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Has habitat heterogeneity promoted phenotypic and ecological sub-structuring among a *Coregonus lavaretus* population in a large Scottish lake?

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Differences in stable-isotope values, morphology and ecology in whitefish *Coregonus lavaretus* were investigated between the three basins of Loch Lomond. The results are discussed with reference to a genetic investigation to elucidate any substructuring or spawning site fidelity. Foraging fidelity between basins of Loch Lomond was indicated by $\delta^{13}C$ and $\delta^{15}N$ values of *C. lavaretus* muscle tissue. There was, however, no evidence of the existence of sympatric morphs in the *C. lavaretus* population. A previous report of two *C. lavaretus* 'species' in Loch Lomond probably reflects natural variation between individuals within a single mixed population.

Key words: Loch Lomond; morphometrics; morphs; stable-isotope analysis; sympatry; thin plate spline.

INTRODUCTION

Substructuring in populations of fishes has been frequently recorded, particularly in postglacial lakes (Skúlason & Smith, 1995; Smith & Skúlason, 1996). Substructuring in extreme cases takes the form of sympatric polymorphism. Arctic charr *Salvelinus alpinus* (L.) morphs, for example show discrete variation in morphological, meristic, behavioural, ecological and life-history traits (Jonsson & Jonsson, 2001). In the U.K., a significant number of *S. alpinus* populations show evidence of substructuring in their gene pool, morphology, ecology and life history (Adams *et al.*, 1998; McCarthy *et al.*, 2004; Garduño-Paz, 2009). In lakes, habitats and feeding resources available to fishes are relatively discrete and can be divided horizontally from the littoral to the pelagic and vertically by depth. Fishes such as *S. alpinus* frequently

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show substructuring based on habitat and food resources (Amundsen, 1988; Adams *et al.,* 1998). Commonly, this polymorphism takes the form of two groups of ecological specialists, such as a littoral-benthic and a pelagic feeder, but can sometimes include a profundal morph. Divergence into pelagic forms foraging on plankton with associated morphological and meristic specialization and benthic forms specializing in foraging on macrobenthos has been observed in *S. alpinus* (Schluter & McPhail, 1993; Robinson & Wilson, 1994) and a number of different fish lineages, including pumpkinseed *Lepomis gibbosus* (L.) (Robinson & Wilson, 1996), creole perch *Percichthys trucha* (Valenciennes) (Ruzzante *et al*., 1998), three-spined stickleback *Gasterosteus aculeatus* L. (Baker *et al*., 2005). More subtle structuring, such as genetic and morphological subpopulations can also be found, *e.g.* in Atlantic salmon *Salmo salar* L. (Verspoor & Cole, 1989).

Population substructuring has also been demonstrated in the European whitefish *Coregonus lavaretus* (L.), which is highly variable across its range (Næsje *et al*., 2004; Ostbye *et al*., 2005; Kahilainen & Ostbye, 2006). The polymorphism in this species often takes the form of differentiation in phenotype, ecology and life history (Bergstrand, 1982; Amundsen, 1988; Kahilainen & Ostbye, 2006), and morphs can represent discrete gene pools (Næsje *et al*., 2004; Ostbye *et al*., 2005). Some *C. lavaretus* populations can support up to five sympatric morphs (Svärdson, 1979; Bergstrand, 1982), which can separate pelagic, littoral and profundal trophic resources (Harrod *et al*., 2010). Apart from habitat and feeding, they most often differ in body size (small and large body forms), shape, growth and gill-raker number (Bergstrand, 1982; Næsje *et al*., 2004; Harrod *et al*., 2010). Similar patterns of divergence are found both in separate lineages of coregonids and in different lakes containing the same *Coregonus* species (Douglas *et al*., 1999, 2005).

Loch Lomond is the largest body of fresh water in Great Britain (*c*. 71 km^2) and was created by glacial encroachment from the north (Slack *et al*., 1957). It has many inflows, the largest of which are the River Falloch in the north and the Endrick Water in the south, but only a single outflow formed by the River Leven in the south. Loch Lomond has three distinct basins: a north basin and a south basin are separated by an intermediate mid basin (Tippett *et al*., 1974). The north basin has the characteristics of a typical highland Scottish lake, in that it is narrow and deep (maximum depth 190 m) and is permanently thermally stratified in the summer months. It is surrounded by hard and often peat-covered metamorphic rocks which yield little in the way of soluble nutrients and so is oligotrophic (Slack *et al*., 1957; Best & Traill, 1994). The south basin has the characteristics of a typical lowland Scottish lake. It is wide and shallow (maximum depth 30 m), has a weak and temporary thermal stratification in the summer months and is surrounded for the most part with soft sedimentary rocks and fertile soils. The catchment is dominated by farmland which is fertilized and is relatively densely populated; therefore, the waters of the southern basin are more mesotrophic. Moreover, the south basin with its myriad of islands provides a heterogeneous environment (Slack *et al*., 1957; Best & Traill, 1994).

Coregonus lavaretus is naturally extant in only seven lakes within the U.K. It is referred to locally as powan (Scotland), schelly (England) and gwyniad (Wales). Within Scotland, *C. lavaretus* are found naturally in only two locations, Loch Lomond and Loch Eck. The large size of Loch Lomond, with its multiple basins of distinct nature and the high level of habitat heterogeneity, would suggest that substructuring within populations is highly likely. Previous studies on British *C. lavaretus* populations suggested the presence of two types of *C. lavaretus* in Llyn Tegid, Wales (Dottrens, 1959). Studies on another coregonid species, the Irish pollan *Coregonus autumnalis* (Pallas), revealed the existence of genetic substructuring in the population of Lough Neagh, Northern Ireland (Ferguson, 1975; Bradley, 2009), though Harrod (2001) found little evidence for ecological differentiation in this stock. The presence of two distinct *C. lavaretus* species in Loch Lomond was first postulated by Parnell (1838). These early observations suggested that two distinct species, differing primarily in head morphology: the long-nosed powan *Coregonus lacepedei* Parnell and the short head powan *Coregonus microcephalus* Parnell, existed within the loch. As a result, there is good reason to expect substructuring in the Loch Lomond *C. lavaretus*.

Population substructuring within a single species may have a number of important implications. First, sympatric populations may represent distinct evolutionary units and are important for the study of speciation. Second, ecological and genetic differentiation within the population may complicate management and conservation of this protected species. Due to differences in their long-term ecology, *e.g.* in habitat use (Harrod *et al.*, 2010), different forms may face uneven susceptibility to pernicious pressures on a particular habitat, spawning site or food resource. Novel conservation refuge populations have previously been established using Loch Lomond *C. lavaretus* and were based on material collected from a single spawning site (Maitland & Lyle, 1992, 1995; Etheridge *et al.*, 2010). If multiple forms coexist and they show assortative spawning, there is the possibility that not all diversity in the Loch Lomond *C. lavaretus* is represented in these refuge populations. To examine the existence of multiple forms of *C. lavaretus* in Loch Lomond, the null hypothesis tested was that there are no significant differences in *C. lavaretus* caught in different areas of Loch Lomond in ecology, life history and morphology.

MATERIALS AND METHODS

FISH COLLECTION

Multi-panel benthic Nordic-pattern gillnets, comprising 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in the three basins of Loch Lomond over the winter of 2005–2006. Nordic nets are not selective for coregonids over the modal size range 78 to 613 mm fork length L_F (Jensen, 1986). In total, 75 gillnets were set overnight in sites in the north, mid and south basins of Loch Lomond (9 November 2005 to 24 January 2006). The nets were set immediately prior to, and during, spawning time, on known or presumed spawning grounds. During this period, a total of 118 *C. lavaretus* were caught (north basin $n = 25$, mid basin $n = 47$ and south basin $n = 46$). Fish were frozen (-20° C) within 4 h of removal from the net.

STABLE-ISOTOPE ANALYSIS

In the laboratory, fish were defrosted and a small piece of white muscle posterior to the head and above the lateral line was removed for stable-isotope analysis. Tissue was dried at constant temperature (50° C for at least 48 h), ground to a fine powder using a grinder (Revel Ltd; www.revelappliances.com) and 0·5 mg of dried ground muscle was packed into pressed 10 mm × 10 mm tin cups and used in simultaneous analysis of stable C and N isotopes. Stable-isotope ratios were determined by continuous flow isotope ratio mass spectrometry at the Max Planck Institute for Limnology, Germany. Stable-isotope ratios are given using the δ notation expressed in ‰ where $\delta = 1000[(R_{\text{sample}}\ R_{\text{standard}}{}^{-1}) - 1]$, where $R = {}^{13}C{:}{}^{12}C$ or ${}^{15}N{:}{}^{14}N$. The reference materials used were secondary standards of known relation to the international standards of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision for a single analysis was ±0·1‰ for $\delta^{13}C$ and ±0·3‰ for $\delta^{15}N$. Examination of C:N values, which are positively correlated with lipid, indicated little need for adjustment of $\delta^{13}C$ values due to variation in lipid concentrations (Kiljunen *et al.*, 2006). During analysis, it was found that age had an effect on stable-isotope analysis results and that the range of ages caught between sites was not similar. In response, fish <4+ years were removed from further analysis to facilitate comparison between basins. All statistical analysis was performed using SPSS v.13 (www.spss.com).

HEAD MORPHOLOGY

Digital photographs of the left side of the fish were taken using a Nikon Coolpix digital camera (www.nikon.com), attached to a copy stand with sufficient lighting and a suitable scale. Thin-plate spline (tps) is a landmark-based geometric technique for the determination of shape. The method provides a powerful analysis of shape independent of size (Rohlf, 1990, 2002; Bookstein, 1991). All morphometric software was downloaded from http://life.bio.sunysb.edu/morph/. For the analysis of morphology, images of 14 individuals from each area were chosen that gave good detail of the head. A total of eight landmarks (Fig. 1) were identified on the head and digitized to co-ordinates using the programme TpsDig. Procrustes superimposition in the programme Coordgen6, with landmarks 1 and 2 as baseline end-points, was used to rotate, translate and scale co-ordinates. On the basis of the co-ordinates obtained, new shape parameters known as partial warp scores were calculated in the programme PCAGen. These new parameters capture spatial variation in a sample and can be used in statistical analysis. The partial warps were reduced by principle components analysis (PCA), and MANOVA was performed on the resulting component scores to examine whether shape differed between fish caught in different localities.

DETERMINATION OF SIZE AND AGE

L_F (to 1 mm) and mass (M) (to 0·01 g) were measured. During the analysis, it was found that the smallest fish had an undue effect on results and so this single individual was removed from further length analysis. Scales were removed from the flank below the dorsal fin for age determination. Three scales were selected which showed no evidence of trauma or reabsorption and impressed onto plastic. A projection microscope was then used to read growth rings on scales to determine age, and the median age (determined from three scales) was taken as the age of the individual to minimize any bias of reading errors. Sex was determined by the visual examination of gonads. The first gill arch was dissected from the left side of the fish

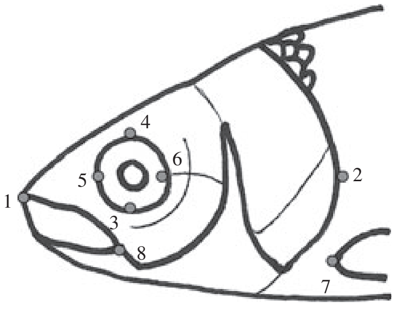

Fig. 1. Eight landmarks (1, snout; 2, protruding lip indicating most posterior point of operculum; 3–6, eye; 7, base of pectoral fin; 8, edge of maxillary) used to examine variation in head shape of *Coregonus lavaretus*, from Loch Lomond, Scotland.

and the gill rakers were counted. The outer surface of the stomach and gut was examined for cysts of the parasite *Diphyllobothrium* spp. and scored for the number of cysts of each individual fish as follows: 0 = 0, 1 = 1–9, 2 = 10–19, 3 = 20–50 and 4 = 50+. Statistical comparisons of these data were carried out using Kruskal–Wallis, while categorical data were tested with χ^2.

The adipose fin was removed and stored in 100% ethanol. Genetic work on this tissue took place in the Marine Scotland Freshwater Laboratory, Pitlochry, and has been reported by Thompson *et al.* (2008). These published data were tested for between-basin differences in allelic richness using ANOVA.

RESULTS

STABLE-ISOTOPE ANALYSIS

There was a significant difference in mean *C. lavaretus* muscle tissue δ^{13}C values between basins (ANOVA, $F_{2,112} = 5{\cdot}09$, $P < 0{\cdot}01$; Fig. 2). Bonferroni *post hoc* comparisons showed that the *C. lavaretus* from the mid basin were significantly depleted in ^{13}C than compared to *C. lavaretus* from the south basin ($P < 0{\cdot}05$), but there was no significant difference between *C. lavaretus* from the north and mid basins ($P > 0{\cdot}05$) or the north and south basins ($P > 0{\cdot}05$). There was also a significant difference between basins in muscle δ^{15}N values (ANOVA, $F_{2,112} = 17{\cdot}68$, $P < 0{\cdot}001$). Bonferroni *post hoc* comparisons showed that *C. lavaretus* from the south basin were significantly more ^{15}N enriched than *C. lavaretus* from both the north ($P < 0{\cdot}001$) and the mid basins ($P < 0{\cdot}001$), but there was no significant difference between *C. lavaretus* from the north and mid basins ($P > 0{\cdot}05$). These differences remained significant when two outliers were removed (Fig. 2). Across all sites, there was a significant difference between sexes in δ^{15}N of muscle tissue (t-test, $t = 2{\cdot}2$, d.f. = 12, $P = 0{\cdot}05$), with males being more δ^{15}N enriched than females, but no significant difference between sexes was found in δ^{13}C (t-test, $t = 0{\cdot}79$, d.f. = 12, $P > 0{\cdot}05$).

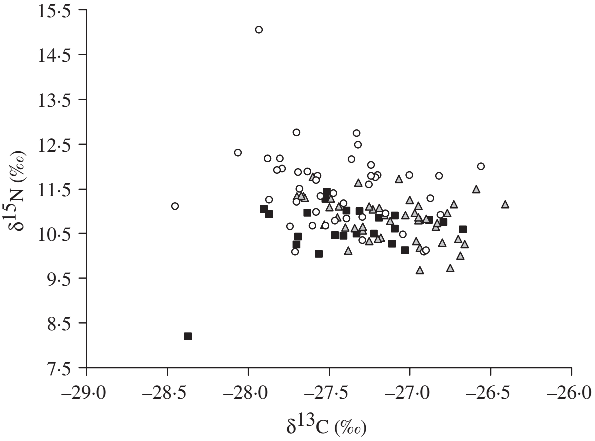

FIG. 2. Variation in δ^{13}C and δ^{15}N of muscle tissue from *Coregonus lavaretus* from the north (■), mid (△) and south (○) basins of Loch Lomond.

Across all sites, there was a significant negative correlation between age and δ^{15}N (Pearson correlation = $-0{\cdot}35$, $n = 114$, $P < 0{\cdot}001$) and a significant positive correlation between age and δ^{13}C (Pearson correlation = $0{\cdot}44$, $n = 114$, $P < 0{\cdot}001$). Further examination, however, found that within sites there was only a significant negative correlation between age and δ^{15}N (Pearson correlation = $-0{\cdot}38$, $n = 44$, $P < 0{\cdot}05$) in the south basin, and a significant positive correlation between age and δ^{13}C in the mid (Pearson correlation = $0{\cdot}49$, $n = 47$, $P < 0{\cdot}001$) and south basins (Pearson correlation = $0{\cdot}43$, $n = 44$, $P < 0{\cdot}01$). When *C. lavaretus* <4+ years were removed from the analysis, only the significant positive correlations between age and δ^{13}C in the mid and south basins remained.

HEAD MORPHOLOGY

Five principle component (PC) scores were derived from partial warp scores, and these explained a total of 77·5% of variation in landmark position. There was no significant difference in combined PC scores, representing head shape, between the three areas of Loch Lomond (GLM, $F_{10,72} = 1{\cdot}7$, $P > 0{\cdot}05$; Fig. 3). There was also no significant difference in number of gill rakers (ANOVA, $F_{2,73} = 1{\cdot}78$, $P > 0{\cdot}05$) between *C. lavaretus* from the three basins in Loch Lomond (Fig. 4). Combining these morphometric and meristic variables did not result in a significant difference between head shape and gill-raker numbers between basins (GLM, $F_{12,40} = 0{\cdot}94$, $P > 0{\cdot}05$).

LIFE HISTORY

There was no significant difference in L_F (Kruskal–Wallis = 1·8, d.f. = 2, $P > 0{\cdot}05$) or M (Kruskal–Wallis = 1·6, d.f. = 2, $P > 0{\cdot}05$) between basins. The relationship between $\log_{10}L_F$ and $\log_{10}M$ could be described with a linear relationship

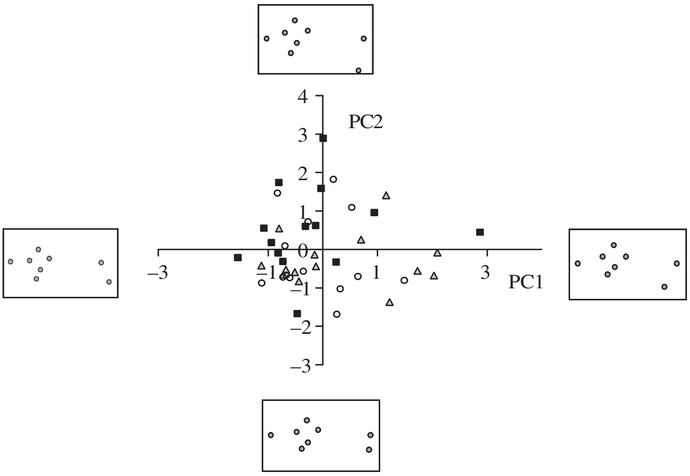

FIG. 3. Variation in PC1 and PC2 scores describing the head shape of *Coregonus lavaretus* from north (■), mid (△) and south (○) basins of Loch Lomond. Most of the variation is explained by changes in the position of the base of the pectoral fin (landmark 7; see Fig. 1) on lower right side of rectangle.

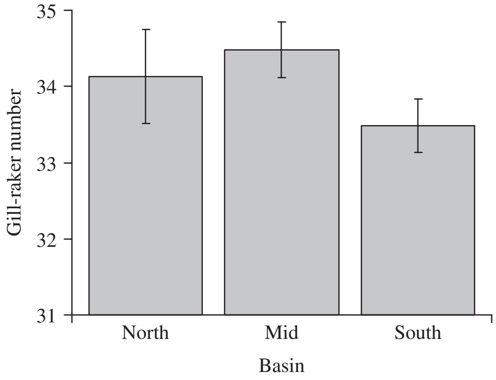

FIG. 4. Mean ± S.E. gill-raker numbers of *Coregonus lavaretus* from north, mid and south basins of Loch Lomond.

in north (ANOVA, $r^2 = 0{\cdot}67$, $F_{1,23} = 46{\cdot}2$, $P < 0{\cdot}001$), mid (ANOVA, $r^2 = 0{\cdot}89$, $F_{1,46} = 371{\cdot}7$, $P < 0{\cdot}001$) and south basins (ANOVA, $r^2 = 0{\cdot}94$, $F_{1,44} = 661{\cdot}6$, $P < 0{\cdot}001$; Fig. 5). There was a significant difference in linear slopes between sites (ANCOVA, $r^2 = 0{\cdot}92$, $F_{2,114} = 3{\cdot}3$, $P < 0{\cdot}05$), however, when an outlier was removed (the smallest fish) there was no longer a significant difference (ANCOVA, $r^2 = 0{\cdot}84$, $F_{2,113} = 2{\cdot}9$, $P > 0{\cdot}05$). *Coregonus lavaretus* samples from the south basin of Loch Lomond had a lower mean age resulting in a near-significant difference in age between basins (Kruskal–Wallis = 6·0, d.f. = 2, $P = 0{\cdot}05$). This was the only part of Loch Lomond where *C. lavaretus* <4+ years of age were caught. If *C. lavaretus* below this age were removed from analysis, there was no significant difference between basins (Kruskal–Wallis = 3·1, d.f. = 2, $P > 0{\cdot}05$).

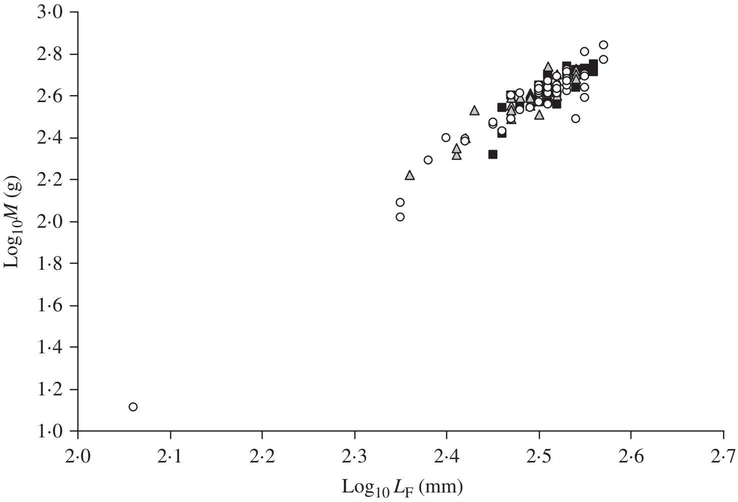

FIG. 5. Relationships between $\log_{10}$ mass (M) and $\log_{10}$ fork length (L_F) of *Coregonus lavaretus* from north (■) ($y = 2{\cdot}7x - 4{\cdot}2$), mid (△) ($y = 2{\cdot}7x - 4{\cdot}1$) and south (○) ($y = 3{\cdot}2x - 5{\cdot}3$) basins of Loch Lomond.

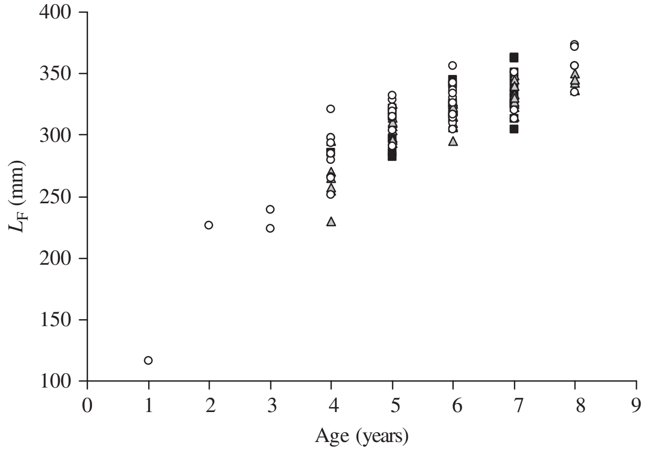

FIG. 6. Relationships between fork length (L_F) at age of *Coregonus lavaretus* from north (■) ($y = 67{\cdot}8x - 4{\cdot}5x^2 + 79{\cdot}6$), mid (△) ($y = 64{\cdot}4x - 3{\cdot}9x^2 + 71{\cdot}5$) and south (○) ($y = 68{\cdot}0x - 4{\cdot}2x^2 + 76{\cdot}3$) basins of Loch Lomond are best described with a quadratic model.

The modal age of *C. lavaretus* in all areas of Loch Lomond was 6+ years. L_F at age of *C. lavaretus* from the north ($r^2 = 0{\cdot}47$, $F_{1,22} = 9{\cdot}75$, $P < 0{\cdot}01$), mid ($r^2 = 0{\cdot}79$, $F_{2,44} = 81{\cdot}57$, $P < 0{\cdot}001$) and south ($r^2 = 0{\cdot}86$, $F_{2,42} = 124{\cdot}63$, $P < 0{\cdot}001$) basins of Loch Lomond were best described with a quadratic relationship (Fig. 6). The resulting curves were not significantly different ($F_{2,114} = 2{\cdot}40$, $P > 0{\cdot}05$).

There was no significant difference in frequencies of males and females caught on the spawning grounds between basins ($\chi^2 = 4{\cdot}59$, d.f. = 2, $P > 0{\cdot}05$). There was no significant difference in scores for infection intensity of *Diphyllobothrium* spp. in *C. lavaretus* between basins of Loch Lomond (Kruskal–Wallis, d.f. = 2, $P > 0{\cdot}05$; Fig. 7). Additionally, there was no significant difference between the prevalence of infected *C. lavaretus* between the north (prevalence = 100%), mid (100%) and south (98%) of Loch Lomond ($\chi^2 = 1{\cdot}58$, d.f. = 2, $P > 0{\cdot}05$).

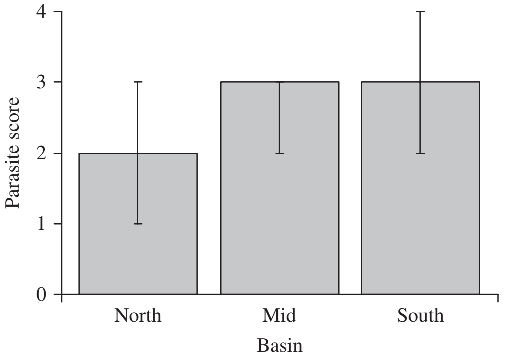

FIG. 7. Median with 25th and 75th percentiles (the upper percentile for the mid basin is the same value as the median) parasite (*Diphyllobothrium* spp.) scores on stomachs of *Coregonus lavaretus* from north, mid and south basins of Loch Lomond.

GENETICS

Utilizing six microsatellites, Thompson *et al*. (2008) used mean allelic richness as a measure of genetic diversity of *C. lavaretus* individuals. Using data extracted from this report, no significant difference was found in genetic diversity between basins (ANOVA, $F_{2,15} = 0{\cdot}005$, $P > 0{\cdot}05$).

DISCUSSION

Morphological, ecological and genetic substructuring of fishes in postglacial lakes is relatively common. These often occur as sympatric foraging specialists, with associated morphological and meristic specializations (Schluter & McPhail, 1993; Robinson & Wilson, 1994). In this study, significant differences in $\delta^{13}C$ and $\delta^{15}N$ of fish from distinct basins of Loch Lomond were found. The isotopic turnover of muscle depends on growth rate, but typically is considered to be *c*. 6 months (Perga & Gerdeaux, 2005; Phillips & Eldridge, 2006). $\delta^{13}C$ values closely reflect the origin of the carbon source the organism has been assimilating, due to limited fractionation: trophic enrichment in $\delta^{13}C$ is typically *c*. 1‰ (Peterson & Fry, 1987; Hobson, 1999). Enriched $\delta^{13}C$ values are indicative of atmospheric C, while depleted $\delta^{13}C$ values are indicative of endogenous (recycled) C sources. Small but significant differences in $\delta^{13}C$ of *C. lavaretus* muscle tissue suggest that fish in the mid basin may be specializing on $\delta^{13}C$-enriched prey such as littoral macroinvertebrates (France, 1995; Post, 2002). Alternatively, the different natures of the loch basins may lead to different isotopic baselines, which are reflected in muscle tissues, for instance $\delta^{13}C$ values in a water body can be influenced by riverine inputs, the surface area of a water body and eutrophication (Post, 2002; Perga & Gerdeaux, 2004). In Loch Lomond, however, levels of dissolved organic C are generally greater in the south basin (Bass, 2007). Trophic enrichment in $\delta^{15}N$ is typically *c*. 3–5‰, at each trophic level, thus the long-term trophic position of consumers can be estimated (Peterson & Fry, 1987; Post, 2002; Sweeting *et al*., 2007). Significant differences in $\delta^{15}N$ of *C. lavaretus* muscle tissue suggest that fish from the south basin are feeding at a higher trophic level. Again, the small differences recorded in *C. lavaretus* $\delta^{15}N$ may also reflect isotopic differences at the base of the food webs in the different basins. For instance, $\delta^{15}N$ values in a water body can be increased by anthropogenic input of nutrients (Constanzo *et al*., 2001). Certainly, $\delta^{15}N$ values of total dissolved N in Loch Lomond are greatest in the south, which was where *C. lavaretus* were most enriched in $\delta^{15}N$ (Bass, 2007).

The stable-isotope values of *C. lavaretus* muscle tissue can be interpreted as fish feeding on different prey items. *Coregonus lavaretus* caught from the mid and south basins of Loch Lomond, however, have been previously described as specializing on plankton (though occasionally also feeding on benthos in winter) (Slack *et al*., 1957; Pomeroy, 1991). Therefore, it seems more likely that these isotopic differences reflect the discrete natures of the north and south basins as opposed to differences in the diet of *C. lavaretus* in these areas. This, in turn, suggests that there is foraging fidelity between basins. While the overlap between individuals suggests that this is not complete, individuals apparently feed in a given basin for months to develop the spatial differences in stable-isotope values in their muscle tissues. There was also a significant difference in $\delta^{15}N$ enrichment between males and females. It is difficult to draw firm conclusions from this, given the small sample size of females

(11 females, 99 males and seven unknown for stable-isotope analysis). Because of the behaviour of spawning *C. lavaretus*, females are usually underrepresented in catches on spawning grounds, and there is no significant difference in the numbers of males and females caught between basins.

Head morphology is associated with efficiency of capturing and processing food and may reasonably be expected to be different when individual fish specialize on particular diets (Gardner *et al.*, 1988; Adams & Huntingford, 2002). There were no significant differences in head shape between fish caught in the different areas of Loch Lomond. Gill-raker number is also linked with different diets; a benthic diet is typically associated with low number of gill rakers, while a pelagic diet is typically associated with a high number of gill rakers (Amundsen, 1988; Lehtonen & Kahilainen, 2002; Harrod *et al.*, 2010). There was no significant difference in gill-raker number between fish caught in the different areas of Loch Lomond. Particular parasite infections are also associated with certain diets and elevated infection intensities of *Diphyllobothrium* spp. infection are associated with feeding on zooplankton (Amundsen & Klemetsen, 1988; Dolezel & Crompton, 2000). There was no significant difference in parasite load between fish caught in the different areas of Loch Lomond, suggesting a similar dependence on pelagic resources. There was also no significant difference in size or growth between fish caught in the different areas of Loch Lomond, providing further evidence for a lack of spatial segregation in the *C. lavaretus* population (Kahilainen *et al.*, 2003). Taken together, these data strongly suggest that there is no phenotypic substructuring within *C. lavaretus* in Loch Lomond, but that there is spatial foraging segregation for a period of months when *C. lavaretus* from each basin forage to a large extent within that basin.

Thompson *et al.* (2008) found *C. lavaretus* mean allelic richness values of 4·9 in the north basin, 4·8 in the mid basin and 4·7 in the south basin of Loch Lomond. This was not significantly different. Thompson *et al.* (2008) also showed that there are no detectable genetic differences between *C. lavaretus* from any pair-wise comparison of basin. Thus, the apparent foraging site fidelity is not reflected in any genetic segregation, and the genetic and morphological information suggests a panmictic population with no spawning site fidelity. This suggests that a number of basin-specific individuals foraging in one basin contributing to spawning in another basin result in gene flow between *C. lavaretus* in the three basins.

There was a difference in age structure between basins, with young fish only being found in the south. Since the samples were caught during the spawning period, it was not expected that immature fish would be captured. While it is possible that the south basin is a better habitat for young fish, it may simply be that immature fish are less likely to be caught on the spawning grounds in the mid and north basins. Since this result was influenced by the capture of a small number of young fish, perhaps by chance, firm conclusions cannot be drawn. There were several outliers in the δ^{15}N values, including two fish that appeared to be feeding up to one trophic level above and below the others. The one fish apparently feeding at the highest trophic level was the youngest (1 year). There was a negative correlation between age and δ^{15}N and a positive correlation between age and δ^{13}C, suggesting that there is an ontogenic shift in feeding. This is common in many fish species in which juveniles are completely zooplanktivorous, while adults include some benthos in the diet, consistent with previous studies on Loch Lomond *C. lavaretus* feeding ecology (Slack *et al.*, 1957; Pomeroy, 1991). These changes are more likely to occur in a monomorphic population to avoid infraspecific competition between age classes (Hessen *et al*., 1986; Sandlund *et al*., 1992).

In conclusion, there was no evidence of substructuring or trophic polymorphism in Loch Lomond *C. lavaretus*, despite the size and complexity of this lake, often considered a prerequisite for the formation of substructuring. Although Parnell (1838) described two distinct *C. lavaretus* species in Loch Lomond, his description was based on only two specimens. According to Day (1884), Parnell later received intermediate forms of these fish, suggesting that these fish reflected natural variation between individuals within a single mixed population. Although Dottrens (1959) similarly proposed the existence of multiple forms of *C. lavaretus* in the single natural Welsh population in Llyn Tegid, their presence has never been confirmed, even following considerable examination and is probably a mistake (Gasowska, 1965; Haram, 1968). The lack of substructuring in Loch Lomond may reflect the species-rich nature of the lake (Adams, 1994). While *C. lavaretus* are likely to have been among the first invaders into Loch Lomond following the last glaciation and may have initially undergone population structuring, the subsequent invasion of other fish species filling the available niches probably precluded this. Importantly from a conservation perspective, *C. lavaretus* collected from one area of Loch Lomond are likely to be representative of the whole population.

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