in any age class, or only did so in one age class toward the end of the population's life span. In both these cases A_M was defined as the age of first breeding. Low breeding frequency in adult age classes indicates that fish do not always breed in successive years (Sparholt, 1985). One population surveyed (Loch Rannoch piscivore) was not included in the analysis because the maturity data were highly irregular (Fraser, 1998).

The adult mortality rate (M_A) was assumed to be constant after maturity and was estimated using catch curve analysis. Catch curve analysis estimates mortality by least-squares regression of the natural logarithm of the number of fish in each age group, starting from the age group that is fully recruited to the sampling gear (Smith *et al.*, 2012). Except for populations where estimates were reported in the source material (N = 9), catch curves were calculated from the available age frequency data using the Chapman-Robson method ('fishmethods' package, Nelson, (2017)). Fish of an exceptional age relative to the population overall were assumed to represent rare individuals or errors in ageing and were excluded from the matrix dimensions and catch curve analysis. M_A was converted to annual survival probability (P, Eq 1) according to the formula $e(-M_A)$ (Ricker, 1975). M_A is viewed here as the natural mortality rate even though some fishing mortality (sport, subsistence, and light commercial) was present, but unquantified, for several populations.

The annual survival rate of juvenile age classes (egg to A_M) was estimated by using iteration to find the value necessary for the population growth rate to be at equilibrium (i.e., $\lambda = 1$) (Vaughan and Saila, 1976; Saether and Bakke, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Wang *et al.*, 2017). Estimation of juvenile survival in this manner was necessary as it was an unknown parameter for all populations sampled, and often for fish in general. The approach also has two useful properties: (1) any effect of variation in λ on elasticities is removed, and (2) irregular estimates in juvenile survival can indicate if estimates of other model parameters are inaccurate (Vélez-Espino, Fox and McLaughlin, 2006). In the case of fish, mortality is high in early life, so it was considered that annual juvenile mortality should be higher than adult mortality (Vélez-Espino, Fox and McLaughlin, 2006).

Population traits

Populations were classified according to the author description as anadromous (ANDR), normal growth resident (NR) or dwarf resident (DWF). Ecological characteristics were noted for resident populations (e.g., littoral feeder, limnetic feeder, piscivore, river or lake-resident) but were not statistically analysed because of the large number of factors with small sample sizes. In this paper, morphotype refers to migratory behaviour and growth form (i.e., ANDR, NR, and DWF) and ecotype to the ecological niche of resident populations. Anadromous Arctic charr are iteroparous and make annual summer migrations to forage in marine waters (Jørgensen and Johnsen, 2014). Dwarfs are characterised by small body size, limited growth after maturation, and the retention of juvenile characteristics (parr marks) throughout life (Adams, 1999). Authors studying six of the lakes sampled considered that two or more putative populations were present and occupied different ecological niches (polymorphic systems). In some cases, lakes were polymorphic but complete data were only available for one population.

The following eight life-history variables were recorded for each population: (1) maximum age (A_{Max}) ; (2) age at maturity (A_M) ; (3) average fecundity (*F*), calculated as the mean of age specific fecundities; (4) the gonad somatic size index (GSI), which measures reproductive effort and is the weight of mature female gonads expressed as a percentage of body weight minus the gonads; (5) length at maturity (L_{Am}) ; (6) the growth completion rate (*K*) of the VBG

function; (7) the adult survival rate (S_A); and (8) the survival probability of juveniles from egg to maturity (S_J), calculated as the product of $P_x < A_M$. In the majority of studies fish length had been measured from snout to tail fork (fork length), whilst a small number had used alternatives (standard or total length) or did not describe their method. Different measurements of length may have introduced a small amount of noise to the L_{Am} data, but this is unlikely to have been significant for the purposes of the study. Where the VBGF had not been calculated by authors, we estimated *K* from mean age at length data using the 'growth' function from the 'fishmethods' package (Nelson, 2017). A PCA was undertaken to summarise the life-history of Arctic charr populations on the periodic continuum that contrasts slow growing, long lived, and late maturing populations against those with opposite traits (Winemiller and Rose, 1992). GSI was not included in the PCA as data were only available for 28 populations.

Relationships between elasticities, population traits and climate

Variation among morphotypes in life-stage elasticities and life-history traits was examined using pairwise Wilcoxon tests with p-values adjusted for multiple comparisons according to Holm (1979). In addition, elasticities of morphotypes were compared by grouping populations according to which life-stage had the highest, second highest, and lowest elasticity score (qualitative elasticities). Fisher's exact test was used to determine if there was an association between the frequency of populations in elasticity ranking groups and morphotype. Because ANDR populations are only found at high latitudes, dwarf and normal growth residents were grouped according to whether they were below or above the latitudinal line that marks the southern limit of ANDR populations (Figure 1). Thus, when comparing elasticities and life-history traits. Resident populations below the ANDR line are referred to as southern and those above the line as northern.

Relationships between the position of populations in three-dimensional elasticity space (i.e., e_R / e_{Sj} / e_{Sa} space) and independent variables (life-history traits and temperature) were examined with Dirichlet regression implemented using the package 'DirichletReg' (Maier, 2014). Dirichlet regression accounts for the non-independence of elasticities (Adler *et al.*, 2014) and allowed relationships between elasticities and independent variables to be plotted in three-dimensional space. For two-dimensional elasticity space (i.e., e_R/e_S space), I used simple linear regression with one elasticity (e_R) as the dependent variable and applied Holm-Bonferroni correction for multiple tests (Holm, 1979). Univariate plots of life-stage elasticities and

independent variables indicated a curved linear relationship might be appropriate in some cases, and therefore, I explored models with and without 2nd degree polynomials. The polynomial model was selected if AIC decreased by more than 2 units relative to the linear model. Natural log and arcsine transformations were implemented for continuous and proportional life-history traits respectively. Relationships between elasticities and climatic temperature (mean annual air surface temperature from 1950 - 2000) were only investigated for NR populations as the distributions of ANDR and DWF populations were heavily skewed toward cold climate regions and the sample sizes were relatively small. The temperature data was acquired at 2.5 km² spatial resolution from the 'worldclim' database *via* the 'raster' package (Hijmans, 2019).

RESULTS

Of the 42 populations sampled, NR populations were the most represented morphotype (northern, N = 9; southern, N = 11), followed by DWF populations (northern, N = 10; southern, N = 1) and ANDR populations (N = 10). Resident populations included NR littoral ecotypes (N = 3), NR limnetic ecotypes (N = 15), NR piscivores (N = 2), stream-resident DWFs (N = 3), and lake-resident DWFs (N = 8). Summary statistics for key model parameters were as follows: S_A , 0.3 to 0.78 (μ = 0.53, SD = 0.11); S_J , 9.1e-4 to 0.018 (μ = 0.002, SD = 0.003); A_{Max} , 6 to 24 years (μ = 12.46, SD = 4.32); A_M , 2 to 11 years (μ = 5.4, SD = 2.34); and F, 49 to 4532 eggs (μ = 1009, SD = 1279). The annual mortality of juvenile age classes calculated for λ = 1 ranged from 0.59 to 4.65 (μ = 1.49, SD = 0.84) and in all populations met the assumption that juvenile mortality should be higher than adult mortality.

For the sampled populations overall, the mean of e_{Sj} was highest ($\mu = 0.59$, SD = 0.11) followed by e_{Sa} ($\mu = 0.24$, SD = 0.08) and lastly e_R ($\mu = 0.16$, SD = 0.06). Ranges in elasticities were substantial, with examples of populations that differed by as much as 0.54 for e_{Sj} (range = 0.22 -0.76), 0.27 for e_R (range = 0.06 -0.33), and 0.42 for e_{Sa} (range 0.12 -0.54). The elasticity of λ to changes in juvenile survival ranked first (i.e., had the highest elasticity) for 38 (93 %) of the populations, of which adult survival ranked second in 26 cases and reproduction ranked second in 12 cases. Adult survival had the highest elasticity and reproduction the lowest elasticity in the other 3 populations.

Comparative life-history and elasticities of morphotypes

The first axis of the PCA modelled 55.4% of variation in life-history traits and corresponded to the periodic life history axis. The end points contrasted populations with late maturation,

long-lives, large body size, and high fecundity (highly periodic), against those with a higher probability of surviving to maturity and a faster growth completion rate (less periodic) (Figure 2). Spearman's rank correlation showed A_M had the strongest relationship with PC1 (rs = 0.92), followed A_{Max} and L_{Am} (rs = 0.83), F (rs = 0.80), K (rs = -0.60), S_A (rs = 0.53) and lastly S_J (rs = -0.51), with all coefficients being significant ($p \le 0.001$) after Holm-Bonferroni correction for 7 tests. The position of populations from northern regions on the periodic axis varied significantly according to morphotype, with ANDR populations located closest to the more periodic end point, followed by NR populations, and lastly DWF populations being the least periodic (Figure 2, Table 1). Univariate analysis of life-history traits showed northern DWF populations differed significantly from other morphotypes in all traits except for GSI, K and S_A , whilst ANDR and northern NR populations only differed in their values for S_J , F and L_{Am} (Table 1).



Figure 2. PCA characterisation of Arctic charr populations on the periodic life-history axis (PC1). Low PC1 values indicate a less periodic life-history. ANDR = anadromous, NR = normal resident, DWF = dwarf resident, K = growth completion rate. S = populations located in southern regions where ANDR populations are absent, N = populations located in northern regions where ANDR populations are present (see Fig. 1).

The position in $e_R / e_{Si} / e_{Sa}$ space of populations according to morphotype is shown in Figure 3. Populations toward the bottom right of elasticity space are those highly sensitive to juvenile survivorship, whilst those toward the top corner are increasingly sensitive to adult survivorship. Movement toward the bottom left corner represents increasing sensitivity to reproduction, and therefore, reduced sensitivity to overall survivorship. Analysis of variance in quantitative elasticities showed the differences between ANDR and northern NR populations in life-history traits and position on the periodic continuum did not translate into significant differences in any of the three life-stage elasticities (Table 1). For northern DWF populations, e_R and e_{Si} were significantly higher and lower, respectively, compared with ANDR and northern NR populations (Table 1). In terms of qualitative elasticities, ANDR and northern NR populations were almost exclusively found in the $e_{Sj} > e_{Sa} > e_R$ group, whereas northern DWF populations were more equally distributed among the three ranking groups (Figure 3). However, a chi square test found there was not a significant association between morphotype and qualitative elasticity group (p = 0.22). Three polymorphic lakes contained populations belonging to different elasticity groups (Loch Rannoch, Lake Iterlaa, and Thingvallavatn). Elasticities for each population can be found in Appendix 6.

	Morphotype $\mu \pm SD$			p-value of pairwise comparison		
	ANDR	NR	DWF	ANDR	ANDR	NR vs
	(N = 10)	(N = 9)	(N = 10)	vs NR	vs DWF	DWF
e _R	0.1 ± 0.02	0.11 ± 0.03	0.19 ± 0.04	0.66	***	**
e sj	0.69 ± 0.04	0.66 ± 0.04	0.51 ± 0.12	0.15	***	**
esa	0.20 ± 0.05	0.22 ± 0.04	0.29 ± 0.12	0.27	0.42	0.42
A _{Max}	15.9 ± 3.9	15.6 ± 2.6	10.6 ± 3.1	0.9	*	**
Ам	7.7 ± 1.4	7.1 ± 2	3.8 ± 1	0.45	***	**
LAm	445 ± 39	247 ± 45	119 ± 23	***	***	***
K	0.12 ± 0.6	0.19 ± 12	0.23 ± 1	0.36	0.056	0.44
F	3122 ± 774	483 ± 219	115 ± 79	***	***	***
GSI	16 ± 6	12.2 ± 3.6	11.2 ± 5.3	0.72	0.72	0.72
S_A	0.55 ± 0.1	0.59 ± 0.12	0.56 ± 0.1	1	1	1
Sj	0.0003 ±	0.002	0.006 ±	***	**	*
	0.0001	± 0.001	0.005			
PLHC	2.60 ± 1.16	0.70 ± 1.28	-1.48 ± 0.68	**	***	**

Table 1. Analysis of variance (Wilcoxon test) comparing the elasticities and life-history traits of Arctic charr morphotypes from northern regions.

ANDR = anadromous, NR = normal growth resident, DWF = dwarf resident. e_R = reproduction elasticity, e_{Sj} = juvenile survivorship elasticity, e_{sa} = adult survivorship elasticity, A_{Max} = maximum age, A_M = age at maturity, L_{Am} = length at A_M (mm), K = growth completion rate, F = fecundity, GSI = gonad somatic size index (%), S_A = annual adult survival probability, S_J = survival probability from egg to A_M , PLHC = periodic life-history continuum (PC1). P-values were adjusted for 3 pairwise comparisons using the Holm-Bonferroni method. * $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$. Sample sizes for GSI were ANDR = 6, NR = 8, DWF = 6.



Figure 3. Triangular ordination of elasticities ($e_R/e_{Sj}/e_{Sa}$ space) for 41 populations of Arctic charr. Adult survivorship had the highest elasticity for encircled populations toward the top corner of the triangle; juvenile survivorship followed by adult survivorship had the highest elasticity for encircled populations closest to the bottom right corner; and juvenile survivorship followed by reproduction had the highest elasticity for encircled populations closest to the bottom left. ANDR = anadromous, NR = normal resident, DWF = dwarf resident, S = populations located in southern regions where ANDR populations are absent, N = populations located in northern regions where ANDR populations are present.

Elasticities-life-history relationships

Linear regression models for elasticities and life-history traits showed the elasticity of reproduction increased as populations tended toward the less periodic end point of the continuum, meaning the elasticity of overall survivorship decreased (Figure 4, Table 2). Dirichlet regression showed elasticities of reproduction and juvenile survivorship moved in opposite directions (increased and decreased respectively) with movement toward the periodic end-point, whereas populations with traits nearer to the middle of the continuum had lower

values for e_{Sa} than the most and least periodic populations. These patterns resulted in a hockey stick shaped curve for A_{M} , L_{Am} , A_{Max} and the periodic continuum when Dirichlet regressions were plotted in three-dimensional $e_R / e_{Sj} / e_{Sa}$ space (Figure 4). The variable A_{Max} captured the negative correlation that was indicated between juvenile survival and reproduction elasticities but were generally poor in explaining variance in e_{sa} (Figure 4). In contrast, S_A was better at capturing movement on the adult survivorship and reproduction axis but had little influence on e_{Sj} and F captured movement on the adult and juvenile survivorship axis but had little influence on e_R (Figure 4, Table 2). Age at maturity followed by A_{Max} were the best trait predictors of elasticities when considering both regression methods together, with L_{Am} , and F having similar explanatory power (Table 2). A relationship between elasticities and S_J and GSI was not evident (Table 2, Figure 4). The Dirichlet regression for K could not be fitted.



Figure 4. Triangular ordination plots depicting Dirichlet regression relationships for the position of Arctic charr populations in $e_R/e_{SJ}/e_{Sa}$ elasticity space and independent variables (life-history traits and climate temperature). A_{Max} = maximum age; A_M = age at maturity; L_{Am} = length at A_M (mm); K = the growth completion rate; F = average fecundity; GSI = gonad somatic size index (%); S_A = adult annual survival probability; S_J = probability of survival from egg to A_M ; PLHC = periodic life-history continuum; T = mean annual air temperature (°C). Lines show the Dirichlet regression predictions.

	Dirichlet regression ($e_R / e_{Sj} / e_{Sa}$ space)			ce)	Regression (e_R / e_S space)		
Model terms	$Z(e_R)$	$Z(e_{Sj})$	$Z(e_{Sa})$	AIC	$t(e_R)$	R^2	р
A_M	-0.32	1.19	-0.48	-278	-4.34*	0.90	< 0.001
Polynomial	-0.37	-0.65	0.74		1.26		
A _{Max}	-4.45*	-4.27*	-4.76*	-229	-2.32*	0.76	< 0.001
Polynomial	4.37*	4.48*	4.91*		1.54		
L_{Am}	-0.31	4.59*	2.54*	-221	0.84	0.44	< 0.001
Polynomial					-1.3		
K		Model not	fitted		4.66*	0.35	< 0.001
Polynomial							
F	1.82	4.14*	2.60*	-215	0.8	0.34	< 0.01
Polynomial					-1.30		
GSI	-2.04*	-2.73*	-2.86*	-127	1.57	0.09	0.27
Polynomial					-1.44		
S_A	-1.62	0.1	0.90	-199	-2.85*	0.45	< 0.001
Polynomial					1.53		
S_J	1.95	1.19	1.56	-186	1.63*	0.06	0.23
Polynomial					-1.04		
PLHC	0.99	3.93*	2.60*	-245	-13.58*	0.83	< 0.001
Polynomial	2.21*	1.71*	2.5*		4.42*		
Τ	-3.04*	-3.8*	-3.40*	-114	3.7*	0.43	< 0.01

Table 2. Regression analysis for life history trait and climate effects on life-stage elasticities.

 e_R = reproduction elasticity, e_{Sj} = juvenile survivorship elasticity, e_{Sa} = adult survivorship elasticity, A_{Max} = maximum age, A_M = age at maturity, L_{Am} = length at A_M (mm), K = growth completion rate, F = fecundity, GSI = gonad somatic size index (%), S_A = adult survival probability, S_J = survival probability from egg to A_M , PLHC = periodic lifehistory continuum (PC1). N = 42 for all models except for GSI (N = 29) and T (N = 20). AIC values are not comparable between models with differing samples sizes. The Z and t values correspond to the standardized effect of independent variables on elasticities in the Dirichlet and simple linear regressions, respectively. Except for T, all predictor variables were log transformed for continuous variables and arcsine transformed for proportional variables (GSI, S_A , and S_J). Polynomials are 2^{nd} degree. * Z or t significant at the 0.05 level. P-values for linear regressions of e_R and life-history traits are after Holm-Bonferroni correction for 8 tests.

Climate gradient, elasticities, and life-history relationships

Mean annual air temperature (*T*) at the locations of NR populations (N = 20) ranged from -14.6 to 7.8°C. In terms of e_R/e_S space, linear regression showed e_R increased with *T* (Table 2), with the model predicting a 0.01 increase in elasticity for every 1 °C increase in temperature (e_R = 0.14 + 0.01 *T*). Dirichlet regression showed e_{Sj} decreased and e_{Sa} increased with *T*, although a plot of observed and fitted data indicated variation was not well described for populations with

a high adult survivorship elasticity and low adult survivorship elasticity (Figure 4). In agreement with the relationships found between elasticities and life-history, population life-history traits became less periodic as *T* increased (Figure 5). Accordingly, *K*, *S_J* and GSI increased with *T* and *S_A*, *A_M*, *A_{Max}*, and *L_{Am}*, and *F* decreased, with relationships after Holm-Bonferroni correction for 9 tests being significant for *A_M*, *A_{Max}*, *L_{Am}*, and the periodic continuum. *S_A* and GSI were significant before correction (p = 0.007 and p = 0.006, respectively).



Figure 5. Linear regression of life-history traits and mean annual air temperature in °C (*T*) for 20 populations of normal growth lacustrine Arctic charr. A_{Max} = maximum age (R^2 = 0.43, p < 0.05), A_M = age at maturity (R^2 = 0.67, p < 0.001), L_{Am} = length at A_M (mm) (R^2 = 0.44, p = 0.05); K = the growth completion rate (R^2 = 0.14, p = 0.93), F = fecundity (R^2 = 0.03, p = 1); *GSI* = gonad somatic size index (%) (N = 16, R^2 = 0.44, p = 0.057); S_A = adult annual survival probability (R^2 = 0.33, p < 0.06), S_J = probability of survival from egg to A_M (R^2 = 0, p = 1); PLHC = periodic life-history continuum (R^2 = 0.52, p < 0.01). P-values were adjusted for 9 tests using the Holm-Bonferroni correction method.

DISCUSSION

The results show the inherent adaptive link between life-history traits and environment (temperature) and demonstrate that climate temperature could be used to infer the elasticity patterns of populations, in particular the relative importance of reproduction and overall survivorship. The importance of survivorship for the population growth rate increased as

temperature decreased, and this trend was mirrored by movement toward the periodic end-point of the life-history axis. A trend toward a more periodic (or slower) life-history at higher latitude, cold climate regions is frequently detected in fish (Heibo, Magnhagen and Vøllestad, 2005; Blanck and Lamouroux, 2007; Parra *et al.*, 2014) and other vertebrate groups (Tökölyi, Schmidt and Barta, 2014; Cabezas-Cartes, Boretto and Ibarguengoytia, 2018; Rachmansah, Norris and Gibbs, 2020), suggesting the relationships reported here between temperature and elasticities are likely to be replicated in other taxa, both at the intraspecific and interspecific level. In agreement with predictions that fish occupying harsher climates divert more resources toward maintenance than reproduction (Morin, Dodson and Power, 1982; Venne and Magnan, 1989; Blanck and Lamouroux, 2007), adult survival rate and longevity decreased with climate temperature whilst GSI increased (GSI and survival nearly significant after Holm-Bonferroni correction). The results lead to speculation that in a warming climate a less periodic life-history will be selected, resulting in populations whose growth rates become increasingly sensitive to reproduction and less sensitive to survivorship.

Despite significant differences in several life-history traits and position on the periodic continuum there was not a clear dichotomy in elasticity patterns between anadromous populations and their descendant normal growth resident forms located at similar latitudes. The comparative analysis of life-history traits showed divergence between these two morphotypes occurred in traits that had a weak effect on elasticities (body length, fecundity, and survival to maturity), rather than age at maturity and longevity, which had stronger effects. The finding that age at maturity and longevity are similar for anadromous and resident Arctic charr contrasts with a previous comparative analysis by Tallman, Saurette and Thera (1996) who identified both traits were significantly higher in anadromous populations. However, their analysis did not separate dwarf strategists, with dwarfs shown here to have a shorter life-span and earlier age at maturation than other morphotypes.

The average elasticities of dwarfs differed significantly from other morphotypes. However, dwarf populations were widely distributed in elasticity space and belonged in each of the qualitative elasticity ranking groups ($e_{Sj} > e_{Sa}$, $e_{Sa} > e_{Sj}$, and $e_{Sj} > e_R$), meaning dwarf characteristics are not necessarily a good indicator of elasticity patterns. This variability in elasticities among dwarf populations might be explained by contrasting selective pressures between the diverse habitat types they occupied, which included headwater streams (e.g., McCart, 1980), the profundal zone of polymorphic lakes (e.g., Smalas, 2013), the shallow littoral zone of polymorphic lakes (e.g., Jonsson *et al.*, 1988; Jonsson, 2000), and monomorphic

alpine lakes (e.g., Rombough, Barbour and Kerekes, 1978). In contrast, elasticities were strongly conserved among anadromous populations, and thus anadromy appears a good predictor of elasticities in Arctic charr. Conserved elasticities might be explained by a high degree of connectivity between anadromous populations from neighbouring rivers or selective constraints imposed by exploiting the marine environment, both of which may act to supress diversification of life-history traits (Bloom *et al.*, 2013; Moore and Harris, 2014).

Comparisons between resident and anadromous morphotypes may have been improved if PPMs could be constructed for a greater number of populations that are geographically proximate, or even better, sympatric. A large proportion (75%) of the resident populations sampled were from Europe but suitable data for anadromous populations were only available from North America. Environmental conditions of these two continents differ in a number of ways, and some genetic differentiation between Arctic charr of these regions has been identified (Brunner *et al.*, 2001). Both factors could influence life-history traits, and therefore elasticities. Analysis of anadromous populations across a wider geographic range may reveal greater diversity in elasticity patterns.

The results agree with two patterns in elasticities that are persistently observed across taxa. First, the elasticity of reproduction never exceeds the elasticity of overall survivorship (Benton and Grant, 1999), and second, the elasticity of reproduction is lower for populations with a slow life-history (more periodic) compared to those with a faster life-history (Saether and Bakke, 2000; van de Kerk et al., 2013). This relationship between the reproduction elasticity and the slow-fast continuum is preserved despite a fundamental difference in the life-history of fish compared with other vertebrate groups. In birds and mammals, fecundity generally decreases with movement toward the slow end point of the continuum (Saether and Bakke, 2000; Dobson and Oli, 2007), whereas the opposite trend is found for ray-finned fish (Winemiller and Rose, 1992; Beukhof et al., 2019). As a consequence, the elasticity of reproduction is positively correlated with fecundity in mammals and birds but negatively correlated in ray-finned fish (Saether and Bakke, 2000; Oli and Dobson, 2003; Stahl and Oli, 2006; Vélez-Espino, Fox and McLaughlin, 2006). Others studying elasticities of ray-finned fish have commented that this negative correlation is somewhat surprising (Vélez-Espino, Fox and McLaughlin, 2006): because elasticities can be interpreted as the strength of selection on fitness components (Stearns, 1992), we might expect that fecundity increases in populations with a high reproduction elasticity in the same way that survival rate and longevity increased in Arctic charr populations with a high survival elasticity. However, fecundity does not

necessarily represent the proportion of the energy budget that is directed toward reproduction, which in fish can be measured using the gonad somatic size index (Vøllestad and L'Abée-Lund, 1994). However, a relationship between GSI and elasticities was not evident in this study (Figure 4, Table 2). Information additional to GSI may be required to quantify reproductive effort of Arctic charr. An important parameter is likely to be variation between populations in the number of rest years between breeding, but this can only be determined accurately using mark-recapture methods (Grainger, 1953; McCart, 1980).

The age at maturity was the life-history trait that explained the most variation in elasticities, and this has generally been the conclusion of studies that have investigated life-stage elasticities independently using traditional regression or correlation analysis (Saether and Bakke, 2000; Oli and Dobson, 2003; Frisk, Miller and Dulvy, 2005; Wang et al., 2017). Dirichlet regression showed a curved relationship in $e_R/e_{Si}/e_{As}$ space: moderately late-maturing populations had low adult survivorship elasticity, high juvenile survivorship elasticity, and low reproduction elasticity; middling maturing populations had more similar values for the two survivorship elasticities and a higher reproduction elasticity; and the earliest maturing populations had opposite patterns in survivorship elasticities and the highest values for the reproduction elasticity. However, in extreme late-maturing populations the trend toward higher juvenile survivorship elasticity and lower reproduction elasticity flattened and slowed, respectively, balanced by a slight increase in adult survivorship elasticity, causing a tick at the end of the curved relationship for age at maturity and $e_R/e_{Sj}/e_{As}$ space (Figure 4). This trend toward greater importance of adult survival in the latest maturing populations suggests a transition in lifehistory tactic. Late-maturing populations, which were often associated with cold climates, may require multiple rest years between breeding due to limited resource availability (Power, Reist and Dempson, 2008), which presumably would increase selection on surviving as an adult. Fecundity, length at maturity, and maximum age provided reasonable explanations for variation in the elasticities of populations with a high juvenile survivorship elasticity but did not capture the transition toward a high adult survivorship elasticity. A greater amount of variation might be explained by developing models which include multiple life-history traits.

Intraspecific variation and implications for management

Elasticities are expected to be similar within species and among closely-related species due to shared evolutionary histories (Oli and Dobson, 2003). However, previous authors have acknowledged that using a closely-related species or a single population from a species to infer
elasticities of data limited populations might not be appropriate when a species exhibits variable life-histories (Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006). This was demonstrated by the range in elasticity values found for Arctic charr ($e_{Sj} = 0.54$, $e_{As} = 0.42$ and $e_R = 0.27$), which were greater or equal to the ranges measured across 10 species of Salmonidae from 5 genera ($e_{Sj} = 0.17$, $e_{Sa} = 0.4$ and $e_R = 0.19$) studied by Vélez-Espino, Fox and McLaughlin (2006). Moreover, these salmonid species were distributed across just two elasticity ranking group (qualitative elasticities) compared to three ranking groups for Arctic charr. Thus, it is indicated that intraspecific variation in elasticities can equal or perhaps exceed that found within families; further investigations are needed to understand if such variability is unique to Arctic charr.

Elasticities are commonly applied at the outset of conservation planning to prioritise life-cycle stages for research and management interventions (Heppell, 1998; Benton and Grant, 1999; Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; van de Kerk *et al.*, 2013). By assessing intraspecific differences in elasticities, I show important consideration needs to be given to the phenotypic life-history state being managed. In particular, earlier maturation in dwarfs and temperate normal growth morphotypes compared with anadromous and high latitude normal growth morphotypes was associated with large differences in elasticities, suggesting population dynamic responses to natural disturbances and management interventions that affect particular life-stages may vary substantially among populations.

Chapter 7. General discussion: Implications for the conservation and management of Llyn Padarn Arctic charr

The programme of research has generated valuable information pertinent to the conservation and management of Arctic charr, a locally threatened species of substantial ecological and socio-economic importance. Population dynamics are fundamental to conservation, with the ultimate goal of management interventions being to reverse or prevent population declines and ensure long-term population viability. To achieve this goal, managers require a thorough understanding of the species habitat requirements, demographic processes, and the efficacy of different management strategies. To this end, Arctic charr at Llyn Padarn have provided an excellent case study due to the range of threats faced by the population, aspects of which extend beyond the target population; the variety of management interventions that have been implemented; and the frequency of scientific and longitudinal monitoring studies relative to most other populations. In this discussion, I consider specifically the implications of the findings for the conservation and management of Llyn Padarn Arctic charr, but there are lessons that are relevant across other populations and species.

Status of Llyn Padarn Arctic charr

Analysis in Chapter 4 showed Arctic charr at Llyn Padarn have recovered in recent years, with population size perhaps returning to levels last measured in the late 1990s, but still indicated to be below the threshold for favourable conservation status according to hydro-acoustic density criteria suggested by Bean (2003). However, the research has provided little evidence that the population could be self-sustaining without continued supplementation. The most recent fyke netting data from 2019 show ~ 70 % of breeding adults are now of stocked origin and the density of juvenile fish in the lake was strongly correlated with the number of fish being stocked in each year, indicating natural recruitment is low. Deep-water oxygen conditions are of poor quality relative to other lakes in the British Isles and disturbances at the Afon y Bala spawning site probably restrict the size of the spawning area and cause high egg and alevin mortality. On the positive side, it was demonstrated that Arctic charr spawn at second location (Penllyn), and that the site is probably more suitable for survival of early life-stages than the Afon y Bala. The CPUE for recent fyke net surveys suggested a possible increase in the number of wild born adults, but there remains considerable uncertainty in these data. As of 2021, the supplementation programme has been paused (pers. comms. Huw Jones, NRW, 2021), and

therefore improving habitat will be vital to facilitate a viable population, especially since the population appears particularly exposed to predicted global climate warming. A 'do nothing' strategy will likely result in population decline, and almost certainly end the ambitions of stakeholders to establish a sustainable recreational fishery.

Improving spawning habitat

Improving the spawning habitat at the Afon y Bala will need a comprehensive understanding of local hydromorphology and the habitat requirements of the population, the latter of which has been greatly improved through the research presented in Chapter 3. A hydromorphology assessment commissioned by NRW identified the deep canalised channel of the Afon Hwch causes high flow velocities during spate events and prevents retention of suitable substrate at the spawning site downstream (Mould and Todd-Burley, 2015). The report recommended the channel depth be lowered to match the depth of the spawning site, anticipating this would (a) reduce flow velocity and gravel mobilisation during spate events and (b) increase the size of suitable spawning habitat (implemented in autumn 2021). However, the study overlooked important literature on Arctic charr spawning habitat and, whilst models aimed to predict habitat suitability under different management scenarios, the definition of good and bad habitat was not made clear. It is recommended that further modelling is undertaken which should aim to identify the necessary conditions to create a spawning area with similar characteristics to those measured at the newly discovered Penllyn site, where fry production was indicated to be good. Accordingly, the geometric mean particle size should be ~ 55 mm, median velocity under normal flow conditions ~ 0.1 ms⁻¹, and depth in the range of 0.5 - 0.8 m (Table 3, Chapter 3). Clearly, flow conditions during spates must be reduced to a level which will not cause substrate scouring and consequent washout of eggs and alevins. At the outset it may be necessary to manually remove and add substrate because particle size recently measured at the spawning site is smaller than desired (Table 3, Chapter 3). To ensure ongoing substrate modifications are not required it will be essential to prevent the continued deposition of fine gravel observed in this study (Figure 8, Chapter 3). Increasing the size of the spawning area in the Afon y Bala would of course be desirable. Opportunities for expansion should focus upstream of the area of highest egg density (Figure 4, Chapter 3), since downstream, eggs will be exposed to high flow velocities and temperature spikes when the bascule gates are opened (Appendix 2). Spawning may already occur upstream, as evidenced by a small number of eggs observed by snorkelling.

The discovery of new and apparently productive spawning site (Penllyn) is an important conservation development and implies the population is more resilient to disturbances in the Afon y Bala than assumed previously. However, it is not known if the site is new for Padarn Arctic charr or just new to science. The difference is potentially significant: if the site has long been utilised then declines prior to supplementation occurred despite the population spawning at Penllyn, whereas if the site has only recently been utilised then this indicates the population is more resilient than before. Irrespective, it is important the site is protected and, if possible, enhanced.

The only obvious localised anthropogenic disturbances at the Penllyn site are swimmers, paddle sports, and dogs which may disturb eggs when entering the water. These disturbances may also affect the Afon y Bala. Managers could choose to control access to spawning areas during the egg incubation and alevin period (November to April). Because the Penllyn site is located above the lake outflow and has a substrate of cobbles with deep interstitial spaces, extreme flow velocities causing significant washout of eggs and alevins are unlikely. The consistent and steady water flow should ensure high oxygen concentrations of the interstitial water and prevent settlement of fine sediment. Algal growth (likely *Cladophora* spp.) was substantial at some of the areas sampled and was negatively correlated with the number of fry captured. Proliferation of algae can increase sedimentation by reducing flow velocity (Potter and Dare, 2003). Algae should be monitored and, whilst taking account of other ecological considerations, controlled if abundance increases. Algal growth is likely to be encouraged by eutrophication and therefore continued management of diffuse and point sources of nutrients (e.g., the wastewater treatment works) is important. Finally, slate spoil was present adjacent to the area where fry emerged. Removing this spoil could increase the size of spawning habitat if it is covering more suitable cobble substrate.

Fixing deep-water hypoxia

Elevated concentrations of eutrophying nutrients, in particular phosphates, are generally a primary cause of deoxygenation in the hypolimnion (Molot *et al.*, 1992; Jacobson, Stefan and Pereira, 2010), as was demonstrated in the spatial analysis of stratified lakes in the British Isles and temporal analysis of Llyn Tegid and Llyn Cwellyn (Chapter 2). However, the link between phosphate concentrations and hypolimnetic oxygen was not evident for Llyn Padarn. Oxygen concentrations were the lowest of the 31 lakes sampled with cold-adapted fish, and in several years, including recently, have approached the 3 mg/l level considered lethal for salmonids

(Davis, 1975). Moreover, the analysis only considered the top two thirds of the lake; examination of DO in the deepest regions showed anoxia (< 2 mg/l) is common. Yet compared to other Arctic charr lakes in Wales and elsewhere in the British Isles, WFD data showed spring phosphate concentrations are reasonable and have not changed significantly since the start of the data series in 2007 (Table 3 and 4, Chapter 2), despite substantial upgrades to the waste water treatment works. Unlike Llyn Cwellyn and Llyn Tegid, linear regression did not detect a response of dissolved oxygen to inter-annual variation in TP (Table 5, Chapter 2). Therefore, assuming water quality monitoring data provide an accurate and complete picture of TP in the lake, it must be considered that other factors are driving hypoxia and that further efforts to reduce TP inputs are unlikely to raise oxygen concentrations to the levels observed at similar lakes. The possible importance of nitrates was discussed in Chapter 2, but another explanation could be high sediment oxygen demand (SOD), perhaps driven by current or past deposition of organic sewage sediments (Miskewitz and Uchrin, 2013). DOC and stratification strength increased over the time-series, although not quiet significantly (Chapter 2, Table 4), which may have counteracted any positive effect of improvements to waste water treatment. It could be that recent TP inputs from the sewage treatment works have been reduced but legacy phosphates in lake sediments are slowly being released (Edlund et al., 2017), or that other external sources of TP (e.g., agriculture) have increased. It is also possible that lower oxygen relative to other lakes is, to some degree, a natural phenomenon caused by characteristics of basin morphometry (Molot et al., 1992; Deeds et al., 2021), and that these characteristics make Llyn Padarn particularly vulnerable to nutrient enrichment and associated deoxygenation. However, sediment profile analysis commissioned by NRW show clearly that eutrophication has increased in recent centuries, and in particular, after operations at the Llanberis sewage works commenced in the 1950s (Bennion et al., 2010). In the absence of depth profiles and nutrient data from before anthropogenic impacts, it will require advanced modelling and further data collection to estimate what natural conditions for the lake should be, and which stressors are driving deoxygenation. Unfortunately, the automated monitoring buoy, which collects depth profile data as well as physico-chemical parameters, has been beset by technical difficulties since deployment in 2011 and has only recently began generating frequent temporal data that will be required for further studies. It is recommended that SOD and legacy phosphorus are measured and included in any future investigations. The case study highlights the difficulty of understanding and fixing hypoxia in degraded lakes and emphasises the need to proactively address the early signs of deterioration detected at nearby Llyn Cwellyn and Llyn Tegid.

Vulnerability to climate warming

One prediction of climate change is that deep-water thermal-refugia habitat will be diminished (Jacobson, Stefan and Pereira, 2010; Jane et al., 2021). The Llyn Padarn population appears particularly vulnerable. First, lake volume and thermal-refugia habitat below the thermocline are only of moderate size (hypolimnion ~ half the depth of Llyn Tegid and Llyn Cwellyn, Chapter 2, Table 3). Second, summer hypoxia in the hypolimnion is likely to force Arctic charr higher up the water column where temperatures are warm. Third, surface air temperature is the warmest of the 31 lakes in the British Isles studied and 1.28 and 2 °C warmer than nearby Llyn Cwellyn and Llyn Bodlyn, respectively (Chapter 2, Table 3). However, the time-series analysis of lakes in Wales did not reveal any significant change to either hypolimnetic temperature or volume (Chapter 2, Table 5). Given the complexity of climate and lake systems, a longer timeseries is probably required to detect the impacts of climate warming on deep-water thermal habitat (e.g., Richardson et al., 2017). It should also be noted that deep-water thermal conditions at Llyn Padarn are considerably cooler than the majority of Arctic charr lakes in Ireland (Chapter 2, Table 3), some of which are known to have healthy populations (e.g., Lough Shindilla, see Kelly et al., (2014)). The comparison suggests the population at Llyn Padarn may persist even if temperatures in the hypolimnion were to increase by as much as 3 or 4 °C, although the warmer Irish lakes have far higher oxygen concentrations and may be subject to fewer additional stressors. It is also possible that Irish Arctic charr are in some way adapted to warmer temperatures (Zillig et al., 2021).

Other pressures

Several threats to Llyn Padarn Arctic charr were not investigated directly in the thesis. Perch have recently arrived at the lake and are potential competitors of charr and predators of juveniles (Morrissey-McCaffrey *et al.*, 2019). Perch trapping surveys undertaken in 2019 suggest the population may be small (Appendix 6), but further studies are necessary. Given the high sensitivity of Arctic charr population growth to juvenile survivorship and possible predation by perch on this life-stage, cropping (i.e., removal) of perch would appear a worthwhile and relatively simple intervention that would also provide information on population status. Angling activity for Arctic charr is not well recorded, and therefore the impact on the population is uncertain. It is strongly recommended that anglers are required to officially report their catch directly to local managers, including information on fish length and number of fishing hours (to calculate catch per unit effort). Length information is essential to

identify the size range of Arctic charr vulnerable to angling tactics. Sensitivity-elasticity analysis indicated the removal of large fish (> 287mm) will have a limited effect on population dynamics (Chapter 5). A minimum size limit for fish taken by anglers could be implemented, as is the case at Windermere where fish < 20 cm must be returned (Winfield, Berry and Iddon, 2019). However, sensitivity-elasticity analysis indicated it would be prudent and worthwhile to raise this size limit, particularly whilst the population status remains unfavourable. Finally, the impact of fish-eating birds on Arctic charr at Llyn Padarn remains unknown.

Sensitivities-elasticities analysis

Resources for conservation are never unlimited, meaning managers wish to know which out of range of possible interventions will be most effective in terms of improving population viability, which can be measured by the population growth rate (λ). Sensitivities and elasticities analysis are a popular and intuitively appealing quantitative approach to inform such decisions: if the survival of a life stage has a large effect on λ relative to other stages then it would seem most worthwhile for managers to target that life-stage (Heppell, Caswell and Crowder, 2000; Vélez-Espino, Fox and McLaughlin, 2006; Manlik, Lacy and Sherwin, 2018). Accordingly, elasticities analysis suggested managers at Llyn Padarn should assign highest priority to interventions improving juvenile survivorship, followed by young adults, fecundity, nest and fry survival equal third, and lastly adults > 287 mm in length. This order was replicated for sensitivities analysis except that fry survival was the most important parameter and fecundity the least (Chapter 5, Table 2).

However, when interpreting sensitivities and elasticities for practical management there are several caveats that must be considered. First, is it feasible to alter a vital rate identified as most important for λ . Traits may have a high sensitivity or elasticity but, in practice, can be tightly constrained (Benton and Grant, 1999; De Kroon, Van Groenendael and Ehrlen, 2000; Manlik, Lacy and Sherwin, 2018). For example, what if juvenile survival, which had the greatest effect on λ at Llyn Padarn, is already close to its natural maximum, whereas egg survival, which was less important for λ , is well below its natural maximum due to anthropogenic disturbance. Clearly, in this case, attempting to increase juvenile survival will be futile and addressing the cause of low egg survivorship will be more worthwhile (Hiraldo *et al.*, 1996; Beissinger and Peery, 2007). However, in the above scenario, it remains true that protecting juvenile survival should be given high priority. It is also possible that juvenile survival could be increased above its natural maximum by supplementing juveniles with captive-reared individuals, as has been

implemented at Llyn Padarn. For some vital rates, notably fecundity and growth, it is difficult to see how management can directly influence these parameters. A feasibility assessment should also consider the difficulty and cost of various management interventions (Manlik, Lacy and Sherwin, 2018). For example, improving the physical characteristics of salmonid spawning habitat may be achieved by simple and relatively cheap interventions (Hendry et al., 2003), whereas improving the oxygen conditions of the lake habitat utilised by juveniles has proven costly, and thus far, ineffective. Finally, summed stages assume the vital rates of individuals in a demographic group are equally affected by disturbances and management interventions, which may not be the case. For example, small juveniles may be vulnerable to predation by a non-native predator, such as perch, but not vulnerable to angling, whereas the opposite may apply to larger juveniles. Conversely, a single management intervention may improve vital rates spanning multiple summed stages. For example, an intervention that improves habitat in the pelagic zone potentially increases the vital rates of all fish that use this habitat, which may include each of the summed stages other than eggs and alevins. As such, interpretation and summing of sensitivities-elasticities may need to be reassessed as more information on lifestage ecology, disturbances, and management interventions is acquired. The stage-specific elasticities and sensitivities presented in Chapter 5 (Figure 3) will be useful in this regard.

In summary, when making a choice as to which vital rate to target by management, it is crucial to understand both the importance of vital rates for λ and also their potential for manipulation in the field (Manlik, Lacy and Sherwin, 2018). Ultimately, this could mean conservation measures focus on parameters with the smaller, rather than the larger, influence on λ (De Kroon, Van Groenendael and Ehrlen, 2000). In this case, sensitivities and elasticities inform managers and the research community that further work is required to develop conservation measures that target the more important life-stage. The comparative analysis of elasticities showed Arctic charr populations are generally most sensitive to juvenile survivorship, yet we know very little about the ecology and vulnerabilities of juvenile Arctic charr. As outlined above, controlling perch and the ensuring juveniles are not taken by anglers are interventions that might be worthwhile, but without measuring the reduction in juvenile survival caused by perch predation or angling it is not possible to quantitatively estimate the effect on λ of managing these pressures.

At the outset of the research, it was recognised that there was considerable uncertainty regarding life-table information for Llyn Padarn Arctic charr, and therefore it was important to investigate models with different parameter values. Life-stage simulation analysis in Chapter

5 showed variation in survival, growth rate and fecundity estimates generally had limited effect on the outcomes of sensitivities-elasticities analysis and inferences for management. However, the comparative analysis of life-history phenotypes showed differences in age at maturity could substantially change quantitative and qualitative elasticities (Chapter 6). Therefore, if recent increases in the size of breeding age fish at Llyn Padarn reflects a change in age at maturity compared to when last measured in the 1970s then it is possible that elasticities and sensitivities calculated using the historic data (Chapter 5) will not be the same for the contemporary population. A strategy of growing fast and maturing early (i.e., a less periodic strategy) may have been adopted, which according to analysis in Chapter 6 would suggest greater sensitivity to fecundity and adult survival (Table 1 and Figure 4, Chapter 6). Alternatively, if the contemporary Llyn Padarn population reaches maturity at a later age than in the 1970s then juvenile survival is likely to be even more important for population growth than calculated in Chapter 5. This being said, analysis in Chapter 6 indicated age at maturity is selected by climate (Figure 5), suggesting plastic changes may be constrained at a local scale. It is possible that Arctic charr at Llyn Padarn are attaining a greater size due to low population density or ecological changes at the lake (Johnston and Post, 2009; Grossman and Simon, 2020), and that age at maturity is the same (or similar) as when last measured in the 1970s. In this scenario elasticities and sensitivities calculated from the historic life-history data should be appropriate for guiding management of the contemporary population. Moreover, increased length at maturity was generally associated with high sensitivity to juvenile survivorship and low sensitivity to reproduction and adult survivorship (Table 1 and Figure 4, Chapter 6). To conclude, there is a high degree of confidence that juvenile survivorship is more important for the population growth rate of Padarn charr than adult survivorship and reproduction.

Population monitoring and data limitations

Continued monitoring of the population will be essential to evaluate the efficacy of future management interventions and the consequences of ending supplementation. Whilst Arctic charr at Llyn Padarn have been monitored more intensively than most other populations in the British Isles, some of the survey methodologies could be improved and missed opportunities for data collection seized. Fyke netting data provide the longest time series but there has been variation in the duration of surveys and number and location of nets deployed. Because spawning occurs over several weeks but the peak spawning period is likely to vary between years dependent on environmental conditions (Frost, 1965; Austin, Essington and Quinn, 2019), differences in the timing and duration of surveys is perhaps most problematic. It would

preferable to set nets throughout the spawning period at the same times in each year, for example, sampling on at least one night in each week starting end of November to the third week of December. By adopting this approach, the week of peak spawning could be confidently identified and CPUE compared directly between years. Furthermore, environmental relationships with temporal patterns in spawning intensity could be modelled to give insights into how parameters such as temperature and flow velocity effect spawning phenology and variation in CPUE estimates between years. Setting the same number of nets each year and in the same location would remove concerns regarding the potential non-linear relationship between CPUE and fishing effort discussed in Chapter 4. Enumerating and recording the catch in each net, rather than a total, would allow calculation of a standard deviation and generate useful information on how catch varies dependent on net location. Finally, it is important that monitoring is undertaken at both spawning sites to provide a complete picture of population dynamics and information on the relative suitability of each site for offspring survival (assuming fish have a natal homing instinct). Regarding missed opportunities for data collection, NRW report that fish have sometimes perished in the nets, although not during the duration of the present research. In future any dead fish should be stored so that age and growth can be assessed later. It may also be possible to acquire otoliths from fish taken by anglers by agreement to store frozen heads. If supplementation is reinstated then striped eggs from each fish could be enumerated in the field (gravimetrically or by image analysis, see Appendix 2) to update information on fecundity and to compare fecundity of stocked and wild fish. Of course, methodologies must be balanced against the desire to minimise stress to captured fish and disturbance of spawning activities and eggs. The environment at the lake and river also presents a number of challenges (in particular high discharge and gravel movements in the Afon y Bala) which have in some years prevented surveys from being executed as planned. Coronavirus restrictions and failure of hydro-acoustic equipment have prevented surveys in the last two years: it is important that an assessment of current population status is undertaken at the next opportunity.

Non-invasive methods are often preferable for sampling threatened populations but do have limitations. Hydro-acoustic surveying is an established method and continuation of the programme would be valuable because of the reasonably long time series. However, there will always be uncertainty regarding the apportionment of echoes to species, although the correlation between the numbers of fry stocked each year and estimates of fish density does provide some assurance that hydro-acoustic data for Llyn Padarn are a good representation of trends in Arctic charr abundance (Chapter 4). Confidence could be further established by assessing correlations with results of direct sampling methods, such as angling CPUE or fyke net CPUE. The latter was not undertaken here because there have been a limited number of years when both hydro-acoustic and fyke netting were undertaken, and because the fyke netting data has its own limitations, as has been discussed. Another non-invasive method that could be adopted is quantitative eDNA sampling which can provide an accurate proxy for population size of lake dwelling salmonids (Lacoursière-Roussel *et al.*, 2016). Some small scale eDNA sampling for Arctic charr has been undertaken at Llyn Padarn since 2019 and these data could provide a baseline for future monitoring.

Non-destructive methods of assessing spawning success will be necessary to evaluate habitat remediation. Investigations in Chapter 3 identified two possible technique that could be implemented: (1) remote egg counting using camera images of the substrate; and (2) emergent fry traps. Camera surveys are useful for identifying the location and extent of spawning sites and may also provide a semi-quantitative estimate of egg density. A key disadvantage regarding quantitative measurements is that eggs are not visible if they have fallen into the interstitial spaces or are buried. Surveying shortly after spawning may mitigate this limitation. An alternative to camera images is counting eggs whilst snorkelling, which has been successfully employed at littoral spawning sites (Low et al., 2011; Igoe and Ruane, 2012). However, the method was found to be impossible at the Llyn Padarn sites because of the flowing water. SCUBA equipment would allow greater control whilst swimming and has been utilised at Llyn Padarn in the past (Milner, 1985; Thomas and Holt, 2011). Cost and health and safety restrictions prevented the use of SCUBA methods here. The emergent fry traps proved to be an effective, reliable, and time-efficient method for monitoring fry recruitment at shallow littoral sites, such as Penllyn, but at the Afon y Bala surveys were challenged by the depth of water and burial of traps during flood events. The issue of depth could be overcome by using a boat to deploy and empty traps. Ideally, emergent fry surveys should be preceded by an assessment of spawning activity (e.g., number of eggs or breeding fish). Without such information it cannot be distinguished whether variation in the numbers of emergent fry is caused by variation in the survivorship of early life-stages or the number of spawning adults. The density of emergent fry measured at Penllyn in 2020 is perhaps a baseline for good spawning success (Chapter 3, Table 4), but further monitoring at Llyn Padarn and other lakes is necessary to support this.

Collection of tissue samples (i.e., fin clips) is relatively straightforward and should be continued to assess future changes in genetic diversity and effective population size now that the supplementation programme has ended. However, the laboratory methodologies used here and previously developed by S. Beck (Beck, 2014) should be reviewed due to the difficulty in accurately scoring several of the markers. These difficulties were probably caused by the multiplex design (concentration or combinations of primers) or sub-optimal PCR conditions (Dewoody, Nason and Hipkins, 2006). Alternative multiplexes for Arctic charr are presented in recent work by Doenz *et al.*, (2019) and Layton *et al.*, (2020). Changing the laboratory methods may reduce comparability between data sets but tissue samples and extracted DNA from 2018 – 2020 have been archived and could be reanalysed.

Conclusion

Arctic charr at Llyn Padarn remain highly threatened by spawning habitat disturbance, deepwater hypoxia, and climate warming, although the discovery of a new spawning site potentially indicates greater resilience than has been previously assumed. Supplemental breeding has been successful in rescuing the population from seemingly imminent extinction, but now that the programme has ended, it is likely the population will once again decline without substantial improvements to habitat. Managers should closely monitor the population and habitat at the lake and be prepared to reinstate supplementation. The priorities for conservation and research at Llyn Padarn should be the factors driving deep-water hypoxia, remediation of spawning habitat, and interventions that protect or increase the survival of fry and juveniles, which could include controlling the perch population and protection of juveniles from angling activities.

Finally, regarding future research more generally, there has been a dearth of long-term monitoring campaigns for Arctic charr populations. Of those that have been undertaken, sampling methods (e.g., hydro-acoustics) have significant limitations and do not target all stages of the life-cycle (Winfield *et al.*, 2010). Long-term monitoring studies across the life-cycle, which are common for many other salmonid species, are necessary to detect and assess the effects of environmental change and disturbances (e.g., TDO7, flooding, angling, perch abundance) on population dynamics, as well as important demographic processes, such as density dependent compensation, which will be necessary to develop predictive models (Elliott, 1993; Jensen and Johnsen, 1999; Wilson, 2003; Gregory *et al.*, 2020). As indicated by sensitivities analysis in the present work, an understanding of juvenile survival will be vital for making accurate predictions of population trajectories. Expanding research of Arctic charr

population dynamics and juvenile survival will require new sampling techniques are developed, since many of the methods used for salmonids that migrate into and out of rivers throughout their life-cycle (e.g., redd counting, electric fishing, and fish counters) are unsuitable when a species does not dig visible redds and mostly occupies the pelagic zone of lakes, as is the case for the majority of Arctic charr populations in the British Isles. Here, I have demonstrated the suitability of swim-up fry traps for sampling Arctic charr at the start of life. The next technical challenge is to identify how emergent fry can be tracked over their life-span. There is enormous enthusiasm amongst the academic research community regarding the evolutionary significance of polymorphism in Arctic charr (Jonsson and Jonsson, 2001; Doenz *et al.*, 2019). Such studies should of course continue, and are relevant to conservation (Bush and Adams, 2007; Mee *et al.*, 2015, Chapter 6), but researchers should expand their work, through collaboration with monitoring agencies, to investigate population dynamics and demographic processes. In acquiring this knowledge, we can increase the probability that these fascinating, charismatic, and valuable fish will persist across space and time.

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Appendix 1: Correlation analysis of indpendent variables used to model thermal oxygen habitat (Chapter 1)



Figure 1. Spearman's rank correlation for predictor variables used in multivariate GAMs for TDO7 in weakly stratified lakes. TP = total phosphorous in spring (μ g/l); DOC = dissolved organic carbon (mg/l); depth = maximum depth (m); TA = average air temperature (°C).



Figure 2. Spearman's rank correlation for predictor variables used in multivariate GAMs for TDO7 in stratified lakes. TP = total phosphorous in spring ($\mu g/l$); DOC = dissolved organic carbon (mg/l); depth = maximum depth (m); TA = average air temperature (°C).

Appendix 2: Impact of thermal pollution from power station discharge on early-life stage survivorship of Llyn Padarn Arctic charr

INTRODUCTION

It is generally recognised that increases in water temperature reduce the survivorship of Arctic charr (*Salvelinus alpinus*) eggs, with multiple studies concurring that mortality increases abruptly at temperatures > 8.5 °C (Kelly *et al.*, 2020). However, often the focus has been on changes to average temperature over the incubation period, rather than the effect of short duration extremes that may occur because of weather anomalies or localised thermal pollution from anthropogenic sources. This study utilised *in situ* egg incubation chambers to test the hypotheses that thermal pollution caused by discharges from the Dinorwig pumped storage hydro-electric power station (hereafter, power station) reduce survivorship of early-life stages (eggs and alevins). The experiment design was compromised by several extreme flood events which resulted in the burial of apparatus and substantial sedimentation of incubation chambers at the Control treatment site. Nether the less, the study provides useful information on hatching times at the Afon y Bala and the temperature regime in the river.

METHODS

Egg incubation chambers (Figure 1) were made from 15 x 20 cm zip close nylon mesh bags with a 1mm mesh aperture. Each bag was half filled with ~500 g of washed pebbles of between 20 and 50 mm diameter. A cylindrical piece of plastic mesh was then inserted inside the bag to prevent the top from collapsing. Bags were distributed evenly between six heavy duty perforated plastic crates (16 bags per crate) that had been partially filled with pebbles for ballast (Figure 1). Three of each crate were positioned at 2 m distance from one another in a line perpendicular to the river current at two experimental sites (Figure 2). Eggs at site 1 experienced ambient river temperatures and eggs at site 2 were exposed to warmer water discharged from the bascule gates of the power station. The precise location of the sites was chosen because they were at depth suitable for wading, had a flat substrate, and were easily accessible form the bank. A TinyTag temperature logger programmed to take measurements at 1 hour intervals was attached to one crate at each site. Average flow velocity measured at the substrate using a Geopacks digital flow meter on the 24th August 2020 was 0.19 m/s at site 1

and 0.15 m/s at site 2. Depths measured on January 20^{th} 2020 were 56 cm and 75 cm at site 1 and 2 respectively.



Figure 1. Apparatus for egg survivorship experiments: (a) perforated plastic crate for holding egg incubation bags (note, the incubation bags shown are larger than those used in the final experiment); (b) Eggs photographed on petri dish for counting; (c) eggs seeded into an incubation bag; (d) a crate excavated from the gravel at site 1 after flooding.



Figure 2. Location of experimental sites for egg survivorship experiments. Site 1: ambient river water temperature; Site 2: eggs exposed to warmer water discharged from the bascule gates of the power station.

Eggs were acquired from Arctic charr captured in fyke nets set overnight in the Afon Hwch on December 5th 2019. Ova were striped in the field from 17 females into 4 separate pots, each of which were fertilised with gametes from ~ 4 males. The procedure was undertaken by NRW (Natural Resources Wales) for their artificial breeding and stocking programme that has been ongoing since 2009. A ¹/₂ teaspoon was used to distribute eggs evenly across incubation bags. Each spoon of eggs was first photographed on a petri dish (Figure 1) to allow precise counting later of the number of eggs added to individual bags (mean eggs bag⁻¹ = 33.54, SD = 4.53). Any dead eggs (i.e. eggs with a creamy appearance) were removed. Seeded incubation bags were kept submerged in a container of natural river water until deployment (Figure 1). The time elapsed between fertilisation and deployment at the experimental sites was ~ 4 hrs.

Survivorship of eggs was assessed at 50, 63, and 75 days after fertilisation. Unfortunately, one crate was lost from site 2 during severe flooding in the first week of the experiment. Thus, survivorship at day 53 was only measured at site 1 and from a single crate. On sampling occasions thereafter, 8 bags were removed in a stratified manner (i.e. every other bag) from each of the two crates remaining at both sites. On day 63 one extra bag was removed in error

from site 1 and on day 75 three bags from site 1 and two bags from site 2 were found to be damaged and therefore not assessed for survivorship. To ensure consistent flow conditions in the crates over the duration of the experiment, the bags removed on day 63 were refilled with their substrate and returned to the crates. Surviving eggs and alevin were released into open incubation bags at a littoral site in Llyn Padarn.

In addition to the loss of one crate at site 2, flooding on day 5 of the experiment caused the near complete burial under gravel of the crates at site 1 (Figure 1). Once it became possible to access the river (9 days after burial) the crates were excavated and the excess gravel emptied. A second issue encountered was infilling of the incubation bags with fine sediment, especially in those bags that were buried at site 1. Accordingly, burial and sedimentation are likely to have confounded comparisons of survivorship between ambient and discharge treatments.

RESULTS

Data retrieved from TinyTag loggers (Figure 3) showed the average temperature experienced by eggs exposed to discharges (site 2) was significantly higher than eggs at site 1, the ambient location (site 2: $\mu = 6.55$ °C, SD = 0.96, max = 9.55; site 1: $\mu = 6.36$ °C, SD = 0.89, max = 8.82; t-test: N = 1800, *p* < 0.001). The temperature at site 2 was greater than site 1 on 1062 of the 1800 hours logged, and exceeded the 8.5 °C threshold considered critical for Arctic charr egg survival on 67 occasions, compared with only 4 occasions at site 1. Temperature peaks above 8.5 °C at site 2 occurred on 14 separate days and ranged in duration from 1-6 hrs ($\mu =$ 3.71, SD = 1.9), with each of these events occurring between December 6th to January 11th (prior to hatching, see below). The most extreme case was December 9th, when temperatures exceeded 9 °C for 6 hrs running. The highest temperature difference between sites was 3.39 °C, recorded on February 11th. Similar differences in temperature were measured in mid-December (Figure 4).



Figure 3. River temperature recorded at 1 hour intervals between 6th December 2019 and 18th February 2020 at two sites in Afon y Bala and Afon Hwch where egg survivorship was assessed. Site 1 eggs experienced ambient river temperatures and site 2 eggs were located downstream of discharges from the power station.

The survivorship of eggs at the ambient site was highest on day 50 (N = 16, M = 90.1%, CI = 70.9 - 98.9 %) and decreased on each sampling occasion thereafter (day 63: N = 17, M = 63.3%, CI = 13.5 - 94.7 %; day 75: N = 12, M = 8.1%, CI = 0 - 49.1 %). Thus, survival rate declined steeply between day 50 and 73 (the period of hatching) compared to between day 0 and 50. Comparing survival between the two sites (Figure 5), survivorship on day 63 was lowest at the discharge site (N = 16, M = 49.4%, CI = 2.9 - 82.4 %) but highest here on day 75 (N = 14, M = 33.3%, CI = 1.8 - 62.6 %). However, Wilcoxon tests showed that differences in egg survivorship between ambient and discharge sites were not significant (day 63: p = 0.540; day 75: p = 0.056). Hatching was first recorded on day 63 (site 1: M = 17.4% hatched, CI = 0 - 87.7; site 2: M = 0.33% hatched, CI = 0 - 69.1), with 100% of live eggs having hatched by day 75. Differences between sites in the percentage of eggs hatched on day 63 were not significant (Wilcoxon test, p = 0.2).



Figure 5. Survivorship of Arctic charr eggs measured on three occasions at experimental sites in the Afon y Bala and Afon Hwch. Site 1 eggs experienced ambient river temperatures and site 2 eggs were exposed to warmer water discharged from the power station.

DISCUSION

Water discharges from the power station increased the average and peak temperatures in the Afon y Bala but did not cause higher mortality rate in eggs. This finding contrasts with a laboratory experiment by Natural Resources Wales (NRW) that replicated temperature regimes measured at the Afon y Bala in 2009 and found that the survivorship of eggs exposed to thermal pollution was lower (42% and 49% at the end of February) than eggs incubated at a consistent 2 - 5.5 °C in a separate hatchery facility (91.8% surviving at the end of February) (Thomas, Griffiths and Hall (2010). One key difference between the studies was the magnitude of temperature spikes. The maximum temperature attained in the experimental tanks was 11.7 °C and the maximum difference between spikes and ambient was 8 °C (NRW, unpublished data), whereas here, the maximum temperature recorded was just 9.55 °C and the maximum difference only 3.39 °C. Moreover, average temperature of the treated tanks in the laboratory (4.1 °C) was lower than at the discharge and ambient site in this study (6.55 °C and 6.36 °C

respectively). Thus, thermal pollution from the power station during the egg incubation period of 2019 - 2020 was not as significant as has been recorded in previous years, but average natural water temperatures were less suitable for egg survival. As has been mentioned, the results of the experiment may have been confounded by sedimentation and burial of egg incubation bags.

One noteworthy finding that was not impacted by flooding and sedimentation was the early hatching time of eggs. The time of 50% hatching is likely to have occurred shortly after day 63, compared to 78 - 86 days reported by Baroudy and Elliott (1994) for Windermere autumn spawning charr eggs incubated in natural lake water, and 94 days in the experiment investigating thermal pollution discussed above (NRW, unpublished data). It is likely that the earlier hatching time was a consequence of warmer temperatures (Jobling *et al.*, 1993). Abnormally fast development at the egg and alevin stage can mean that first feeding occurs before food becomes abundant in spring and may increase the risk of emergent fry encountering harsh weather conditions (Skoglund, Einum and Robertsen, 2011).

Appendix 3: Multiplex design (Chapter 4)

		Allele size				
Locus	Primer Sequence (5'-3')	(bp)	Repeat motif	Dye	Multiplex	Primer concentration (uM)
Ssa406	F: AGGTGGGTCCTCCAAGCTAC	404-466	(GT)14	NED	1	0.22
	R: ACCCGCTCCTCACTTAATC					
Ssa85	F: AGGTGGGTCCTCCAAGCTAC	147-200	(GT)14	NED	1	0.1
	R: ACCCGCTCCTCACTTAATC					
SsaD48	F: GAGCCTGTTCAGAGAAATGAG	139-300	TAGA	FAM	1	0.18
	R: CAGAGGTGTTGAGTCAGAGAAG					
SalF56	F: TGCAGTTCCACAATATATCCC	180-220	(TG)25	VIC	1	0.1
	R: AAGGGCACACTCAGATTTTG					
SalJ81	F: CAGCATAATCACTCCCGC	105-179	(GT)33	PET	1	0.1
	R: GAAAGCTACCTTGCGTGC					
SalD39	F: GGGGAGTCTGTGTTAAGTTGG	194-320	(GT)3(AC)23	FAM	2	0.1
	R: TGAATGGACGTTCCTCTGAC					
SalP61	F: CACTTATTAACGCCCACTCCC	100-140	(CA)18	VIC	2	0.07
	R: TTCACAACCACAGGAAAGAACTC					

Table 1. Multiplex design for Arctic charr in North Wales using 7 microsatellite loci according to Beck (2014).

Appendix 4: Reasons for not including SSa406, Ssa85, SalD39, and SalJ81 loci in the microsatellite analysis (Chapter 4)

SSa406: After completing laboratory work, it was noticed that the primer sequence reported by Beck (2014) for locus SSa406 was identical to locus Ssa85, as shown in Table 1 of Appendix 3.

Ssa85: Because of the misreporting of Ssa406, the Ssa85 primer was present in multiplex 1 at a far higher concentration than required (i.e., Ssa406 + Ssa85 = 0.33 um, see Table 1 of Appendix 3). The higher concentration of primer appeared to produce significant PCR artefacts for SSa85.

SalD39: The marker exhibited 'hedgehog peaks', meaning homozygotes could not be distinguished. In addition, the marker did not amplify for a large proportion of individuals.

SalJ81: Insufficient amplification in samples from S. Beck.

Appendix 5: Data sources used to construct PPMs for Arctic charr populations (Chapter 6)

Table 1. Data sources for life-history information used to construct PPMs for 41 populations of Arctic charr.

Population	NA	N_M	N_F	Source
NR southern region				
Llvn Padarn PL (1)	773	> 35	≈ 52	Butterworth (1980)
Llvn Peris PL (2)	867	> 45	≈ 59	Butterworth (1980)
Loch Builg PL (4)	876	157	38	Barbour (1984):
8 ()				Unpublished data (Scottish National
				Heritage, 2008)
Loch Doine PL (5)	651	154	49	Barbour (1984)
Loch Mealt BEN (6)	290	53	29	Barbour (1984)
Vangsvatnet PL (8)	382	≈ 382	≈ 100	Jonsson and Hindar (1982b, 1982a)
Loch Rannoch BEN (9)	≈ 81	≈ 81	≈ 42	Fraser (1998)
Loch Rannoch PL (9)	≈ 60	≈ 60	≈ 19	Fraser (1998)
Atnsjø PL (10)	983	983	62	Hegge, Dervo and Skurdal (1991)
Løna PL (12)	451	236	12	Jonsson and T Østli (1979)
Llyn Cwellyn PL (21)	50	50	4	Sullivan (1976)
DWF southern region				
Vangsvatnet (8)	>167	167	10	Jonsson and Hindar (1982b, 1982a)
NR northern region				
Vatnshlidarvatn PL (17)	>165	165	11	Jonsson (1996, 2002)
Thingvallavatn BEN (7)	644	644	175	Jonsson et al., (1988)
Thingvallavatn PL (7)	4224	4224	404	Jonsson et al., (1988)
Thingvallavatn PI (7)	224	224	26	Jonsson et al., (1988)
Iterlaa PI (3)	576	217	18	Sparholt (1985)
Tarnvatn PL (27)	157	157	26	Kjaer (2018)
Skogsfjordvatn PL (15)	608	593	35	Smalas (2013)
Skogsfjordvatn PI (15)	218	217	13	Smalas (2013)
Keyhole Lake PL (22)	3475	1424	127	Hunter (1969)
DWF northern region				
Thingvallavatn (7)	746	746	7	Jonsson <i>et al.</i> , (1988)
Candlestick Pond (14)	40	40	13	Rombough, Barbour and Kerekes (1978)
Skogsfjordvatn (15)	89	89	23	Smalas (2013)
Vatnshlidarvatn (17)	>167	167	10	Jonsson (1996, 2002)
Big lake (23)	209	59	18	McCart (1980)
Skovatn (27)	51	51	13	Kjaer (2018)
Iterlaa (3)	286	91	11	Sparholt (1985)
Shublik Springs (25)	165	82	18	McCart and Craig (1973); McCart (1980)
Un-named Springs (25)	141	43	17	McCart and Craig (1973); McCart (1980)
Babbage River (16)	502	77	75	Bain (1975)
ANDR northern region				
Fraser River (11)	533	≥ 96	17	Dempson and Green (1985)

Sylvia Grinnell (13)	806	340	23	Grainger (1953); Ick (2010)
Fish Hole Creek (16)	204	241	27	Bain (1975)
Lake Quingu (18)	>324	109	≈ 60	Loewen (2008)
Lake Quasigiat (19)	>172	105	≈ 60	Loewen (2008)
Lake Iqalugaarjuit (20)	>172	130	≈ 60	Loewen (2008)
Kavik River (26)	111	81	28	McCart (1980)
Firth River (27)	281	161	20	McCart (1980)
Sagavanirktok Rv. (28)	1098	395	49	McCart (1980)
Canning River (25)	>286	247	96	McCart (1980)

Morphotype: ANDR = anadromous, NR = normal growth resident, DWF = dwarf resident. PL = pelagic ecotype, BEN = benthic ecotype, PI = piscivorous ecotype. N_A = sample size for age and length data, N_M = number of fish examined for stage of maturity, N_F = number of female fish sampled to assess fecundity. N_A and N_M are for female fish except where data for both sexes were combined. Approximations of N were made when not clearly stated by the author. Numbers in parenthesis refer to population location shown in Figure 1, Chapter 6.

Appendix 6: Perch trapping surveys at Llyn Padarn 2019

INTRODUCTION

Perch have been reported at Llyn Padarn since approximately 2010. Perch are not native to the lake and are potential predators and competitors of native salmonids, including the threatened Arctic charr population (Allen, 1935; Morrissey-McCaffrey *et al.*, 2019). The survey presented here was a pilot study. Further studies of the population were intended but it was decided the population was perhaps too small to catch a sufficient number of individuals. The data presented could provide a baseline for any future monitoring.

METHODS

Un-baited traps have proven a highly effective method for sampling perch at Lake Windermere (Mills and Hurley, 1990). Perch willingly swim into the traps in spring when they migrate to littoral habitats to spawn, perhaps out of curiosity, or because the traps provide a surface to deposit eggs. I was unable to acquire the same perch traps as used at Windermere so instead used similar round collapsible funnel traps which are cheap and readily available (Figure 1). Two sizes of traps were acquired: one $\sim 30 \times 60$ cm and the other $\sim 40 \times 80$ cm. The traps had two entrances. One entrance was closed with a cable tie and the second was adapted to prevent otter entrapment by fixing a plastic mesh ring ~ 75 mm in diameter (Figure 1). The traps had a diamond shaped mesh with dimensions of approximately 1.5 cm.



Figure 1. Funnel type traps used to survey perch (left) and the adapted opening to prevent otter entrapment (right)

Seven traps were deployed in a line at 3 locations shown in Figure 2, with a distance of ~ 10 m between each trap. These sites were ~ 5 to 10 m in depth which has been the depth range targeted by surveyors at Windermere. Small and a large traps were evenly distributed between the sampling locations. The surveys ran from 26^{th} April to 28^{th} of May with traps being checked approximately every 6 – 7 days. Captured perch were euthanised by overdose with clove oil followed by exsanguination (Fernandes *et al.*, 2016). All other species were immediately returned to the lake. Weights and lengths (fork length) were recorded for perch only.



Figure 2. Location of traps deployed at Llyn Padarn

RESULTS AND DISCUSSION

A total of 9 perch were captured. Most were captured at site B (n = 6), with no perch captured at site C. The catch per unit effort (catch divided by days fished and number of traps set) excluding site C is summarised Table 1. The mean fork length and weight of perch was 98 mm and 49 g, respectively (Table 2). Perch were the most frequently captured fish, followed by eel, brown trout, stickleback, and Atlantic salmon (Table 3).

Date	Days fished	Traps set	Perch	CPUE
02/05/2019	6	14	2	0.02381
09/05/2019	6	13	2	0.025641
15/05/2019	6	13	1	0.012821
21/05/2019	6	11	3	0.045455
28/05/2019	7	13	1	0.010989
Mean				0.023743

Table 1. Perch catch per unit effort (CPUE) for site B and C

Table 2. Lengths and weights of perch captured at Llyn Padarn in May 2019

Date	Location	Fork length	Weight (g)
02/05/2019	В	22*	173*
02/05/2019	В	7.5*	5.1*
09/05/2019	В	85	7
09/05/2019	В	79	6
15/05/2019	А	183	80
21/05/2019	В	85	7
21/05/2019	А	113	17
21/05/2019	Α	91	9
28/05/2019	В	215	134

*Measured after thawing

Table 3.	Composition	of fish	species	captured	in traps
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Species	Frequency
Brown trout	15 % (n = 3)
Salmon smolt	4.5 % (n =1)
Eel	23 % (n =5)
Stickleback	14 % (n = 3)
Perch	42 % (n = 9)



Figure 3. A large perch (22 cm) captured at Llyn Padarn

Table 4 shows the CPUE for perch at Windermere North basin. Whilst the materials, shape, and dimensions of traps used at Windermere (Figure 4) are different to those used a here, it seems unlikely that these differences would account for the very low perch CPUE at Llyn Padarn compared to Windermere (mean CPUE = 0.02 and 5.10, respectively). It is therefore indicated the perch population at Llyn Padarn in 2019 was considerably smaller than has been recorded at Windermere. The largest perch captured were comparable in length to Windermere (Allen, 1935). Importantly, perch are obtaining a length where predation on fish, which may include small Arctic charr, is likely (Allen, 1935; Morrissey-McCaffrey *et al.*, 2019).

Date	Days fished	Traps set	Perch	CPUE
11-May	7	50	1040	2.97
14-May	3	50	1292	8.61
18-May	4	50	1199	6.00
22-May	4	50	1108	5.54
26-May	4	50	796	3.98
29-May	3	50	526	3.51
Mean				5.10

Table 4. Catch of perch at Lake Windermere North Basin reported by Bagenal (1972)



Figure 4. Windermere perch trap. Essentially it consists of three semi-circular fencing-wire hoops (c. 57 cm in height) covered with half-inch (1-3 cm) hexagonal wire netting, to give a trap with a flat base c. 76 x 67 cm. At one end is a funnel directed inwards for c. 44 cm to an opening 8-5 cm in diameter; at the other end is a door for the removal of the catch.

Location	Morph/ecotype	Reproduction	Juvenile	Adult
			survivorship	survivorship
Lake Padarn	NR	0.202	0.606	0.192
Lake Peris	NR	0.231	0.462	0.307
Lake Iterlaa	PISC	0.080	0.641	0.279
Lake Iterlaa	DWF	0.134	0.402	0.465
Lake Builg	NR	0.213	0.639	0.148
Lake Doine	NR	0.209	0.627	0.163
Lake Mealt	NR	0.335	0.335	0.330
Lake	DWF	0.176	0.527	0.298
Thingvallavatn				
Lake	NR/LIT	0.096	0.674	0.229
Thingvallavatn				
Lake	NR/PEL	0.170	0.680	0.150
Thingvallavatn				
Lake	NR/PISC	0.121	0.607	0.272
Thingvallavatn				
Lake Vangsvatnet	NR/PEL	0.152	0.607	0.242
Lake Rannoch	NR/LIT	0.254	0.507	0.239
Lake Rannoch	NR/PEL	0.328	0.328	0.344
Lake Atnsjo	NR	0.139	0.697	0.164
Fraser River	ANDR	0.115	0.693	0.192
lake lona	NR	0.203	0.610	0.187
Frobisher Bay	ANDR	0.066	0.661	0.273
Lake Vangsvatnet	DWF	0.184	0.551	0.266
Candlestick Pond	DWF	0.276	0.552	0.173
Lake	NR/PEL	0.162	0.646	0.192
Skogsfjordvatn				
Lake	DWF	0.213	0.426	0.360
Skogsfjordvatn				
Lake	NR/PISC	0.095	0.666	0.238
Skogsfjordvatn		0.100	0.7.10	0.010
Upper Babbage	RR	0.188	0.563	0.249
River		0.107	0.000	0.177
Fish Hole Creek	ANDR	0.137	0.686	0.177
Lake	NR/PEL	0.152	0.610	0.238
Vatnshlidarvatn	DWE	0.000	0.000	0.540
Lake	DWF	0.226	0.226	0.549
Valusinuarvalii		0.091	0.646	0.274
Lake Quiligu	ANDR	0.001	0.040	0.274
Lake Quasigiat		0.092	0.041	0.207
Lake Iqaiugaarjuit		0.110	0.038	0.255
Lake Cwellyn	rel Dei	0.252	0.304	0.244
keynole Lake	IPEL	0.075	10./4/	10.178

Appendix 7: Elasticities of Arctic charr populations (Chapter 6)

Big Lake	DWF	0.124	0.619	0.257
Shublik Springs	RR	0.208	0.624	0.168
Unamed Spring	R-R	0.208	0.625	0.166
Kavik River	ANDR	0.145	0.724	0.131
Firth River	ANDR	0.100	0.699	0.201
Sagavanirktok	ANDR	0.109	0.763	0.128
River				
Canning River	ANDR	0.122	0.734	0.143
Lake Tarnvatn	PEL	0.112	0.670	0.219
Lake Skovatn	DWF	0.181	0.542	0.277

NR = normal growth resident; DWF = dwarf resident; ANDR = anadromous; RR = river resident dwarf; LIT = littoral feeding ecotype; PEL = pelagic feeding ecotype; PISC = piscivorous ecotype