

Master's Thesis

**Resource use of brown trout (*Salmo trutta*) colour
morphs in a Norwegian coastal watercourse**

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Resource polymorphism is common in salmonid fishes and often increases diversity of the population, for instance by supporting individual variation in diet, habitat use and growth. Brown trout (*Salmo trutta*) can exhibit different life history traits often related to adaptation to littoral and pelagic resource use or to freshwater and marine feeding habits. Brown trout can also show variation in body colour, but the ecological basis for colour morphs, such as difference in trophic niches, remains poorly understood. Carbon, nitrogen and sulphur stable isotope analyses were conducted to investigate potential differences in long-term assimilated diets of three brown trout morphs that differed in body colour (brown, intermediate, silver). Based on the results of isotopic mixing models, the main prey items in all morphs were zooplankton and three-spined sticklebacks (*Gasterosteus aculeatus*). There were size related niche shifts from pelagic zooplankton diet to sticklebacks and from freshwater resources towards marine food sources in all colour morphs at the natural lengths of 250–300 mm except brown morph switched to marine food sources at the natural length of 400mm. Based on results of isotopic variation, the niche regions were overlapping among all colour morphs and silver morph had smallest niche size, however it was not significantly different. Understanding resource polymorphism within a population gives us insight to the possible harms the morphs in the population might face due to possible environmental changes.

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Lohikalojen resurssien käytössä on yleisesti vaihtelua, mikä lisää populaation monimuotoisuutta esimerkiksi lisäämällä vaihtelua yksilöiden ruokavaliossa, elinympäristön käytössä ja kasvussa. Taimenilla on erilaisia elinkierto-ominaisuuksia usein liittyen sopeutumiseen litoraalin benttisten ja pelagiaalin planktisten ravintokohteiden sekä makeanveden ja meriveden elinympäristön käyttöön. Taimenten ihonväriyksessä voi olla vaihtelua, mutta värimuotojen ekologisista lokeroista ei tiedetä paljoa. Taimenen värimuotojen (ruskea, välimuoto ja hopea) ravinnonkäyttöä tutkittiin norjalaisessa rannikkovesistössä hiilen, typen ja rikin vakaiden isotooppien avulla. Taimenten pääasialliset ravintokohteet olivat eläinplanktonit ja kolmipiikit (*Gasterosteus aculeatus*). Kaikki muodot osoittivat kasvunaikaisia ravinnonkäytön muutoksia pelagiaalin eläinplanktonista kolmipiikkeihin ja makeanveden ravintokohteista meriravintokohteisiin 250–300 mm pituisina paitsi ruskea muoto siirtyi meriravintokohteisiin 400 mm pituisena. Vaihtelu eri muotojen vakaiden isotooppien arvoissa osoitti, että eri muotojen ekologisten lokeroit olivat päällekkäiset ja niiden ekologisten lokeroitien koissa ei ollut merkittäviä eroja, vaikka hopealla muodolla oli muodoista pienin ekologinen lokero. Populaation resurssien käytön laajuus auttaa ymmärtämään mahdollista vaaroista, joita eri muodot voivat kohdata mahdollisten ympäristömuutoksien seurauksena.

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1 INTRODUCTION

The habitat that an individual, subpopulation, population or species occupies and uses as feeding and reproduction grounds is known as niche. The usage of different niches by different groups of individuals within a population is known as resource polymorphism and can occur due to phenotypic and genetic differences within the population (Skúlason & Smith 1995; Skúlason et al. 2019). In a population, group of individuals exhibiting the same phenotype are called morphs. Salmonid fishes are known to show resource polymorphism, which is often related to feeding on littoral benthic versus pelagic planktonic prey (Olson et al. 2019) or migratory feeding habits (Skúlason & Smith 1995). Resource polymorphism can occur within populations living in habitats that have opportunities for niche expansion through migration, decreased resource competition between species or increased resource competition within species (Skúlason & Smith 1995, Martin & Pfennig 2010, Woods et al. 2012, Skúlason et al. 2019). Generally, resource polymorphism enhances the survival of fish populations due to exploitation of different resources and time of reproduction and is seen as something positive for the population (Roches et al. 2021). On the other hand, the lack of resource polymorphism in a population makes the population reliant on certain resources and this can lead to overuse of the resources and can therefore threaten the survival of the population (Paull et al. 2012, Skúlason et al. 2019). Resource polymorphism, phenotypic variation and genetic variation are all linked meaning that when resource polymorphism is observed among different phenotypes it is most likely also observed among the genotypes of these morphs and vice versa. Variations in phenotype and genotype determine the variation in resource use (diet and habitat) of populations (Roches et al. 2021). Variation in population resource use is known as the niche width of a population. Many salmonid populations have migratory individuals ranging from lakes and rivers to marine environments and they can change their feeding habits based on the environment and food availability (Skúlason & Smith 1995). If this change in fish feeding habits happens to many fish simultaneously, it can have negative effects on the community such as overconsuming food sources or adding more nutrient load to the community than before the change in their feeding habits (Hoar 1988, Westley et al. 2013, Harding and Reynolds 2014, Sitters et al. 2015).

A salmonid fish brown trout (*Salmo trutta*) can have different life history strategies within the same waterbody, such as sea migratory and freshwater resident individuals (Boel et al. 2014, Wollebaek et al. 2018). Life history choices are driven by environmental factors such as food availability and predation (Nevoux et al. 2019) but may also be influenced by biological factors such as inherited parental life history strategies (Ferguson et al. 2019). Besides deciding between feeding environments brown trout are also known to undergo changes in their diet as they grow larger commonly referred to as ontogenetic niche shifts (Jensen et al. 2012, Sánchez-Hernández and Cobo 2018, Sánchez-Hernández et al. 2021). Food availability and growth rates control both the time of ontogenetic diet shift and

migration of brown trout (Jensen et al. 2008, Jensen et al. 2012, Jones et al. 2015). Brown trout is an opportunistic feeder (Piria et al. 2022) but it typically feeds on littoral benthic invertebrates (Jensen et al. 2012), although some individuals may also feed on pelagic planktonic prey (Sánchez-Hernández and Amundsen 2015). At sea, brown trout mostly stay near the estuary, but some individuals also use pelagic areas (Eldøy et al. 2015). Large brown trout feed on pelagic fishes at sea, but smaller individuals mostly feed on benthic invertebrates (Knutsen et al. 2001).

Brown trout are known to exhibit different body colours ranging from dark brown to light silver within the same population. The body colour is determined by environmental conditions such as substrate colour (Westley et al. 2013). Lighter substrate colour results in lighter coloured brown trout and darker substrate to darker coloured brown trout. Fitting body colour with the substrate colour makes the fish harder to spot and is a way for the fish to avoid predation (Hoar 1988, Westley et al. 2013). This also means that different coloured brown trout within a population may use different habitats based on the substrate colour of the habitat. Brown trout migrating to sea are known to have more silver body colour than the freshwater counterparts to avoid predation (Hoar and 1988). During the spawning season in fall brown trout usually change body colour to a darker spawning outfit (Jacquin et al. 2017). Darker brown trout can also have better antioxidant defence and can lead to better reproduction rates than the lighter counterparts (Parolini et al. 2018). Darker male individuals are also known to invest on average more in reproduction leading to more offspring and therefore dark colour in brown trout males can indicate better fitness (Jacquin et al. 2017).

The feeding habitat and diet of an individual can be studied from their body tissues using, for instance, ratios of carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$) and sulphur ($^{34}\text{S}/^{32}\text{S}$) stable isotopes, typically expressed as isotope delta values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) in per mil (‰) (Layman et al. 2012). Differences in photosynthetic pathways in different parts of the lake can lead to spatial differences in carbon isotopes ratios of photosynthetic organisms (France 1995, Briones et al. 1998). These differences in isotope ratios of habitats can be used to identify which habitats a fish has obtained resources from (DeNiro and Epstein 1978, Peiman et al. 2017, Ruokonen et al. 2019). For instance, $\delta^{13}\text{C}$ values are typically higher in littoral primary producers (benthic algae and macrophytes) than in pelagic phytoplankton (DeNiro and Epstein 1978). Nitrogen stable isotopes become more enriched in ^{15}N per trophic level (~ 3.4 ‰) and can therefore be used to determine the trophic level of an individual in a food web (Minagawa & Wada 1984, Post 2002). Sulphur stable isotope values at sea (+21 ‰) (Böttcher et al. 2007, Matsubayashi et al. 2017) are higher than in freshwaters (-15–+20 ‰) (Nehlich 2015, Matsubayashi et al. 2017) and can therefore be used to differentiate marine and freshwater feeding. In aquatic environments sulphur isotopes get enriched in ^{34}S around 1.9 ‰ every trophic level (McCutchan et al. 2003). Sulphur isotopes have also been used to determine if food source is from sediment or water column (Croisetière et al. 2009) and the $\delta^{34}\text{S}$ values differ among different primary producers due to different photosynthetic pathways (Layman et al. 2012).

On a population level the resource use and niches of brown trout has been studied widely (Boel et al. 2014, Wollebaek et al. 2018). There are also indications that different colour morphs utilize different habitats, meaning there could be differences in their niches (Hoar 1988, Westley et al. 2013). However, concrete evidence on the extent of different brown trout colour morph niche segregation and width is still lacking.

Two central-Norwegian coastal lakes hold a brown trout population consisting of freshwater and marine brown trout with individuals ranging in body colours from brown to silver. Carbon, nitrogen and sulphur stable isotopes were used to study the trophic niches of three brown trout colour morphs, i.e., their assimilated diets, ontogenetic (size-related) niche shifts and among-individual isotopic variation ("niche width"). The brown trout morphs were visually categorized as silver, intermediate or brown based on their body colour. The study questions were

- 1) What are the long-term assimilated diets of different brown trout colour morphs?
- 2) What are the ontogenetic niche shifts of different brown trout colour morphs?
- 3) How overlapping and wide isotopic niches the brown trout colour morphs have?
- 4) Are there any temporal niche shifts of different brown trout colour morphs?

Based on previous studies (Jensen et al. 2012, Eldøy et al. 2015), I predicted that all brown trout morphs will mostly feed on littoral invertebrates. Because there are some indications that brown trout body colour and habitat are connected (Hoar 1988, Westley et al. 2013), I expected to see differences in diets of brown trout colour morphs. However, I expected the $\delta^{13}\text{C}$ values would stay the same for all morphs when they grew larger due to the expectation that they mostly feed on littoral resources at all lengths as suggested by other studies (Grey 2001, Jensen et al. 2012). In general, brown trout change to piscivory when they grow larger (Jensen et al. 2012, Sánchez-Hernández and Cobo 2018, Sánchez-Hernández et al. 2021) and I anticipated that the $\delta^{15}\text{N}$ values would get higher with increasing fish natural length in all colour morphs indicating ontogenetic niche shifts to higher trophic levels. There are some indications that larger brown trout individuals are more sea dwelling than smaller ones (Jonsson 1985, Jonsson & Gravem 1985, Peiman et al. 2017) and I expected that in all morphs the $\delta^{34}\text{S}$ values would get higher as the natural length of the fish increases indicating feeding more on marine food sources. The high variation in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of different colour morphs would suggest larger niche regions. I expected the niche regions of different colour morphs would not have much overlapping because there are some indications that different coloured brown trout would use different habitats with different substrate colours (Westley et al. 2013). However, I expected that there could be possible differences in the niche sizes of different colour morphs as other studies have

indicated that resource polymorphism can lead to more opportunistic or specialized feeding leading to different variability in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values among different morph groups (Piggott et al. 2018). Brown trout can undergo seasonal changes in use of marine and freshwater habitat and dietary resources (Jonsson and Jonsson 2002). From the studied brown trout, the stable isotopes from muscle tissue indicate the diet from the past summer months and from liver the past few weeks (Heady & Moore 2013). The fish samples were collected on fall when sea brown trout move back to freshwaters, and I expected some recent changes to lower $\delta^{34}\text{S}$ values between muscle and liver tissues in brown trout due to switching from marine to freshwater resources. Also, brown trout have been recorded to feed more on pelagic zone during summer months due to food availability (Jonsson & Graven 1985, Schei & Jonsson 1989) and I expected that the $\delta^{13}\text{C}$ values would be lower in muscle tissues than liver tissues indicating recent change from pelagic to littoral feeding.

2 MATERIALS AND METHODS

2.1 Study location

Brown trout and invertebrate samples were collected between 7.9.2021 – 10.9.2021 from two coastal oligotrophic lakes, Storvatnet (63.604°N 9.639°E) and Litlvatnet (63.616°N 9.663°E) situated in Fremstad catchment in central Norway (Figure 1). Storvatnet has a surface area of 2.9 km², an average depth of 8 m, a maximum depth of 16 m and a drainage area of 22.1 km², whereas Litlvatnet has a surface area of 0.5 km², an average depth of 0.5 m, a maximum depth of 3 m and a drainage area of 26.9 km² (Norges vassdrag- og energidirektorat (NVE) 1998). Storvatnet and Litlvatnet are located 6 m and 5 m above sea level, respectively, and are connected by a small stream Fremstadelva (0.8 km), with Litlvatnet connected to the sea by a stream Heggaelva (1.0 km). The fish species observed in these lakes consist of brown trout, three-spined stickleback (*Gasterosteus aculeatus*) and Atlantic salmon (*Salmo salar*).

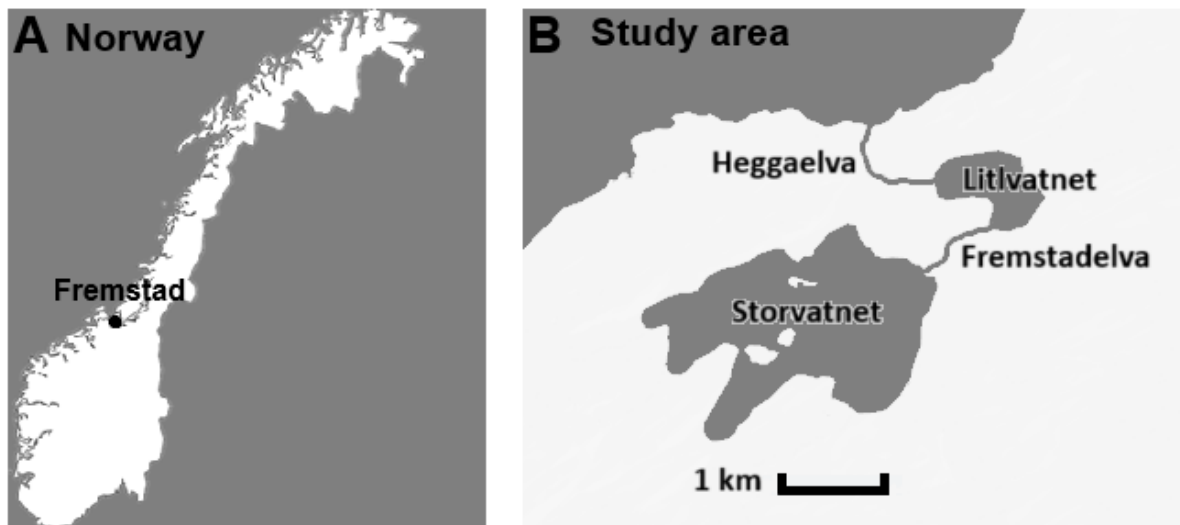


Figure 1. Locations of the Fremstad catchment (A) and Storvatnet and Litlvatnet connected by stream Fremstadelva and flowing to the sea via Heggaelva (B). Maps are acquired and modified from Google Maps.

2.2 Sample collection

Brown trout were collected for stable isotope analysis (SIA) to estimate their long-term assimilated diets and isotopic niches. Nordic multi-mesh bottom gillnets and standard single-mesh gillnets were used in the littoral zone (0–8 m) and Nordic floating and Swiss National Science Foundation (SNSF) gillnets in the pelagic zone (0–6 m) to collect brown trout. The mesh sizes for Nordic bottom gillnets were 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm and they were 30 m long and 1.5 m high. Standard gillnets had mesh sizes of 21, 22.5, 26, and 29 mm and they were 30 m long and 1.5 m high. The mesh sizes for Nordic floating gillnets were the same as for the Nordic bottom gillnets, however, Nordic floating gillnets were 30 m long and 6 m high. For SNSF floating gillnets the mesh sizes were 10, 12.5, 16, 19, 19.5, 24, 30, 35 and 45 mm and they were 52 m long and 6 m high. Overall, 44 Nordic bottom gillnets, 15 standard gillnets, 8 Nordic floating gillnets and 4 SNSF gillnets were set on Storvatnet during a four-day sampling period and 31 Nordic bottom gillnets, and 12 standard gillnets were set on Litlvatnet during a two-day sampling period.

The brown trout body colour (brown, intermediate, silver) was visually estimated by three different people. The natural length (in mm) and wet mass (in g) was measured, and muscle and liver samples were taken for SIA in the field lab. Muscle samples were collected from 272 fish caught from Storvatnet and 120 fish from Litlvatnet. From those individuals 138 liver samples were collected from Storvatnet brown trout and 35 from Litlvatnet. Muscle samples from a total of 90 individuals across the different colour morphs were chosen for SIA, with liver samples being also analysed from a sub-set of 30 individuals (Table 1). Due to the lack of silver morphs in Litlvatnet, more muscle and liver samples from individuals

caught from Storvatnet were chosen for compensation. The individuals were chosen across the range of natural lengths of each colour morphs. However, only larger silver individuals were caught from Litlvatnet.

TABLE 1. Summary of the natural length (mm; mean, range (min–max)) and number of muscle and liver (in brackets) SIA samples from brown trout morphs in Storvatnet and Litlvatnet.

Morph	Lake					
	Storvatnet			Litlvatnet		
	N	Mean (mm)	Range (mm)	N	Mean (mm)	Range (mm)
Brown	15 (5)	278	130–471	15 (5)	259	104–527
Intermediate	15 (5)	217	84–365	15 (5)	278	116–534
Silver	20 (7)	226	108–470	10 (3)	429	304–560
Sum	50 (17)			40 (13)		

Littoral benthic, pelagic planktonic and marine invertebrate samples and prey fish were collected for SIA to allow graphical representation of the study system food webs and to have data of supposed fish food sources for isotopic mixing models. From lakes, littoral benthos samples were collected with a kick net (mesh size 500 μm) in shallow areas (0–1 m) or with a benthic sledge (mesh size 243 μm and sieved through a sieve bucket with a 500 μm mesh size) in deeper areas (2–5 m). Prey fish from Litlvatnet were also caught with a kick net in shallow. Kick net was used to collect samples rising from the bottom due to turbulence made with kicking the sediment. Sledge, on the other hand, was dragged across the bottom in littoral zone capturing anything caught on its' way. Zooplankton samples were collected from the lakes and marine coastal areas with a plankton net (collected with mesh size 50 μm and sieved through mesh sizes 50, 100 and 200 μm) by several vertical hauls throughout the uppermost (0–15 m) water column. Marine littoral benthos samples were collected by hand-picking or with a kick net (mesh size 500 μm) in shallow areas (0–1 m).

Invertebrate samples were categorized as littoral benthos and zooplankton, and other source samples were three-spined sticklebacks as prey fish and marine littoral benthos and marine zooplankton as marine invertebrates. A total of 128 invertebrate samples were obtained from freshwater (Storvatnet: 51, Litlvatnet: 77) and 33 from marine habitats. From these samples, 51 (Storvatnet: 15, Litlvatnet: 20, marine: 16) were chosen for SIA, with the samples covering different habitats and species found in the study system (Table 2). No three-spined sticklebacks were caught from Storvatnet, thus only Litlvatnet prey fish were included. The prey fish were chosen of different sizes (23–32 mm).

TABLE 2. The means and ranges of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values and the sample sizes of different brown trout food sources chosen for stable isotope analysis from the study system.

Location	Group	Resource	N	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
				Mean	Min-max	Mean	Min-max	Mean	Min-max
Storvatnet	Littoral benthos	<i>Baetidae</i>	3	-20.9	-21.6- -20.3	4.2	4.0-4.4	6.7	6.4-6.8
		<i>Ephemeroptera</i>	2	-27.2	-28.6- -25.8	4.8	4.7-5.0	7.9	7.8-7.9
		<i>Planorbidae</i>	2	-18.9	-19.3- -18.5	4.9	4.6-5.1	7.0	6.8-7.1
		<i>Potamopyrgus</i>	2	-22.8	-23.4- -22.2	5.1	4.4-5.8	7.0	6.3-7.8
		<i>Radix</i>	1	-19.0	-	4.1	-	6.4	-
	Zooplankton	<i>Cladocera</i>	2	-29.4	-29.4- -29.3	4.0	3.9-4.0	8.2	8.1-8.2
Litlvatnet	Zooplankton	Zooplankton mix	3	-30.6	-30.6- -30.5	5.7	5.6-5.9	7.4	7.3-7.6
		Littoral benthos	<i>Baetidae</i>	3	-24.4	-25.3- -23.6	4.7	4.5-5.1	3.5
	<i>Ephemeroptera</i>	2	-22.4	-24.9- -19.9	4.1	3.4-4.8	2.1	2.0-2.3	
	<i>Planorbidae</i>	1	-20.4	-	5.6	-	-0.1	-	
	<i>Potamopyrgus</i>	2	-23.5	-23.6- -23.4	6.2	6.2-6.3	2.4	2.4-2.4	
	<i>Radix</i>	2	-18.4	-18.8- -18.1	3.5	3.3-3.7	-0.2	-0.7-0.3	
	Prey fish	Three-spined stickleback	5	-23.8	-24.0- -20.6	9.7	8.6-10.2	3.0	-1.2-6.4
	Zooplankton	Zooplankton mix	5	-30.4	-30.5- -30.4	5.6	5.4-5.6	5.6	5.3-6.1
Marine	Littoral benthos	<i>Gammarus</i>	6	-17.6	-19.0- -16.6	6.4	5.4-7.4	19.5	18.9-19.8
		Marine snail	6	-17.2	-18.6- -16.6	8.5	8.0-8.8	19.0	18.2-19.4
		<i>Polychaeta</i>	1	-18.1	-	10.6	-	17.3	-
	Zooplankton	Zooplankton mix	3	-21.4	-23.0- -18.7	6.5	6.2-6.7	15.5	14.6-16.8

2.3 Methods

All samples were stored in Eppendorf tubes and freeze-dried at the Norwegian University of Science and Technology (NTNU) for 48 hours, frozen and then sent to the University of Jyväskylä where they were kept frozen until they were homogenized and weighed for SIA. Selected samples were ground into a homogeneous powder inside the tubes using a metallic bar and 1.900–2.100 mg of the powder was transferred into tin cups. Cups were rolled into tight balls using tweezers and placed in a 96-well sample tray. After every five samples, internal laboratory standards were weighed and analysed, including pike (*Esox lucius*) muscle tissue, Birch leaf (*Betula* sp.), NBS123 of known stable isotope ratio for each, and sulphanimide was used as a standard for elemental composition.

The carbon, nitrogen and sulphur isotope analyses were conducted at the University of Jyväskylä using an Isoprime 100 (Isoprime Ltd, UK) continuous flow stable isotope ratio mass spectrometer connected to a Elementar Vario PYRO Cube (Elementar Analysensysteme, Germany) element analyser.

2.4 Stable isotope modelling and statistical analyses

All data analyses and visualisations were conducted in R version 4.3.1 (R Core Team 2023). Two biplots figures were created using `plot_data()` function from the MixSIAR package (Stock & Semmens 2016, Stock et al. 2018) to visualise the food source and brown trout morph $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values in the same plots. The biplots represent the different food source $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ mean values with standard deviations and are plotted with brown trout $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values where $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values were plotted against $\delta^{13}\text{C}$ values. Bayesian Mixing Models MixSIAR package (Stock & Semmens 2016, Stock et al. 2018) was used to estimate proportions of potential food sources in long-term diets of different brown trout morphs. MixSIAR compares the isotope values of consumers (here brown trout morphs) to those of potential food sources (here zooplankton, littoral benthos, prey fish and marine invertebrates) and produces Bayesian estimates of prey proportions (posterior distributions). MixSIAR model was run with normal settings (three parallel chains of 100 000 length, burn-in of 50 000 and uninformative prior) and by setting trophic fractionation factors of 0.4 ± 1.3 ‰ for $\delta^{13}\text{C}$, 3.4 ± 1.0 ‰ for $\delta^{15}\text{N}$ and 1.9 ± 0.51 ‰ for $\delta^{34}\text{S}$ (Post 2002, McCutchan et al. 2003). The MixSIAR results present the mean proportions of different sources in the diets of brown trout colour morphs, as well as standard deviations and 2.5 %–97.5 % credible intervals for the dietary estimates.

Generalised linear mixed effects models (GLMM) were run in nlme package (Pinheiro & Bates 2000, Pinheiro et al. 2023) to test the effects of morph and length on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values in brown trout as well as the two-way interaction of morph*length to see potential differences among morphs in length and isotope interactions. The GLMMs were conducted with `lme()` function where lake was set as a random factor to account for the dependency of observations (fish) from the same lake. The length and isotope relationships were visualised for different

brown trout colour morphs using `geom_smooth` function with `loess` (locally estimated scatterplot smoothing) method in `ggplot2` package (Wickham 2016).

NicheROVER package (Swanson et al. 2015) was used to model isotopic niche regions of different colour morphs based on individual variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values. NicheROVER uses a Bayesian approach to create in this case 10 random niche regions for each morph. The probability of overlapping isotopic niches (based on 2.5 %–97.5 % credible intervals) was calculated using 10 000 random draws of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of muscle tissues of each brown trout morphs using Markov chain Monte Carlo (MCMC) algorithm. The estimated posterior distributions of niche sizes were created by drawing 1000 values by MCMC algorithm for each morph. Coda package (Plummer et al. 2006) was used to calculate the 95 % credible intervals for niche sizes of different colour morphs. Coda is used for handling MCMC data and was used to create highest posterior density intervals. It was used for 1000 random MCMC draws of niche sizes for each morph.

The temporal niche shifts of different brown trout colour morphs were tested with paired, two-tailed t-tests comparing the muscle and liver $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values, with $p < 0.05$ values considered as statistically significant shifts. The results were visualized with `ggpaired()` function in `ggplot2` package (Wickham 2016) where the muscle and liver $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of brown trout individuals were connected with lines and the distribution of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values were visualised as boxplots for each brown trout colour morph.

3 RESULTS

3.1 Diet and ontogenetic niche shifts of brown trout morphs

In visual inspection of isotope biplots the $\delta^{13}\text{C}$ values showed clear differentiation between pelagic zooplankton and littoral benthic invertebrates (Figure 2, Table 2). The $\delta^{15}\text{N}$ values showed clear differences in trophic levels of zooplankton and sticklebacks, whereas zooplankton and littoral benthos showed overlapping $\delta^{15}\text{N}$ values (Figure 2A). Marine sources also overlapped with zooplankton and littoral benthos but also with sticklebacks in $\delta^{15}\text{N}$ values. The $\delta^{34}\text{S}$ values differed markedly between marine and freshwater sources (lake zooplankton, littoral benthic invertebrates and sticklebacks) (Figure 2B).

The three brown trout morphs showed no significant differences in assimilated diets, based on overlapping 95 % credible intervals of dietary proportions estimated with MixSIAR (Figures 2-3, Table 3). The most consumed resources for all brown trout morphs were zooplankton and sticklebacks, whereas littoral benthos was consumed only little (Figure 3, Table 3). Silver morphs seem to have had more tendency towards marine food sources than other morphs, however, the distributions were overlapping (Figure 3, Table 3).

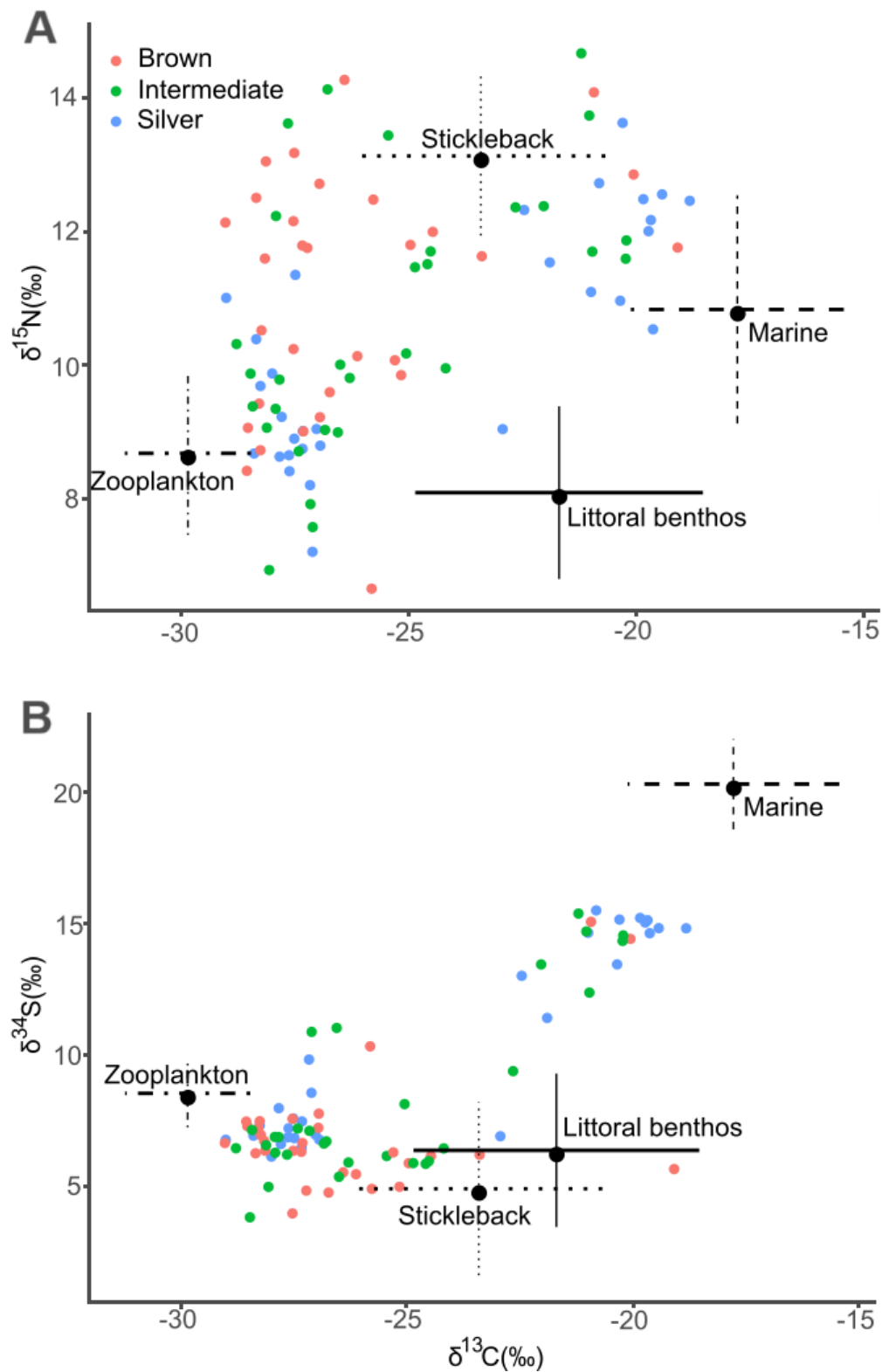


Figure 2. Stable isotope biplots showing the fractionation corrected mean \pm SD $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (A), and $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values (B) of different food sources of brown trout, as well as the isotope values of individual brown trout, with colour morphs separated in different colours. Here the isotope data from both lakes are pooled in the same figures.

TABLE 3. MixSIAR results of proportions of different food sources in the diets of each brown trout colour morph. The mean, standard deviation (SD) and lower and upper Bayesian credible intervals (95 % CI) of estimated dietary proportions are presented.

Prey source Colour morph	Mean	SD	95 % CI	
			Lower	Upper
Zooplankton				
Brown	0.45	0.07	0.31	0.59
Intermediate	0.42	0.08	0.26	0.56
Silver	0.32	0.09	0.14	0.49
Littoral benthos				
Brown	0.08	0.05	0.003	0.19
Intermediate	0.07	0.06	0.002	0.22
Silver	0.13	0.12	0.002	0.40
Sticklebacks				
Brown	0.38	0.06	0.26	0.51
Intermediate	0.39	0.07	0.26	0.53
Silver	0.32	0.09	0.13	0.51
Marine				
Brown	0.08	0.04	0.02	0.16
Intermediate	0.12	0.05	0.02	0.22
Silver	0.22	0.06	0.09	0.33

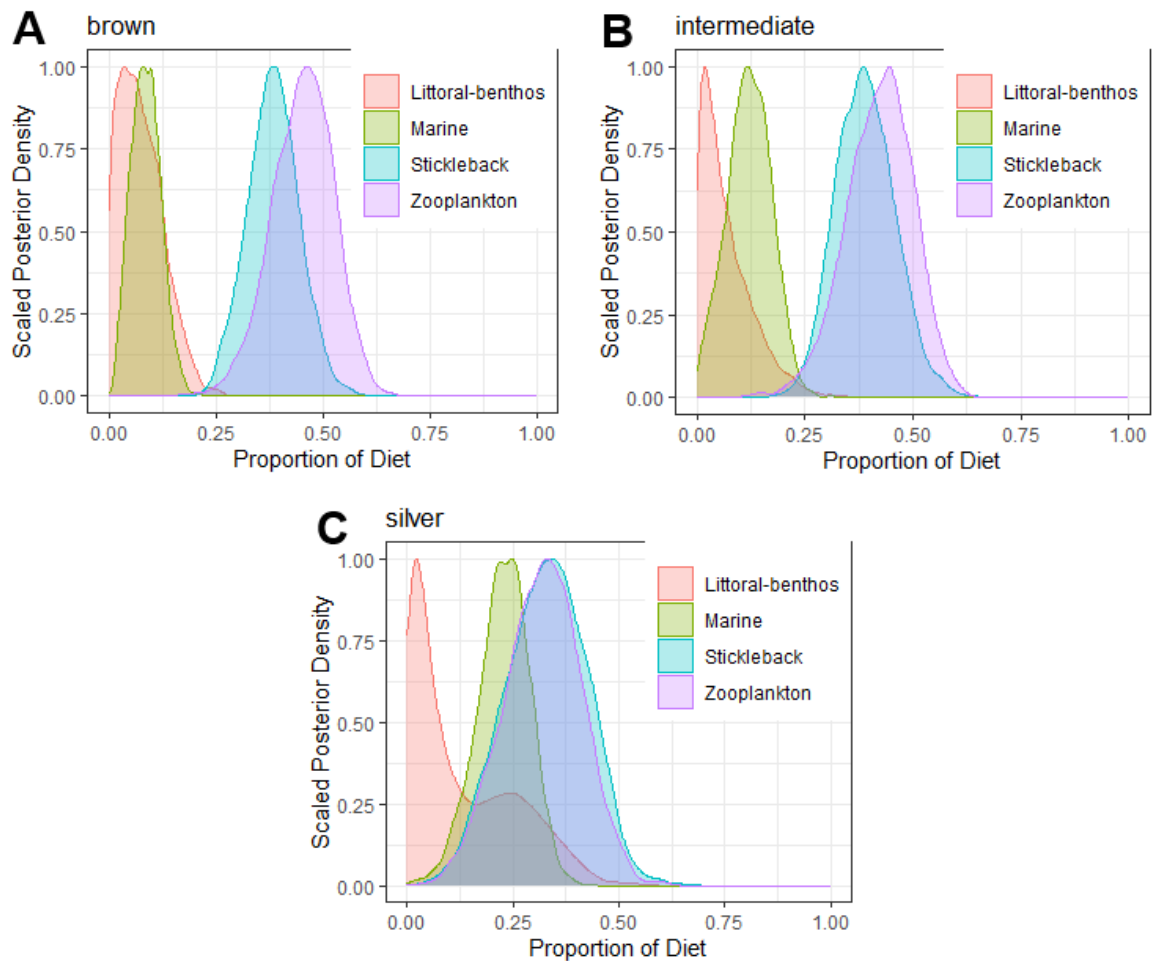


Figure 3. MixSIAR results showing the proportions (i.e., posterior distributions) of different food sources in the assimilate diets of brown (A), intermediate (B) and silver (C) colour morphs of brown trout.

The GLMM results showed no significant difference among differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values between brown trout colour morphs (Table 4). However, there were significant positive relationships between the natural length and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of brown trout colour morphs. The increase in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values was typically evident at natural lengths of 250–300 mm for all morphs, except that the increase in $\delta^{34}\text{S}$ values of brown colour morph happened at around 400 mm natural length, with the pattern being largely driven by the high $\delta^{34}\text{S}$ values of the two large individuals (Figure 4). The significant morph*length two-way interaction indicate that the effect of natural length on $\delta^{34}\text{S}$ values varied between the brown and silver colour morphs, with silver morph shifting to higher $\delta^{34}\text{S}$ values at a smaller size (Table 4).

TABLE 4. GLMM results showing main effects of brown trout colour morph (brown, intermediate, silver) and natural length and their two-way interactions (morph*length) on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of brown trout muscle tissue. The means, standard errors (SE), degrees of freedom (df), t-values and p-values are given for each model parameter, with statistically significant ($p < 0.05$) parameters highlighted in bold.

Predictor	Estimate	SE	df	t	p
$\delta^{13}\text{C}$					
Intercept	-29.26	1.82	83	-16.07	<0.001
Brown - Intermediate	1.28	1.21	83	1.06	0.292
Brown - Silver	1.07	1.29	83	0.83	0.411
Intermediate - Silver	-0.22	1.16	83	-0.19	0.853
Length	0.01	0.003	83	3.51	<0.001
Brown*Length - Intermediate*Length	-0.001	0.004	83	-0.26	0.797
Brown*Length - Silver*Length	0.003	0.004	83	0.70	0.483
Intermediate*Length - Silver*Length	0.004	0.004	83	1.05	0.297
$\delta^{15}\text{N}$					
Intercept	7.53	0.55	83	13.76	<0.001
Brown - Intermediate	0.15	0.71	83	0.22	0.830
Brown - Silver	-0.53	0.74	83	-0.71	0.480
Intermediate - Silver	-0.68	0.68	83	-1.00	0.321
Length	0.01	0.002	83	7.29	<0.001
Brown*Length - Intermediate*Length	-0.0007	0.003	83	-0.29	0.771
Brown*Length - Silver*Length	-0.002	0.002	83	-0.79	0.434
Intermediate*Length - Silver*Length	-0.001	0.002	83	-0.51	0.610
$\delta^{34}\text{S}$					
Intercept	4.80	1.20	83	4.01	<0.001
Brown - Intermediate	-0.65	1.57	83	-0.41	0.680
Brown - Silver	-1.06	1.57	83	-0.65	0.515
Intermediate - Silver	-0.41	1.49	83	-0.28	0.782
Length	0.009	0.004	83	2.11	0.038
Brown*Length - Intermediate*Length	0.009	0.006	83	1.57	0.121
Brown*Length - Silver*Length	0.01	0.005	83	2.59	0.011
Intermediate*Length - Silver*Length	0.005	0.005	83	1.01	0.316

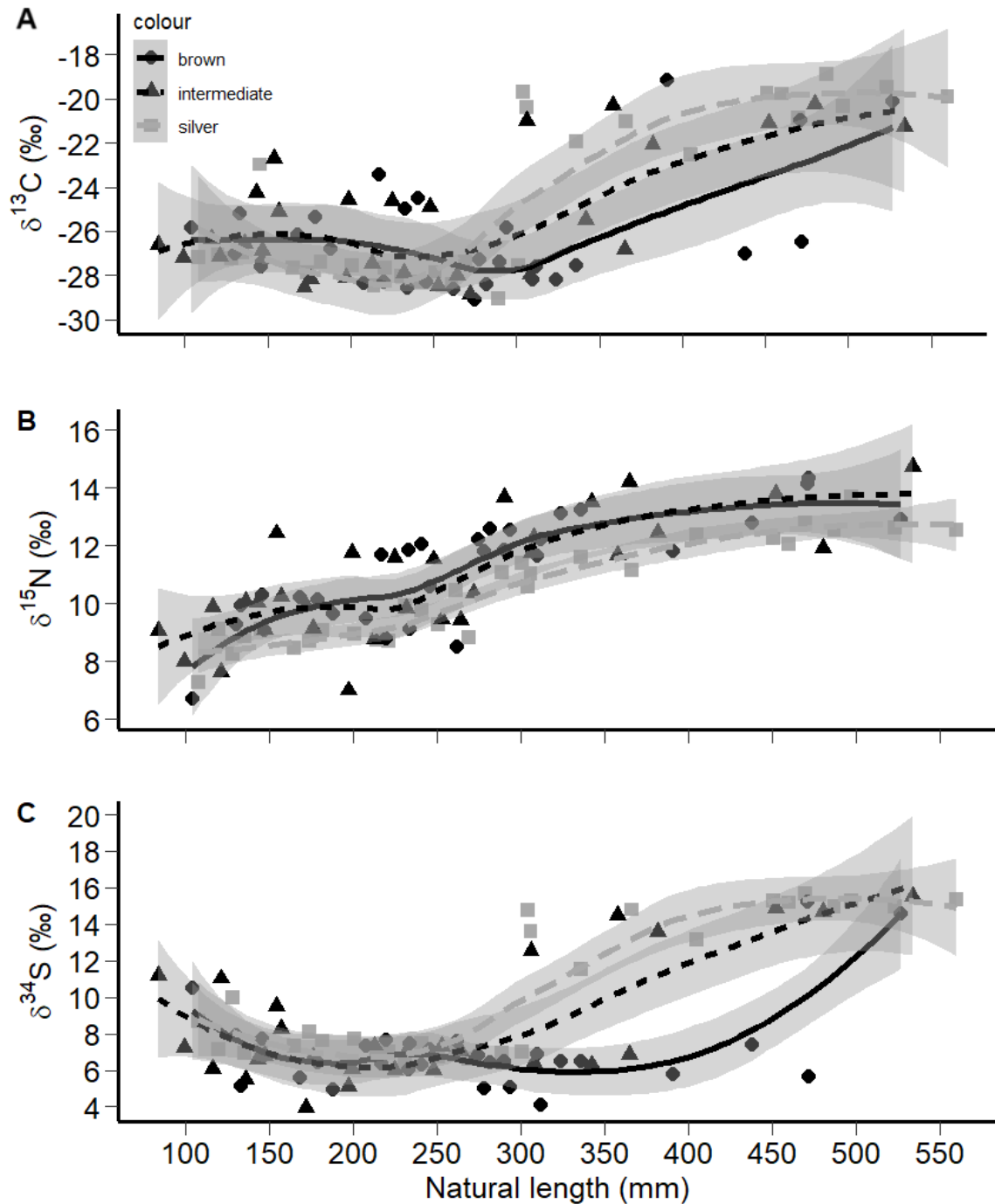


Figure 4. The $\delta^{13}\text{C}$ (A), $\delta^{15}\text{N}$ (B) and $\delta^{34}\text{S}$ (C) values of brown trout muscle tissues plotted against natural length for each colour morph using `geom_smooth` function with `loess` method in `ggplot2` package. The grey shaded areas present 95 % confidence intervals for loess curves.

3.2 Isotopic niche overlaps and widths

Based on `nicheROVER` analysis, there were no significant differences in the isotopic niches of brown trout colour morphs, as indicated by the overlapping of the niche regions (Figure 5, Table 5). The posterior distributions of niche area estimates also

overlapped among colour morphs (95 % Bayesian credible intervals for the niche area estimates for brown: 500–1190, intermediate: 480–1130 and silver: 250–600), indicating no significant differences in the isotopic niche widths based on coda analysis (Figure 6).

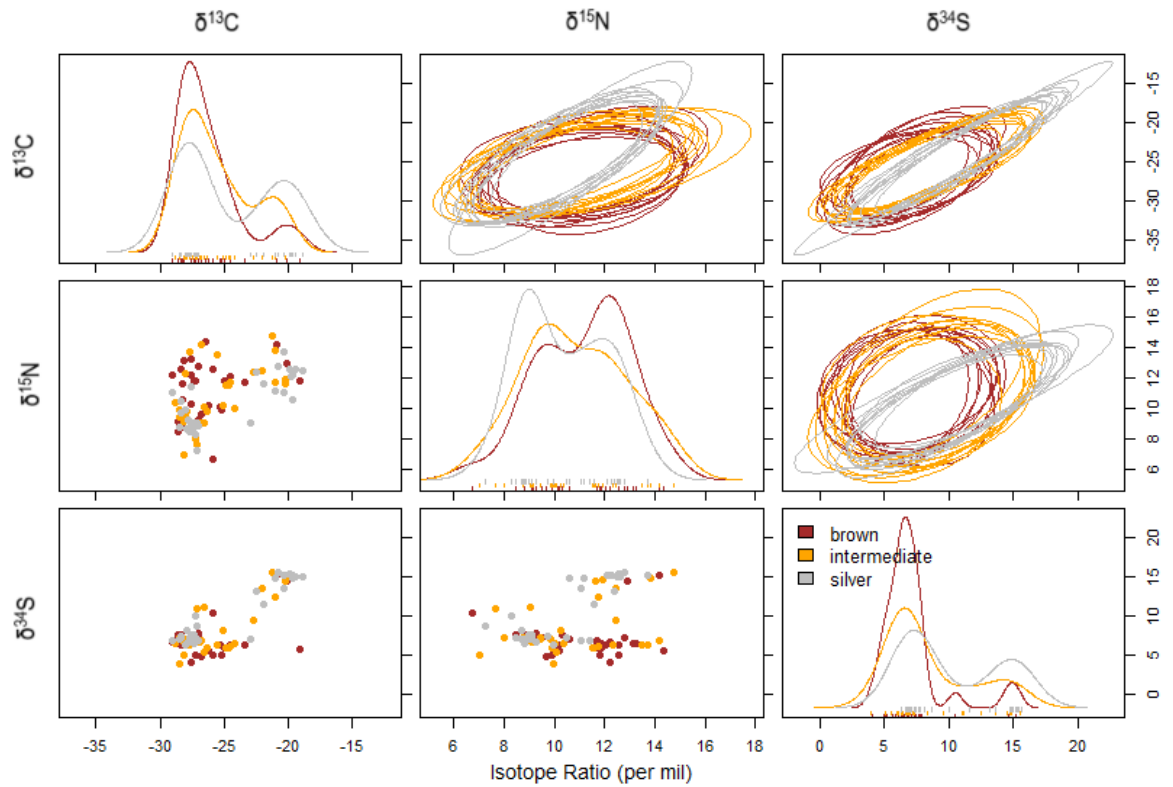


Figure 5. Two-dimensional scatterplot of all individual observations (dots), their one-dimensional density plots (lines) and two-dimensional elliptical projections of trophic niche regions made with ten random projections (ellipses) produced in nicheROVER from $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of brown trout muscle tissue.

TABLE 5. The mean probabilities and lower and upper 95 % Bayesian credible interval of posterior distributions of probabilities of isotopic niche region overlaps among different colour morphs of brown trout produced in nicheROVER analysis.

Colour morphs	Mean	95 % CI	
		Lower	Upper
Brown - Intermediate	0.82	0.66	0.94
Brown - Silver	0.41	0.26	0.61
Intermediate - Brown	0.83	0.66	0.95
Intermediate - Silver	0.60	0.42	0.78
Silver - Brown	0.72	0.53	0.89
Silver - Intermediate	0.88	0.74	0.98

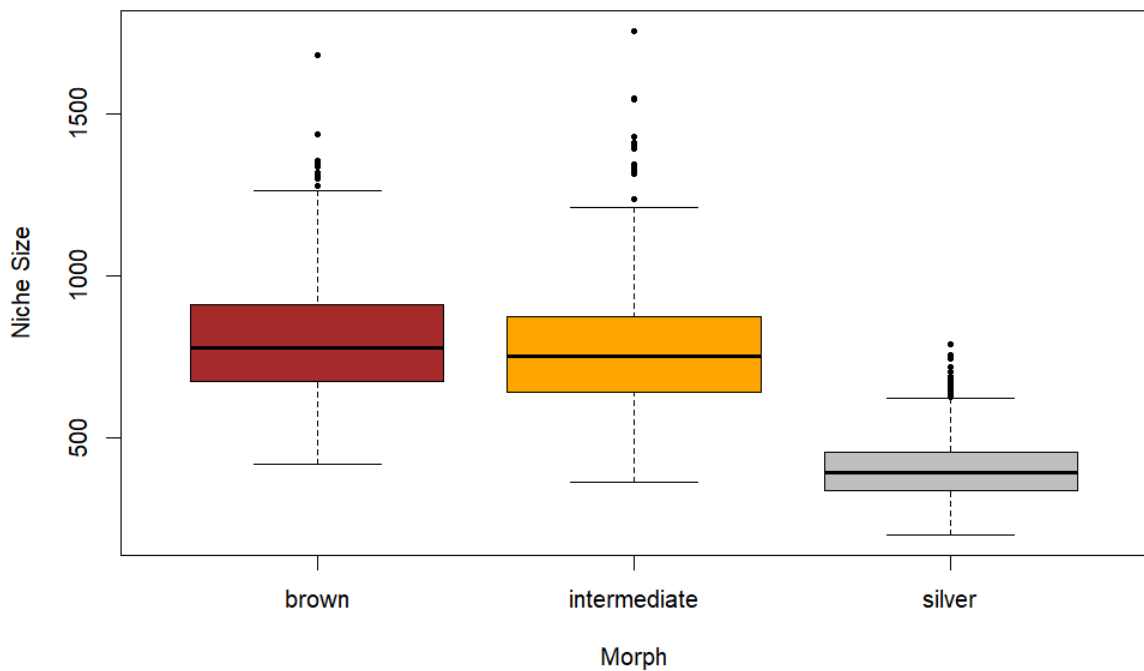


Figure 6. Posterior distributions of isotopic niche areas for each brown trout colour morph based on the variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of muscle tissue modelled in nicheROVER package. Boxes show where 50 % of the modelled data points are, with the median value presented as a line inside the box. The whiskers represent the variability of the minimum and maximum data points and dots represent outlier values.

3.3 Temporal niche shifts in brown trout colour morphs

Paired t-test results showed significant differences in muscle and liver $\delta^{15}\text{N}$ values for all brown trout colour morphs and in $\delta^{34}\text{S}$ values for intermediate and silver morphs (Table 6, Figure 8). Muscle tissue had significantly higher $\delta^{15}\text{N}$ values than liver tissue for all morphs, whereas the $\delta^{34}\text{S}$ values were significantly higher in liver than in muscle tissues of intermediate and silver morphs (Table 6). The temporal niche shifts were consistent across all individuals in $\delta^{15}\text{N}$ values of intermediate and silver morphs and in $\delta^{34}\text{S}$ values of silver morph, whereas in $\delta^{15}\text{N}$ values of brown morph individuals there were two individuals where the $\delta^{15}\text{N}$ values were higher in liver tissue than in muscle tissue and in $\delta^{34}\text{S}$ of intermediate morph there was one individual that had higher $\delta^{34}\text{S}$ values in muscle tissue than in liver tissue (Table 6).

TABLE 6. Results of paired t-tests comparing the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of muscle and liver tissues in brown trout colour morphs. Mean differences in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of muscle and liver tissues (Mean dif.), degrees of freedom (df), t- and p-values, as well as the upper and lower confidence limits (95 % CI) for parameters are shown, with statistically significant differences ($p < 0.05$) highlighted in bold.

Isotope value						95 % CI	
Colour morph	Mean dif.	t	df	p	Lower	Upper	
$\delta^{13}\text{C}$							
Brown	-0.37	-1.66	9	0.131	-0.88	0.14	
Intermediate	-0.06	-0.18	9	0.864	-0.81	0.70	
Silver	-0.15	-0.68	9	0.515	-0.67	0.36	
$\delta^{15}\text{N}$							
Brown	-0.84	-2.41	9	0.039	-1.62	-0.05	
Intermediate	-1.61	-5.11	9	<0.001	-2.32	-0.90	
Silver	-1.29	-5.89	9	<0.001	-1.79	-0.80	
$\delta^{34}\text{S}$							
Brown	1.45	1.29	9	0.228	-1.08	3.98	
Intermediate	0.84	2.94	9	0.016	0.19	1.48	
Silver	1.34	3.10	9	0.013	0.36	2.32	

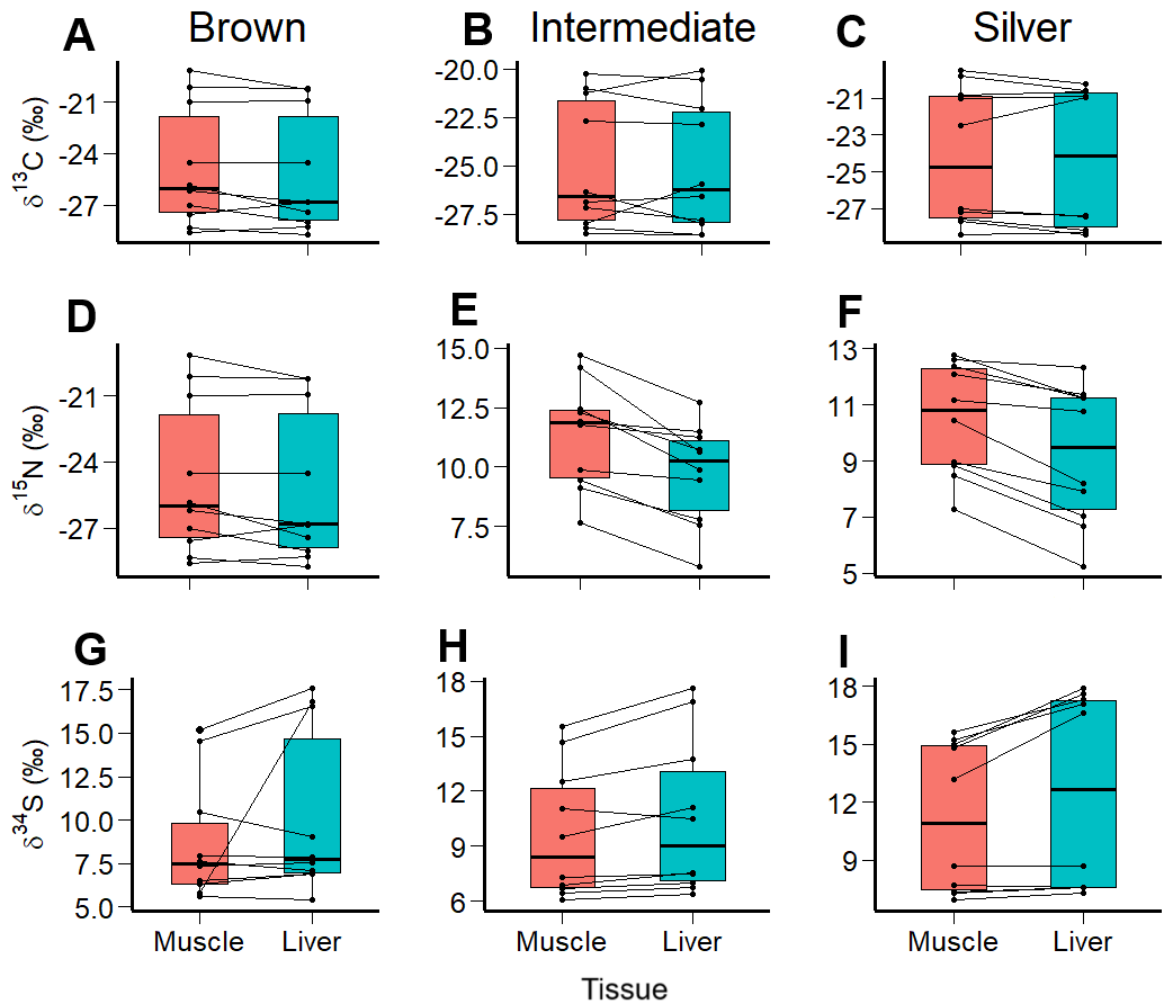


Figure 8. Temporal niche shifts in brown trout colour morphs based on differences in $\delta^{13}\text{C}$ (A, B, C), $\delta^{15}\text{N}$ (D, E, F) and $\delta^{34}\text{S}$ (G, H, I) values of muscle and liver tissues reflecting long-term (over a few months) and short-term (few weeks) diets, respectively. The lines connect muscle and liver $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of fish individuals. Boxes show where 50 % of the data points are located, lines inside the boxes represent medians and whiskers represent the observed minimum and maximum values.

4 DISCUSSION

This study tested if three colour morphs of brown trout show evidence for resource polymorphism in two Norwegian coastal lakes, Storvatnet and Litlvatnet, using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ data of trout muscle and liver tissues and potential freshwater and marine food resources. The results indicate no significant differences in the diets of different colour morphs, but ontogenetic niche shifts were evident based on significant positive relationships between natural length and muscle $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of brown trout. On a population level, the size related niche shifts happened at the natural length of 250–300 mm for all morphs and isotopes, except

that the $\delta^{34}\text{S}$ values of brown morph increased later at the natural length of 400 mm, however this was driven by two individuals suggesting a weak relationship between the $\delta^{34}\text{S}$ values and length for brown morph. No significant differences were observed in the position or size of isotopic niche areas of brown trout colour morphs. Temporal niche shifts were observed in $\delta^{15}\text{N}$ values for all morphs and in $\delta^{34}\text{S}$ values in intermediate and silver morphs, based on differences in isotope values of brown trout muscle and liver tissues reflecting long- and short-term trophic niches, respectively (Heady & Moore 2013).

4.1 Diet and ontogenetic niche shifts

Contrary to the first hypothesis, the mixSIAR results suggest two major food sources for all colour morphs, zooplankton and sticklebacks, and barely any littoral benthic invertebrates consumed, even though generally brown trout are known to consume littoral benthic invertebrates (Jensen et al. 2012). MixSIAR results indicate some consumption of marine resources in all colour morphs, however silver morph tended to use more marine resources than the other morphs. A stomach content analyses done previously for brown trout in Storvatnet showed that these fish consumed approximately 16 % of littoral benthos across all morphs which was almost two times greater than estimated here based on SIA (approximately 9 % between all morphs) (Ekren 2023). However, Ekren (2023) assessed recent diet differences whereas this study used stable isotopes to reveal long-term assimilated diets and also contained brown trout from the nearby lake Litlvatnet. In Ekren (2023) study, zooplankton was the dominant prey item for all the morphs, which supports the results that brown trout in this system mostly consume zooplankton. The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values in brown trout muscle tissues indicated that around 37 % of brown trout diet (among all morphs) consisted of sticklebacks, contrary to the stomach analyses by Ekren (2023) where around 9 % of the stomach contents consisted of fish. However, since this study consisted of two lakes it is possible that sticklebacks are less common in Storvatnet than in Litlvatnet. Since the muscle samples were collected in fall, the stable isotope ratios of the muscle tissue reflect the long-term assimilated diets from the past summer months. Zooplankton abundance in Nordic lakes is usually higher during summer and fall months (Hindar & Jonsson 1982) and therefore it is possible that the number of zooplankton being consumed by these brown trout was due to food availability and there could be seasonal variation in their diets.

The stable isotope data suggest minor differences in trophic niches of the three morphs. Brown trout are known to start spawning in fall (Riedl and Peter 2013, Larios-López et al. 2015) and thus it is possible that some of them were already changing their spawning outfits (i.e., becoming darker) when they were caught in September (Jacquin et al. 2017). It is possible that some brown trout would have been classified differently on other times of the year when they are, on average, silverier in colour.

All trout morphs showed ontogenetic niche shifts as indicated by the significant positive effect of length on isotope values (Jensen et al. 2012). The increased $\delta^{13}\text{C}$ values indicate that the food source changes from pelagic to more

littoral as the trout grow (Jensen et al. 2012, Sánchez-Hernández et al. 2017). Trout seemed to shift from pelagic zooplankton to sticklebacks and littoral benthos and/or to marine sources with increasing length based on $\delta^{13}\text{C}$ values and $\delta^{34}\text{S}$ values that best distinguished freshwater and marine food sources. The $\delta^{15}\text{N}$ values indicate a shift to a higher trophic position with increasing length (Sánchez-Hernández & Cobo 2018, Brown-Vuillemin et al. 2020). Because marine organisms have on average higher $\delta^{34}\text{S}$ values than freshwater organisms as observed here and in other studies (Nehlich 2015), it suggests that brown trout used marine food sources as they grew larger despite their body colour. However, the change in $\delta^{34}\text{S}$ values happened at a larger size (ca. 400 mm) in brown colour morph than in the other morphs (250–300 mm). However, it should be noted that this shift was largely driven by two large individuals of brown colour morph, which were likely male sea trout with spawning outfits. The size related changes in brown trout towards piscivory has been observed around the sizes of 250–300 mm previously (Kahilainen & Lehtonen 2003, Jensen et al. 2012). There is also some previous evidence of salmonids exhibiting size related shifts from pelagic feeding to littoral feeding, however this was recorded at smaller length (150 mm) (Jardine et al. 2005).

4.2 Morph isotopic niche overlap and widths

Results from nicheROVER shows that the niches of different brown trout colour morphs overlapped with one another. This was against the hypothesis that different colour morphs would use different niches. Based on MixSIAR and nicheROVER results the body colour of brown trout is not a clear indication of ecologically distinct morphs in this system. It seems that brown trout colour morphs do not explain the variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of the population. Overlapping niches of phenotypically and genetically different brown trout morphs have been observed before (Peiman et al. 2017, Verspoor et al. 2019). However, there is also evidence of distinctive niches between phenotypically different brown trout morphs (Piggott et al. 2018). Results from coda analyses indicates that silver morphs had a smaller isotopic niche area than the other morphs, whereas brown and intermediate morphs had similar niche size, however the niche size was not significantly smaller in silver morphs than in brown or intermediate morphs. Previously brown trout have been observed to have morphs with different niche sizes (Piggott et al. 2018).

4.3 Temporal niche shifts in colour morphs

Contrary to the fourth hypothesis, no statistically significant differences were observed in the $\delta^{13}\text{C}$ values of liver and muscle tissues, indicating no temporal shifts from pelagic to littoral feeding or vice versa for any of the morphs (Peiman et al. 2017, Ruokonen et al. 2019). The $\delta^{15}\text{N}$ values in liver were lower than in muscle indicating seasonal shifts to lower trophic levels (Post 2002). However, there is evidence that the enrichment in ^{15}N is lower in liver than in muscle tissue without diet changes, indicating that the difference could be due to tissue type and not recent changes to lower trophic levels (Chen et al. 2012). Intermediate and silver

morphs had approximately 0.8 ‰ and 1.3 ‰, respectively, higher $\delta^{34}\text{S}$ values in liver than muscle tissues indicating recent (past weeks) changes to feeding more on marine sources (Matsubayashi et al. 2017). However, since the fish were caught during the time of the year when they usually migrate from sea to freshwaters to spawn (Jonsson and Jonsson 2002), it is an unexpected finding that they only recently had changed to feed more on marine food sources and the change in $\delta^{34}\text{S}$ values between muscle and liver tissues was also considerably small. The enrichment in ^{34}S between liver and muscle tissues has not been studied in brown trout, however, it has been studied in whitefish and the findings suggest that same diet in broad whitefish (*Coregonus nasus*) lead to lower $\delta^{34}\text{S}$ values in liver than muscle tissues (Hesslein et al. 1993). Therefore, difference between $\delta^{34}\text{S}$ values of muscle and liver tissue probably cannot be explained by tissue type.

4.4 Study limitations and suggestions for future research

This study provides insights into resource polymorphism of brown trout in a Norwegian coastal watercourse using stable isotope analysis. The results indicated minor differences in trophic ecologies of the three brown trout colour morphs, which all seemed to shift to a higher trophic position and to use more marine prey resources with increasing natural length. However, there is still need for future research including 1) sampling at different times of the year to see brown trout morph diets before they start allocating resources for reproduction, 2) telemetry study to investigate fish movements between marine, riverine and lake habitats to understand more about their behaviour in this system, 3) genetic study to see if there are genetic differences between the colour morphs that could indicate resource polymorphism, and 4) enrichment of ^{34}S in brown trout muscle and liver tissues since information is currently limited to one fish species.

4.5 Conclusions

Resource polymorphism is linked with phenotypic and genetic differences in a population meaning that when resource polymorphism is observed among different phenotypes it is also observed in the genetic differences of the morphs. This study investigated potential resource polymorphism in phenotypically different brown trout individuals (i.e., colour morphs) in a Norwegian coastal watercourse, but found little evidence for trophic niche segregation. Knowing if a population exhibits resource polymorphism and what niches different morphs utilize is vital for the conservation of the population because different morphs can have so distinctive resource usages that the protection of a certain morph will not protect all morphs. Therefore, it is important to study this population more to prevent harm for any potential morphs in the system.

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