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Winter ecology of specialist and generalist morphs of European whitefish, *Coregonus lavaretus*,
in subarctic Northern Europe

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Abstract:

European whitefish is a model species for adaptive radiation of fishes in temperate and subarctic lakes. In northern Europe the most commonly observed morphotypes are a generalist (LSR) morph and a pelagic specialist (DR) morph. The evolution of a pelagic specialist morph is something of an enigma however as this region is characterised by long, dark winters with pelagic primary production limited to a brief window in late summer. We conducted the first winter-based study of polymorphic whitefish populations to determine the winter ecology of both morphs, we combined seasonal diet and stable isotope analysis with several proxies of condition in three polymorphic whitefish populations. The generalist LSR morph fed on benthic and pelagic prey in summer but was solely reliant on benthic prey in winter. This was associated with a noticeable but moderate reduction in condition, lipid content and stomach fullness in winter relative to summer. In contrast, the DR whitefish occupied a strict pelagic niche in both seasons. A significant reduction in pelagic prey during winter resulted in severe decrease in condition, lipid content and stomach fullness in DR whitefish in winter relative to summer, with the pelagic morph apparently approaching starvation in winter. We suggest that this divergent approach to seasonal foraging is associated with the divergent life history traits of both morphs.

Significance statement:

We provide the first evidence of winter ecology of polymorphic whitefish populations in Europe.

The environmental conditions facilitating the evolution and maintenance of these polymorphic populations are an area of wide ecological interest and our findings underline the significance of winter ecology in shaping their divergence.

Introduction

Adaptive radiation of ancestral into multiple species has repeatedly occurred in different fishes from the tropical to the polar regions (Schluter, 2001; Seehausen & Wagner, 2014; Skúlason *et al.*, 2019). In the northern hemisphere, adaptation typically occurs along a benthic to pelagic gradient with morphs, also termed ecotypes, species or forms, developing foraging morphology to increase their efficiency at consuming benthic invertebrates or zooplankton (Bolnick & Ballare, 2020; Jonsson & Jonsson, 2001; Kahilainen & Østbye, 2006), but salmonids can also show divergence towards piscivory and profundal benthivory (Skoglund *et al.*, 2015; Tamayo *et al.*, 2020). Such adaptive radiations are typically associated with the presence of a vacant niche within the species habitat. For example, the adaptive radiation of cichlids in East African Rift lakes is thought to result from the absence of a competitors in newly created lake ecosystems (Seehausen, 2006). In the absence direct competition from specialist species, a few generalist species rapidly evolved into a diverse assemblage of phenotypes, and ultimately new species (Seehausen, 2006). Similarly, in northern Europe evidence of adaptive radiation in European whitefish (*Coregonus lavaretus*) and Arctic charr (*Salvelinus alpinus*) is seen as a consequence of the high niche availability after glacial retreat (Chavarie *et al.*, 2015; Harrod *et al.*, 2010; Jonsson & Jonsson, 2001).

This pattern of adaptive radiation following glacial retreat is especially true of whitefish populations in large northern Fennoscandian watercourses draining to the Barents Sea (Häkli *et al.*, 2018; Kahilainen & Østbye, 2006). As the ice sheets retreated these systems were isolated from southern watercourses and many competing species due to historical contingency and provided ecological opportunity for whitefish radiation. The most likely ancestor was a

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generalist type of whitefish that subsequently diverged into different morphs (Kahilainen & Østbye, 2006; Østbye *et al.*, 2006). Generally, divergence into pelagic and benthic morphs has occurred in large and deep lakes located lower elevation in the watercourses with slightly higher productivity (Siwertsson *et al.*, 2010). Furthermore, the original fish community in these systems was not colonised by competing fishes specialised for pelagic feeding, e.g. vendace, *Coregonus albula* (Kahilainen *et al.*, 2007). The absence of a pelagic specialist resulted in a vacant niche for adaptive radiation of planktivorous morph, a niche which European whitefish, *Coregonus lavaretus* adapted to fill.

Whitefish populations in this region are characterised by the presence of up to four distinct morphs within a single system (Kahilainen *et al.*, 2014; Thomas *et al.*, 2017). A widely distributed generalist morph, termed the large sparsely rakered morph (hereafter LSR) due to its feeding apparatus, is commonly believed to be the original morph of whitefish (Kahilainen & Østbye, 2006). The LSR morph, typically reaches a maximum size up to 60cm and 20 years of age, and is characterised by branchial arch containing 20-30 gill rakers typically circa 2.5 mm in length (Kahilainen *et al.*, 2011). Where present, the zooplanktivorous whitefish morph, hereafter referred to as the densely rakered morph (abbreviated to DR), is smaller and much shorter lifespan than the LSR morph, and is characterised by having 30-40, long (c. 4.3 mm) gill rakers on each branchial arch (Kahilainen *et al.*, 2011). Some lakes in the region also contain a profundal morph and a large pelagic morph, adapted for surface feeding (Kahilainen *et al.*, 2011).

The LSR and DR morphs in this region have been extensively studied over the last two decades (Kahilainen & Lehtonen, 2002; Thibert-Plante *et al.*, 2020). However, the presence of a pelagic specialist in the region remains something of an enigma. Lakes in subarctic Fennoscandia covered by a thick layer of ice and snow for approximately half of the year (Hayden *et al.*, 2014a, 2015). As these lakes are situated at approximately 69°N there is no sunlight for between late November and mid January, resulting in minimal pelagic productivity for at least 6 months of the year. There is a brief period of intensive pelagic production in these lakes in mid to late summer (Kahilainen *et al.*, 2009). Twenty-four-hour sunlight and weak thermal stratification result in a bloom of phyto- and zooplankton, typically between July and August (Forsström *et al.*, 2005; Hampton *et al.*, 2017). This pelagic resource window coincides with the principal growing season for fish in the region and provides essential fatty acids to whitefish, fuelling their growth and gonad development through subsequent months (Keva *et al.*, 2019). It is plausible, though as yet untested, that DR whitefish obtain sufficient benefit from maximising their capacity to consume high quality pelagic prey during this brief window of maximum prey abundance to sustain them through the lean winter months.

In order to develop a more complete understanding of the winter ecology of planktivorous whitefish we compared the diet and condition of DR and LSR whitefish in three subarctic lakes in northern Finnish Lapland. We sampled both morphs and their pelagic and benthic prey resources during the open water season in August-September and repeated sampling in March while the lakes were under thick ice-cover. We examined three aspects of winter ecology of whitefish morphs drawn from our overarching prediction that DR whitefish would primarily forage on pelagic prey throughout the year, increasing the risk of winter starvation relative to

generalist LSR whitefish. We measured Fulton's condition factor, elemental C:N ratio and stomach fullness, of both morphs in both seasons to assess inter-morph variation in biological condition during winter. We then compared seasonal changes in the diet, inferred from stomach content and stable isotope analyses, of both morphs to determine the winter diet of the DR morph, and ascertain whether it differed from the LSR morph. Finally, we examined seasonal trends in stable isotope derived niche width, of both morphs to determine seasonal changes in the trophic ecology of both morphs

Method

Sample collection

We tested these hypotheses on polymorphic whitefish populations in three subarctic lakes in northern Fennoscandia. Ropijärvi (hereafter L. Ropi - 68.41 °N, 21.35 °E) is situated on the Tornio-Muoniojoki watercourse, whereas Muddusjärvi (L. Muddus - 69.04°N, 26.94°E) and Vastusjärvi (L. Vastus - 69.07 °N, 27.11 °E) are on the Paatsjoki watercourse. All lakes are suited below the treeline and their catchment consist of birch or pine forest and peatland. The predominant land use in the region is eco-tourism and reindeer herding (Hayden *et al.*, 2019). All lakes are characterised by a cold-water adapted fish fauna dominated by salmonid fish including whitefish, Arctic charr, and brown trout, *Salmo trutta* (Hayden *et al.*, 2017). All lakes were sampled once in late summer (L. Ropi - September 2011, L. Muddus and L. Vastus - September 2014) and again in spring before ice off (L. Ropi - March 2012, L. Muddus and L. Vastus - March 2014). Fish and invertebrates were sampled in all cases, sampling methodology followed Hayden et al (2015). Each lake was subdivided into littoral (shoreline to compensation depth i.e. 1% surface light left), profundal (compensation depth to deepest point in each lake) and pelagic

(compensation depth to surface) zones as outlined in (Hayden et al. 2019). Zooplankton were sampled using a 50 µm plankton net vertically hauled from 14 or 20 m to lake surface at the deepest point in L. Vastus and Ropi, but in L. Muddus we selected the deepest location (20 m) in the northern part of main basin. Three replicate tows were taken during each sampling event. Zooplankton were stored in lake water until return to the laboratory where they were frozen at -20°C. Zooplankton were then counted and the zooplankton density ($n L^{-1}$) was estimated by dividing the number of zooplankton in each tow by the towed volume. Benthic invertebrates (BMI) were collected using an Ekman grab (sampling area 272 cm²), three replicate samples were taken from littoral (1m depth) and profundal (14 or 20m depth) habitats during each sampling event. Benthic invertebrates were sorted to family level and BMI density at each habitat and season were calculated. A representative bulk zooplankton sample as well as BMI samples of each family were frozen for stable isotope analysis. Fish were sampled using benthic and pelagic gill nets. Each net series consisted of seven 1.8 x 30m panels (knot to knot mesh size: 12, 15, 20, 25, 30, 35 & 45mm) and a 1.5 x 30m Nordic multimesh net comprised of twelve equidistant panels (mesh sizes 5 - 55mm). In each lake three replicate nets were set in littoral, profundal and pelagic (floating above profundal zone) zones. In summer, nets were set overnight for c. 10-12 hrs, whereas in winter soak time was extended to 24-48 hrs due to lower fish activity. Whitefish were identified to morph based on body shape and gill raker morphology (Kahilainen *et al.*, 2011; Kahilainen & Østbye, 2006). LSR whitefish has silvery coloration, robust body shape, downwards mouth position and gill rakers are short, widely spaced and few in numbers (Kahilainen & Østbye, 2006). DR whitefish has darker colouration, slender body shape, pointed head, equal jaw length and gill rakers are long, densely spaced and numerous (Kahilainen & Østbye, 2006). All DR and LSR whitefish sampled were weighed (wet mass, ±0.1

g), measured (fork length, $\pm 1.0\text{mm}$) and Fulton's condition factor (k) was determined for each individual following the formula,

$$k = \frac{M}{L^3} \times 100$$

where M is total mass in grams and L is total length in cm. A representative subsample ($n=50$) were frozen for diet and stable isotope analysis.

Laboratory analysis.

The first left gill arch was dissected from all whitefish sampled and gill rakers were counted under preparation microscope. Stomachs were dissected from DR and LSR whitefish morphs. Stomach fullness was estimated on a scale of 0-10 where 0 represents empty and 10 represents a fully distended stomach. Prey items were identified to family level and the relative contribution of each family to the full stomach content was estimated using the points method (Swynnerton & Worthington, 1940). A 1cm^3 muscle plug was dissected from each whitefish and oven dried at 60°C for 48 hrs or freeze-dried at -50°C for 48 hrs. Similar plugs of liver tissue were obtained from all whitefish sampled in L. Muddus and L. Vastus, liver samples were not collected from whitefish in L. Ropi.

Zooplankton, benthic invertebrate were similarly dried and subsequently all samples were ground to a fine powder. Subsamples were weighed ($1 \pm 0.1\text{ mg}$) into tinfoil capsules and submitted to the Stable Isotopes in Nature Laboratory, University of New Brunswick, Canada, for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis. Elemental carbon and nitrogen composition of each sample was assessed using a Carlo Erba NC25000 elemental analyser. Carbon and nitrogen isotope ratios were conducted using a Thermo Finnegan DeltaPLUS Advantage mass spectrometer (Thermo Finnegan, Bremen Germany). Isotope ratios are

presented relative to international standards ($\delta^{13}\text{C}$ - Vienne Pee Dee Belemite; $\delta^{15}\text{N}$ - atmospheric N_2). Repeat analysis of several in house standards indicated that instrumental error was below 0.1‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Carbon isotope ratios of fish muscle and liver tissues were arithmetically corrected to remove the influence of lipid using the tissue specific models defined by Logan et al. (2008).

Data analysis

Seasonal changes in resource availability

We used linear mixed effects models to examine seasonal changes in the density of zooplankton, littoral and profundal BMI. In each case, season was the sole fixed effect and lake was treated as a random effect. Models were performed using the ‘*nlme*’ package in R. Model residuals were inspected using the ‘*performance*’ package in R to ensure that we did not violate the assumptions of each model.

Seasonal changes in body condition

We used linear mixed effects models to examine seasonal differences in the seasonal changes in Fulton’s condition factor (k), elemental C:N ratio, a proxy for lipid content (Post, 2002) and stomach fullness of each morph. In each case, season, morph and the season \times morph interaction were fixed effects and lake was treated as a random effect. Model residuals were inspected using the ‘*performance*’ package in R to ensure that we did not violate the assumptions of each model.

Season changes in diet and resource use

We assessed seasonal changes in diet of both morphs using stomach content and stable isotope analysis. Prey items identified in the stomach of each whitefish were assigned to benthic or pelagic prey categories following Hayden et al. (2019). We then used a linear mixed effects model to assess differences in the contribution of pelagic prey items to the diet between morphs and seasons, whereby the proportion of pelagic prey in the diet was a response variable, morph, season and the morph * season interaction were fixed effects and lake was a random effect. Mixed effect models were performed using the 'nlme' package in R.

We used permutational analysis of variance (PERMANOVA) to determine season and morph related differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios of whitefish. We first created Bray-Curtis similarity matrix from the bivariate isotope data for each whitefish sampled. Carbon isotope ratios was multiplied by -1 to meet the requirements of a Bray-Curtis matrix. Variation within this matrix associated with three fixed effects (morph, season, tissue) and all possible interactions was determined using the vegan package in R (Oksanen *et al.*, 2014). Though this approach does not account for random effects morph, season and tissue were nested within Lake. The model assessed statistical significance of each factor based on 9,999 permutations.

We used the two source - one tracer mixing model defined by Post (2002) to estimate seasonal changes in the contribution of pelagic primary production to DR and LSR whitefish morphs. We used the mean values of littoral BMI and zooplankton collected in each lake as lake specific littoral and pelagic food web endmembers respectively. In each we assumed trophic fractionation of $\delta^{13}\text{C}$ between prey and fish to be 1‰ (Post, 2002). We initially intended to use a three-source model accounting for the possible contribution of profundal derived material to each morph, but this approach was confounded by zooplankton and profundal invertebrates having similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios (Supporting Fig. 1). Separate mixing models were performed for

muscle and liver tissue. As above we then used mixed effects linear models to assess seasonal variation in the contribution of pelagic resource to liver and muscle tissue datasets of DR and LSR whitefish morphs.

Seasonal changes in isotopic niche width

We used the SIBER package (Jackson *et al.*, 2011) to assess the isotopic niche width of each morph population sampled, with separate analyses conducted for muscle and liver datasets. In each case we calculate the Bayesian estimate of a standard ellipse encompassing all the isotope values of each population. We assessed seasonal variation among and between morphs in each lake by comparing the 95% credibility intervals on each ellipse.

Results

In total, we sampled 3,235 whitefish, of which 598 were retained for diet and isotope analysis (Supporting Table 1). We obtained fewer fish during the winter sampling events (summer - 2,392; winter - 843), although the size range and distribution was similar between seasons (Supporting Table 1). Across all samples, LSR whitefish (mean \pm SD: 22.6 \pm 5.7 cm) were approximately 7 cm larger than DR whitefish (14.7 \pm 2 cm). The DR whitefish morph had a greater number of gill rakers (mean 34.2) than the LSR morph (mean 24.1).

Seasonal changes in resource availability

We observed a pronounced decrease in zooplankton density in winter relative to summer, whereas seasonal variation in benthic macroinvertebrate (BMI) density was less clear (Fig. 1).

Zooplankton density differed among lakes but in all cases winter density was a small fraction of that observed in summer (Fig. 1; $F_{1,14} = 22.9$, $P < 0.001$). In contrast, seasonal variation in littoral BMI density was below statistically identifiable levels ($F_{1,14} = 1.2$, $P = 0.3$), whereas seasonal variation in profundal BMI density was marginally significant ($F_{1,14} = 5.7$, $P = 0.03$) due to pronounced variation observed in L. Muddus (Fig. 1)

Seasonal changes in biological condition

We assessed seasonal changes in the biological condition of each morph using three measures of condition. Fulton's condition factor (k) of both morphs was lower in winter than in summer, across all lakes (Fig. 2a). However, the relative decrease in DR whitefish exceeded that observed in LSR whitefish (Fig. 2a, Table 1). Across all lakes, Fulton's mean (\pm SD) condition factor in DR whitefish decreased from $0.67 (\pm 0.06)$ in summer to $0.58 (\pm 0.05)$ in winter, exceeding the decrease in LSR whitefish (summer, 0.73 ± 0.06 ; winter, 0.67 ± 0.07 ; Table 1). Elemental C:N ratio, a proxy for lipid content, was assessed for both muscle and liver tissue of both morphs. Liver typically had a higher C:N ratio value than muscle, no clear differences were evident between the morphs (Fig. 2c). A small but statistically significant decrease in winter (3.2 ± 0.1) relative to summer (3.3 ± 0.1) was observed in DR whitefish, but no difference was seen in LSR whitefish (winter, 3.2 ± 0.1 , summer, 3.2 ± 0.1 ; Fig. 2b; Table 1). Similarly, no seasonal shift was seen in C:N ratios of DR whitefish liver (winter, -4.0 ± 0.3 ; summer, -4.0 ± 0.2), and the C:N ratio in LSR whitefish liver was actually higher in winter (4.3 ± 0.9) than summer (4.1 ± 0.2 ; Fig. 2c; Table 1). Stomach fullness decreased in both morphs in winter, though as with the Fulton's condition factor, the decrease was considerably more pronounced in DR whitefish (Fig. 2d; Table 1).

Seasonal changes in diet and resource use

We observed considerable difference in the relative amount of pelagic prey in the stomach content of both whitefish morphs (Fig. 3). The DR morph almost exclusively consumed pelagic prey in summer (mean \pm SD = 86 \pm 31%), while the proportion of pelagic prey consumed decreased in winter (72 \pm 44%), it was still the main component of DR diet (Fig. 3). In contrast, LSR whitefish predominantly consumed benthic prey in summer (mean \pm SD = 72 \pm 42%) and winter (91 \pm 27%), though in both seasons the intra-population variation in diet was greater in LSR than DR morphs (Fig. 3). These observations were supported by the mixed effects model which identified significant variation among morphs ($F_{1,1965} = 804.1$, $P < 0.001$), seasons ($F_{1,1965} = 111.7$, $P < 0.001$), and the morph * season interaction ($F_{1,1965} = 49.3$, $P < 0.001$), indicating that seasonal variation in diet differs between morphs when assessed across all three study lakes.

PERMANOVA of whitefish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios identified significant variation associated with lake, morph, season, tissue and several interactions (Fig. 4, Table 2). The greatest amount of variation was explained by lake ($r^2 = 0.22$) and morph ($r^2 = 0.15$), with season accounting for a much smaller amount of variation ($r^2 = 0.03$). Notably, the interactions between tissue* season and morph*season were also significant, indicating that seasonal differences are morph and tissue specific (Table 2). We observed greater seasonal variation in liver than in muscle, notably in DR whitefish with winter samples being ^{15}N -enriched relative to summer in both morphs, though the enrichment was more pronounced in DR morph (Fig. 4). There was some evidence that DR whitefish muscle in L. Vastus was ^{13}C -depleted in winter relative to summer but this trend was not observed in other lakes. Similarly, some individual LSR whitefish

in L. Ropi were ^{13}C -depleted in winter relative to summer, but that was not replicated in either of the other lakes.

Estimates of pelagic resource reliance inferred from stable isotope mixing models also showed considerable variation among lakes, morphs and seasons, though the trends were not as clear as in the stomach content data (Fig. 5). Across all lakes, DR whitefish were primarily reliant on pelagic resources in both summer (mean \pm SD = $56 \pm 23\%$) and winter ($60 \pm 17\%$), whereas LSR whitefish were more reliant on benthic primary production in both seasons (summer: $29 \pm 30\%$; winter: $41 \pm 30\%$; Fig. 5). A linear mixed effects model indicated that the majority of variation within the data was related to species ($F_{1,855} = 240$, $P < 0.001$) and tissue type ($F_{1,855} = 53.7$, $P < 0.001$), with season ($F_{1,855} = 30.1$, $P < 0.001$) and the season * species interaction ($F_{1,855} = 13.6$, $P < 0.001$) having a smaller, though still statistically significant effect. All other interaction terms were not statistically significant and are not considered further.

Seasonal changes in isotopic niche width

The LSR whitefish morphs had larger isotopic niche width than DR morphs in most lake and tissue combinations, the only exception being in L. Ropi (Fig. 4, Supporting Fig. 2). The isotopic niche width of each morph was comparatively consistent between seasons. The isotopic niche determined from LSR whitefish muscle values was greater in winter than in summer, but in all other cases the 95% credibility intervals of summer and winter datasets overlapped (Fig. 4, Supporting Fig. 2).

Discussion

Seasonal contrasts between summer and winter are pronounced in subarctic regions of the globe, 24 hours sunlight drives intense primary production in late summer whereas winter is considered to be an extended period of dormancy and low productivity in aquatic ecosystems (Studd *et al.*, 2021). We measured the effect of this variation on the trophic ecology and biological condition of pelagic specialist and generalist morphs of whitefish in three lakes in Northern Finland. Both morphs displayed highly divergent responses to seasonal variation. As expected, DR whitefish were primarily reliant on pelagic prey in summer, but contrary to our initial prediction, relied on pelagic prey in winter too, essentially driving them towards seasonal starvation as evident in the marked decrease in condition and stomach fullness observed in winter. In contrast, LSR whitefish displayed a generalist trophic ecology, though primarily reliant on benthic prey, and showed modest decreases in condition and stomach fullness in winter.

Our study had three principal predictions, the first of which, that DR whitefish would show a greater decrease in condition in winter than LSR whitefish was supported by most of the data. Seasonal decrease in Fulton's condition factor was greater in DR than LSR whitefish, in line with our initial expectation. However, contrary to our expectation patterns observed in elemental C:N ratio of muscle and liver tissue were not as clear. Elemental C:N ratios in both tissues of both morphs varied slightly between seasons, but there was no clear trend in the data, suggesting that whitefish are still laying down lipid reserves in late summer or using stored visceral lipids throughout the winter (Luzzana *et al.*, 1996). The absence of a clear pattern in the elemental C:N ratio indicates that the reduced condition factor values observed in winter reflect a decrease in protein or muscle content rather than simply a decrease in lipid reserves, suggesting that DR whitefish may be approaching starvation by late winter (see also Hayden *et al.*, 2015; Keva *et al.*, 2017) Several biomarkers, such as stable isotopes, total mercury and fatty acids,

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from slow growing subarctic whitefish suggest that year-round patterns are characterized by post-spawning starvation under lake ice and rapid increase of condition in open water summer (Hayden *et al.*, 2014a; Keva *et al.*, 2017, 2019). Such starvation effects were further supported by the elevated $\delta^{15}\text{N}$ values observed in the liver of DR, and to a smaller extent LSR whitefish, during winter. Such elevations in $\delta^{15}\text{N}$ are associated with starvation as a consumer begins to assimilate its body tissue and detected in subarctic whitefish in winter (Doi *et al.*, 2017; Keva *et al.*, 2017). Further, the C:N ratios observed in both tissues are close to the 3:1 ratio characteristic of pure protein, suggesting that lipid content of both tissues is low in both summer and winter (Post *et al.*, 2007). In general, whitefish muscle contain little lipids in general, and other lipid reserves such as visceral fat are likely more important in year round lipid dynamics (Keva *et al.*, 2019; Luzzana *et al.*, 1996; Thomas *et al.*, 2019). This aspect of our results is inconclusive however, as we also observed an increase in the $\delta^{15}\text{N}$ values of zooplankton, likely related on copepod dominated zooplankton community, in winter relative to summer (Hayden *et al.*, 2014a), which could also explain the increased $\delta^{15}\text{N}$ values in DR whitefish. Comparative experimental studies on fish physiological responses versus trophic ecology are essential to resolve the major mechanism of year-round changes of stable isotope ratios. Further investigation, potentially using hormonal markers to identify starvation would be interesting in this regard, and may provide some insight into the impact of winter on the biological condition of both morphs (Bar, 2014).

Our second prediction, that DR whitefish would rely on pelagic resources in both summer and winter was roundly supported by the data. While LSR whitefish shifted from a generalist diet in summer to one almost exclusively dominated by benthic prey in winter, the increase in benthic prey in DR whitefish stomach was marginal. In addition, DR whitefish stomach fullness

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decreased dramatically in winter, indicating that these pelagic specialists continue to rely on scarce zooplankton in winter rather than switching to benthic prey. Interpreting the relative contribution of pelagic and benthic prey from the stable isotope data presented in our results is challenging. Profundal benthic invertebrates predominantly assimilate pelagic derived carbon, which can result in their having similar $\delta^{13}\text{C}$ values to zooplankton (Hayden *et al.*, 2013).

Therefore, it is difficult to say with certainty whether the winter pelagic contribution to DR whitefish is through foraging on zooplankton or increased contribution of profundal prey. The most likely scenario is that both of these are correct as the increased benthic contribution to the diet of DR whitefish was not reflected by a decrease in estimated pelagic resource use. When viewed in unison the stable isotope and diet data for DR whitefish are consistent with our expectation that this morph is primarily reliant on pelagic prey in both seasons.

The specialist nature of DR whitefish was further confirmed by our analysis of our third prediction, that DR whitefish would increase their isotopic niche in winter, whereas the isotopic niche of LSR whitefish would decrease. In contrast to this expectation the overriding result from this aspect of the study was the generalist/specialist trophic ecology of both morphs is consistent between seasons. The isotopic niche width of DR whitefish changed marginally between seasons, with no clear pattern evident between lakes or tissue types. If DR whitefish were, as we expected, incorporating greater amounts of benthic prey, this would lead to an increase in their isotopic niche, similar to the larger isotopic niche seen in generalist LSR whitefish (Harrod *et al.*, 2010; Thomas *et al.*, 2017). The absence of any discernable trend in the data suggest that this is not the case. Similarly, the isotopic niche of LSR whitefish was consistent between seasons and tissues, commensurate with their characterisation as a benthic generalist in both seasons. One of the key underlying factors is that turnover rate of stable isotope ratios is slow growing subarctic

fish in very slow and may take years, especially for muscle tissue (Hayden *et al.*, 2014a; Thomas & Crowther, 2015)

Our results provide some new insight into the ecology of both morphs but should be examined in the context of the study systems. The seasonal variation in LSR whitefish diet observed here is lower than that's recorded in monomorphic whitefish populations, i.e. populations only consisting of the LSR morph, in this region. Previous work has shown that LSR whitefish in those conditions feed extensively on zooplankton during the summer bloom (Hayden *et al.*, 2014a, 2015). This pelagic feeding has been shown to provide LSR whitefish with essential fatty acids such as DHA and EPA, necessary for gonad development later in the year (Keva *et al.*, 2019). However, in polymorphic populations, the presence of DR whitefish restrict LSR whitefish from extensive feeding on zooplankton (Hayden *et al.*, 2014b), likely explaining the prevalence of benthic prey in the diet of LSR whitefish detailed here, while also providing some insight into the life history characteristic of DR whitefish which occupy that pelagic niche in polymorphic systems.

In comparison to LSR whitefish, the DR morph employs an r-strategy (Kahilainen *et al.*, 2005, 2003). DR whitefish mature earlier than LSR whitefish, DR whitefish typically mature in their third year whereas in LSR whitefish this does not occur until the fifth year (Kahilainen *et al.*, 2017). In addition, life expectancy is much shorter for DR whitefish (max. 6-7 years) than LSR whitefish (15-20 years; Thibert-Plante *et al.*, 2020). This strategy, in combination with the dietary and biological variation detailed here, may explain how DR whitefish persist in such seemingly inhospitable conditions. This life history strategy does not require DR whitefish to grow to large size or maintain growth for many years, rather it is sufficient for them to consume enough high-quality prey in later life to reach maturation and maximise their reproductive output

(Thibert-Plante *et al.*, 2020; Vesterinen *et al.*, 2021). The DR whitefish spawn in autumn of their third year and subsequent mortality is very high (Kahilainen *et al.*, 2017; Thibert-Plante *et al.*, 2020). In addition to resource limitation, DR whitefish is the main prey for piscivorous fish, especially pelagic brown trout, further elevating mortality and likely boosting r-selective life-history traits (Kahilainen & Lehtonen, 2002; Thibert-Plante *et al.*, 2020; Thomas *et al.*, 2017). Excluding LSR whitefish from the pelagic niche would allow DR whitefish to exploit the summer plankton bloom and assimilate the high-quality fatty acids fuelling their own gonad development (Hayden *et al.*, 2014a; Keva *et al.*, 2019). In contrast, slow growing and late maturing LSR whitefish forage on perhaps lower quality, but consistently available and abundant benthic invertebrates (Hayden *et al.*, 2014a; Keva *et al.*, 2019; Thomas *et al.*, 2019).

Our study was primarily focussed on whitefish, but our findings have broader implications for the understanding of subarctic lakes during winter. A traditional narrative that these ecosystems are ‘dormant’ during winter has been repeatedly challenged in recent years. Notably, some recent work has demonstrated that pelagic primary production continues under lake ice, especially in temperate regions where levels of sunlight remain sufficient to fuel plankton growth (Hampton *et al.*, 2017; Ozersky *et al.*, 2021). Our data provide further support for this highlighting how pelagic specialist DR whitefish can survive winter, albeit with extreme impacts to their body condition, while occupying an almost exclusively pelagic niche. We observed an increase in $\delta^{15}\text{N}$ values of zooplankton by approximately 2-5‰ in winter relative to summer. This is equivalent to one trophic level and supports previous findings in this region that zooplankton community is dominated by copepods in winter, emphasising the complexity of seasonal changes in food web structure. Though not included in our analysis here some field observations lend further support to this. We captured several large piscivorous brown trout in

pelagic set gill nets in L. Ropi and L. Muddus. Stomach content analysis of these trout indicated that they had recently consumed DR whitefish. As such, not only are DR whitefish maintained through winter they are also integral part of a winter pelagic food chain, consisting of herbivorous zooplankton, carnivorous zooplankton, DR whitefish and brown trout (Kahilainen *et al.*, 2009; Thomas *et al.*, 2019).

The presence of pelagic specialist DR whitefish in lakes with low pelagic primary production for 6-8 months of each year remains an enigmatic puzzle. Unlike the generalist LSR whitefish morph, pelagic specialist whitefish maintain a pelagic niche throughout the year, but this has a major impact on the condition potentially even resulting in starvation. We suggest that the perseverance of DR whitefish in these systems is associated with selection towards a *r* strategy, whereas reliance on more constantly available benthic resources drives towards K-selective strategy. This hypothesis needs to be further developed but highlights the insight which can be gained from winter ecology in subarctic regions.

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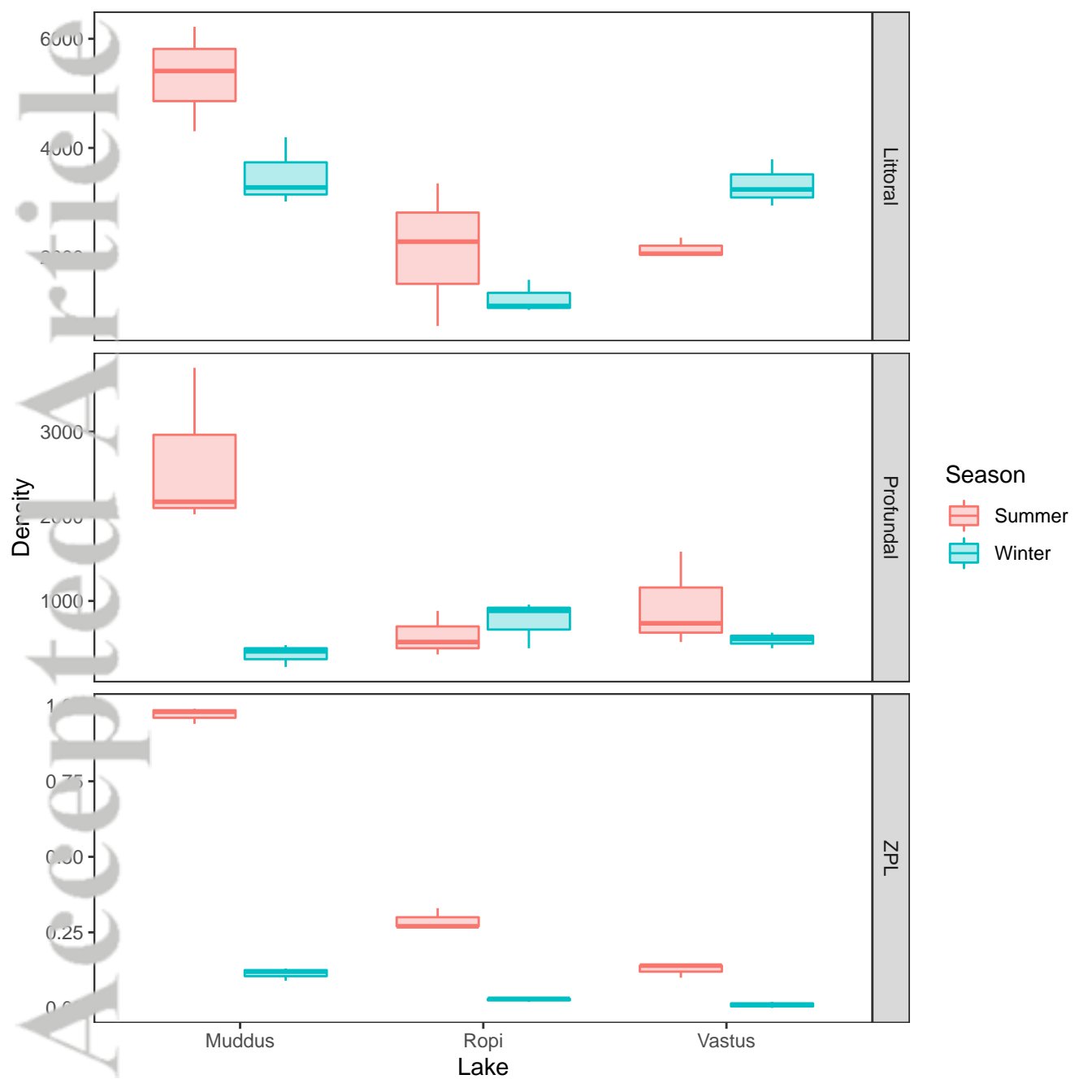
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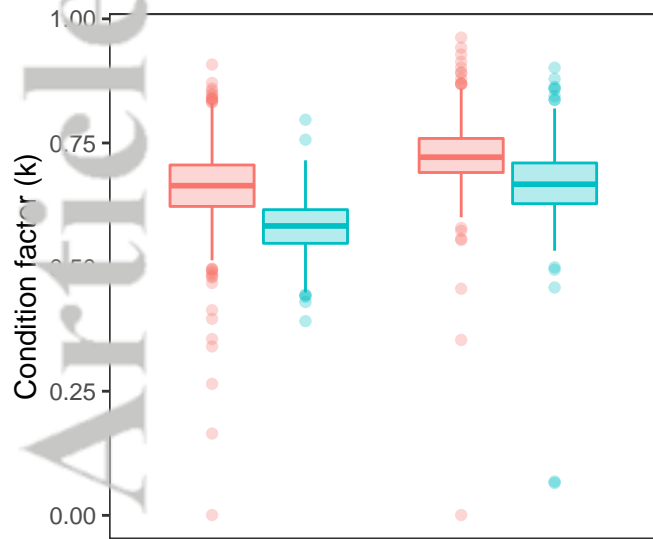
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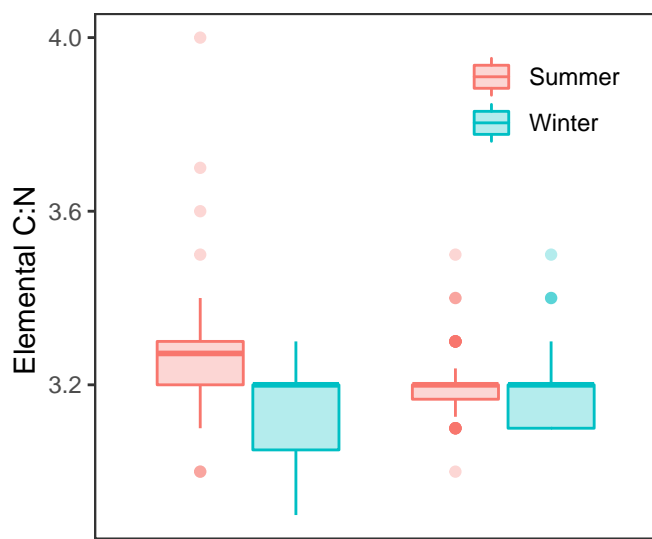
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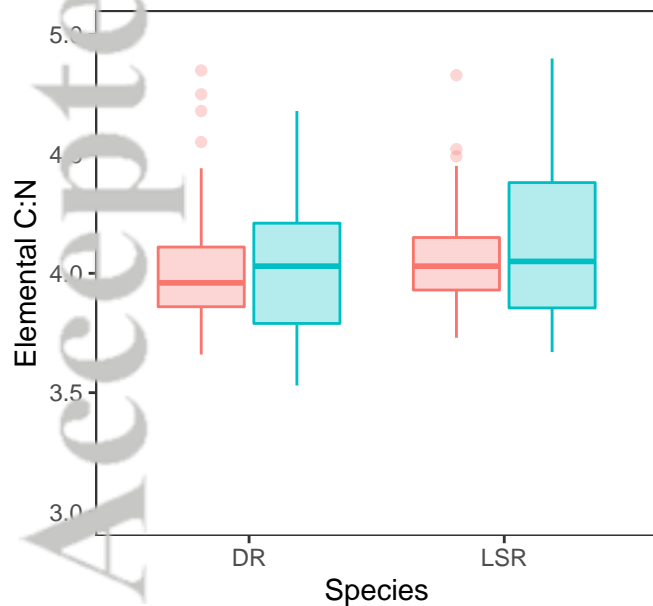
(a) Fulton's Condition factor



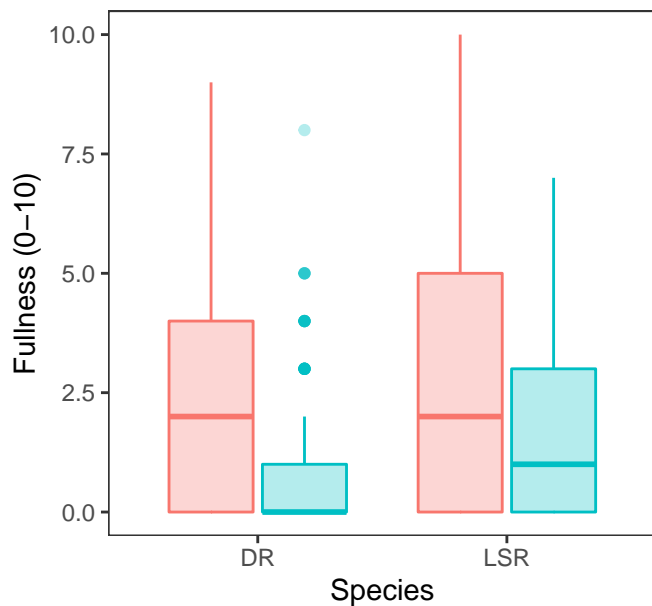
(b) C:N ratio in muscle

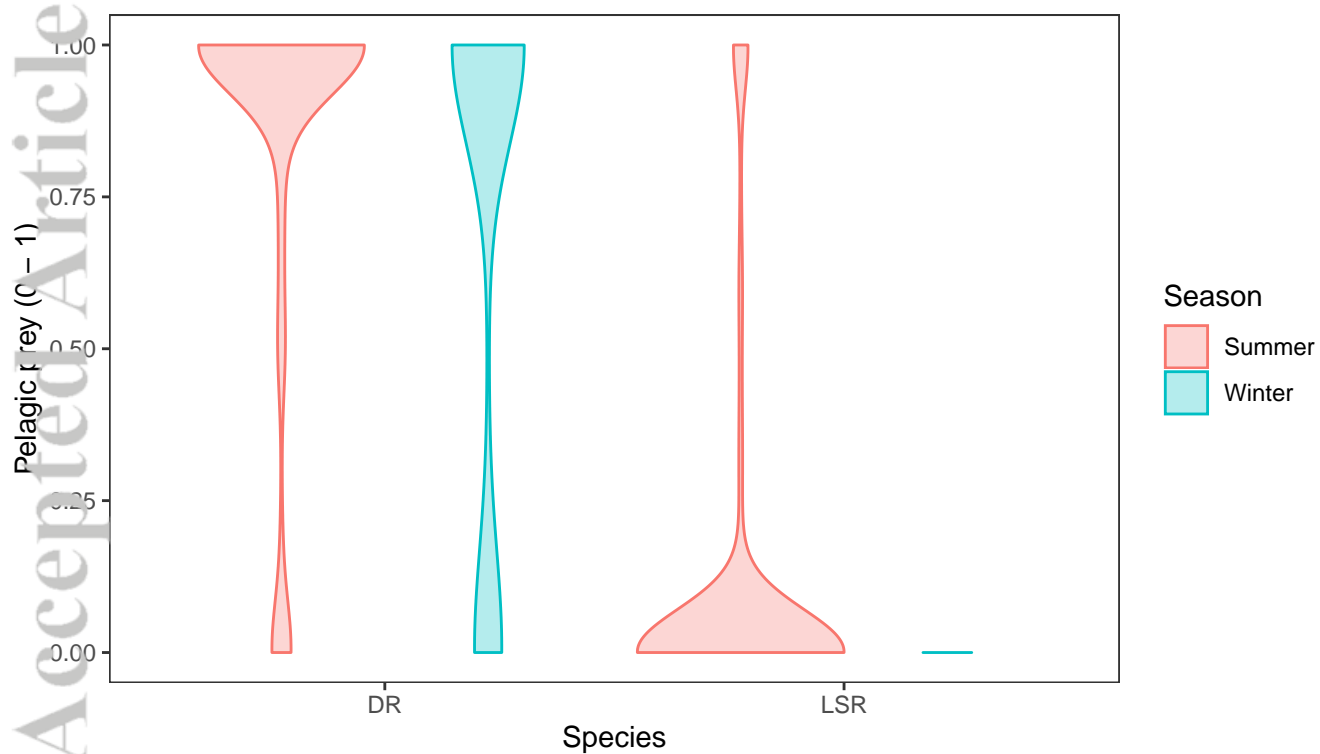


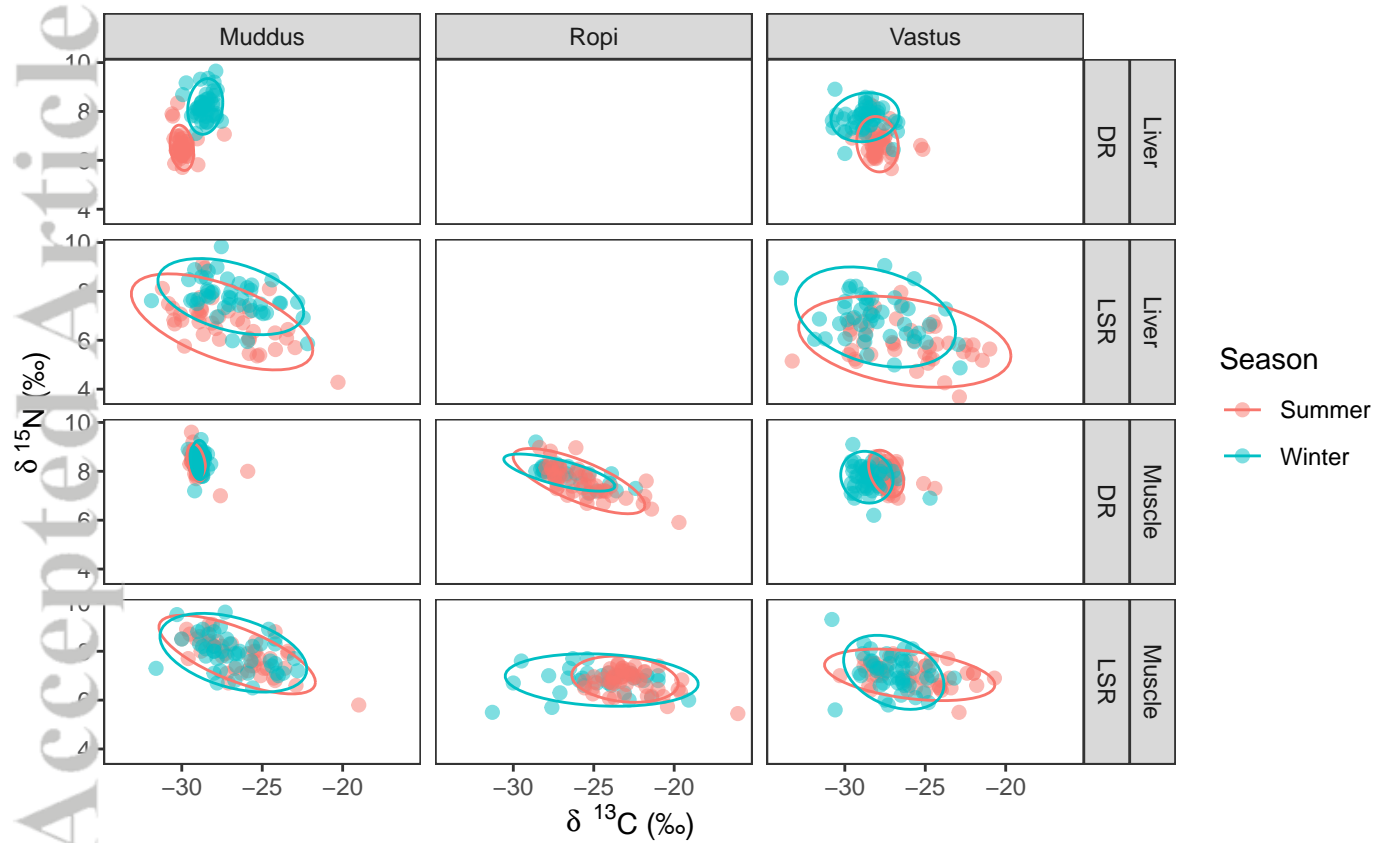
(c) C:N ratio in liver



(d) Stomach fullness







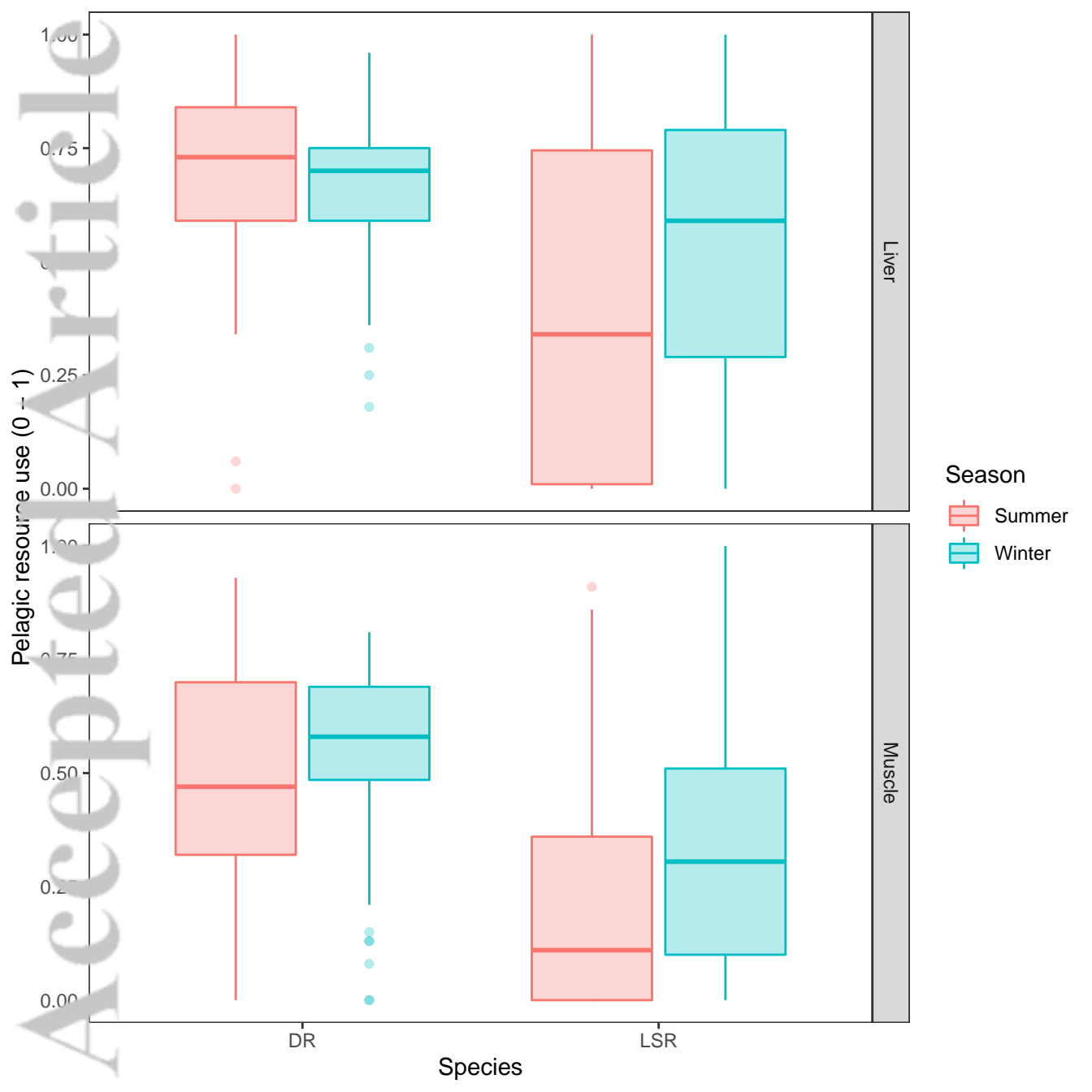


Figure 1. Seasonal changes in the density of littoral and profundal benthic macroinvertebrates (ind m⁻²) and zooplankton (ind L⁻¹) in three lakes in Northern Fennoscandia. Solid lines, shaded boxes and whiskers denote median, interquartile range and 1.5 times the interquartile range respectively

Figure 2. Seasonal changes in the biological condition of DR and LSR morphs of European whitefish sampled in three lakes in Northern Finland. Comparisons of biological condition across four metrics are presented. Note that liver C:N ratio data are not available for L. Ropi.

Figure 3. Violin plots outlining seasonal variation in the relative contribution of pelagic prey items in the stomach content of DR and LSR whitefish morphs in three lakes in Northern Finland. Note that no pelagic prey were observed in the stomach content of LSR whitefish sampled in winter.

Figure 4. Scatterplots detailing the seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios in muscle and liver tissue of DR and LSR whitefish in three subarctic lakes in Northern Finland. Standard ellipses are fitted to the data for illustrative purposes. Liver data were not collected for L. Ropi.

Figure 5. Boxplots plots detailing seasonal variation in the relative contribution of pelagic carbon to DR and LSR morphs of European whitefish. Values representing muscle and liver tissues are presented. Liver data were not collected form L. Ropi.

Significance statement:

We provide the first evidence of winter ecology of polymorphic whitefish populations in Europe. The environmental conditions facilitating the evolution and maintenance of these polymorphic populations are an area of keen ecological interest and our findings outline the ecological mechanisms underpinning radiation in these harsh northern environments.

Table 1. Seasonal changes in Fulton's condition factor, elemental C:N ratio of liver and muscle, and stomach fullness observed in DR and LSR morphs of European whitefish. Summary of mixed effects model assessing variation between season, morphs and the season \times morph across three study lakes. Lake was included as a random effect in the model.

Metric	d.f.	Intercept	Season	Morph	Season \times Morph
Fultons (k)	1,3233	1269.3***	1190.4***	528.1***	8.1***
C:N - muscle	1,513	21342.6***	60.7***	5.5*	37.1***
C:N - liver	1,343	5372.2***	7.4**	10.2**	5.9*
Fullness	1,1965	40.9***	119.9***	21.3***	9.9**

*** P < 0.001, **P < 0.01, *P < 0.05

Table 2. Results of PERMANOVA testing seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios of DR and LSR whitefish morphs in three lakes in northern Finland. Degrees of freedom (d.f.), R^2 , Pseudo F and P values are presented for all factors and interactions. Statistically significant factors and interactions are highlighted in bold.

	d.f.	R^2	F	P
Lake	2	0.22	190.98	<0.001
Morph	1	0.15	261.77	<0.001
Season	1	0.03	55.26	<0.001
Tissue	1	0.04	65.99	<0.001
Lake:Morph	2	0.02	19.99	<0.001
Lake:Season	2	0.01	12.63	<0.001
Morph:Season	1	0.00	5.62	0.011
Lake:Tissue	1	0.00	3.82	0.044
Morph:Tissue	1	0.00	2.12	0.113
Season:Tissue	1	0.01	25.54	0.001
Lake:Morph:Season	2	0.00	1.48	0.247
Lake:Morph:Tissue	1	0.00	-0.84	1
Lake:Season:Tissue	1	0.00	0.27	0.708
Morph:Season:Tissue	1	0.00	1.87	0.167
Lake:Morph:Season:Tissue	1	0.00	0.51	0.543
Residual	847	0.49271		
Total	866	1		