ORIGINAL ARTICLE

Lack of trophic polymorphism despite substantial genetic differentiation in sympatric brown trout (*Salmo trutta*) populations

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Abstract

Sympatric populations occur in many freshwater fish species; such populations are typically detected through morphological distinctions that are often coupled to food niche and genetic separations. In salmonids, trophic and genetically separate sympatric populations have been reported in landlocked Arctic char, whitefish and brown trout. In Arctic char and brown trout rare cases of sympatric, genetically distinct populations have been detected based on genetic data alone, with no apparent morphological differences, that is "cryptic" structuring. It remains unknown whether such cryptic, sympatric structuring can be coupled to food niche separation. Here, we perform an extensive screening for trophic divergence of two genetically divergent, seemingly cryptic, sympatric brown trout populations documented to remain in stable sympatry over several decades in two interconnected, tiny mountain lakes in a nature reserve in central Sweden. We investigate body shape, body length, gill raker metrics, breeding status and diet (stomach content analysis and stable isotopes) in these populations. We find small significant differences for body shape, body size and breeding status, and no evidence of food niche separation between these two populations. In contrast, fish in the two lakes differed in body shape, diet, and nitrogen and carbon isotope signatures despite no genetic difference between lakes. These genetically divergent populations apparently coexist using the same food resources and showing the same adaptive plasticity to the local food niches of the two separate lakes. Such observations have not been reported previously but may be more common than recognised as genetic screenings are necessary to detect the structures.

KEYWORDS
body shape, geometric morphometrics, gill rakers, population genetic structure, stable isotopes, stomach content

1 | INTRODUCTION

Sympatric populations, that is populations that show genetic differentiation despite lack of obvious barriers to gene flow, have been documented in several species of freshwater fish including perch, walleye, rainbow smelt and several salmonid species (Bergek & Björklund, 2007; Dupont, Bourret, & Bernatchez, 2007; Ferguson & Taggart, 1991; Lu & Bernatchez, 1999; Østbye, Næsje, Bernatchez, Sandlund,

& Hindar, 2005; Pigeon, Dodson, & Bernatchez, 1998; Wilson et al., 2004). Such genetic structuring is typically detected based on phenotypic differences, which are often coupled to food niche separation—so-called trophic polymorphism (Wimberger, 1994). Trophic and genetically separate sympatric populations have been reported in salmonid fishes such as Arctic char, brown trout and whitefish inhabiting postglacial lakes in the northern hemisphere, so-called landlocked populations (e.g. Ferguson & Mason, 1981; Gowell, Quinn, & Taylor, 2012; May-McNally, Quinn, Woods, & Taylor, 2015; Power, Power, Reist, & Bajno, 2009; Præbel et al., 2013). In Arctic char and brown trout, a few, rare cases of sympatric, genetically distinct populations have been detected based on genetic data alone (Ryman, Allendorf, & Ståhl, 1979; Wilson et al., 2004), without apparent morphological differences hinting about the genetic substructure, that is the structures are "cryptic."

One of the most recent documentations of cryptic genetic structure is the occurrence of two brown trout populations coexisting sympatrically in two very small (<0.3 km^2), interconnected mountain lakes in central Sweden (Palmé, Laikre, & Ryman, 2013). These lakes—Lake Östra Trollsvattnet and Lake Västra Trollsvattnet—are part of a long-term regional genetic monitoring study initiated in the 1980s (the Lakes Bävervattnen Project; Jorde & Ryman, 1996; Laikre, Jorde, & Ryman, 1998; Palm & Ryman, 1999; Palm, Laikre, Jorde, & Ryman, 2003; Charlier, Palmé, Laikre, Andersson, & Ryman, 2011; Charlier, Laikre, & Ryman, 2012; Palmé et al., 2013). The study area is located in a remote region of a nature reserve with very limited human disturbance, including no stocking and only limited fishing. During the first years of monitoring, samples from Lake Östra Trollsvattnet and Lake Västra Trollsvattnet were compared to each other, but no divergence was observed suggesting only one population existed in these two lakes. After several years of sampling, however, heterozygote deficiencies indicated substructuring within each of the lakes (Jorde & Ryman, 1996), and more recent statistical tools provided evidence of two sympatric brown trout populations occurring at about equal frequencies in both lakes (Palmé et al., 2013). The populations do not exhibit any obvious phenotypic differences, and individual fish cannot be classified to either population by visual inspection; yet, they show a high genetic divergence (F_{ST}) of about 0.1 and appear to have existed in stable sympatry for at least 20 years (Palmé et al., 2013).

Given the difficulty to detect sympatric, cryptic fish populations, such structures of biodiversity may be more common than anticipated and it is important to understand what mechanisms support their existence. Can such populations represent separate ecological roles and for instance use different food resources even though obvious morphological divergence has not yet developed? Here, we use the Lakes Trollsvattnen cryptic and sympatric brown trout as a case study for a detailed mapping to address this question using a wide range of tools.

Trophic polymorphism has often been detected by analysing stomach contents in addition to some coarse morphological examination, such as visual inspection of body shape and coloration, measures of body length and gill raker count (Ferguson & Mason, 1981; Præbel et al., 2013; Walker, Greer, & Gardner, 1988). In some cases, more comprehensive methods, including stable isotope analysis and geometric morphometric body shape analysis, have also been used (Gowell et al., 2012; Hirsch, Eckmann, Oppelt, & Behrmann-Godel,

2013; Siwertsson et al., 2013; Woods, Young, Skúlason, Snorrason, & Quinn, 2013). However, to our knowledge, no study has used a combination of all of these methods previously. We examine diet, cryptic morphology, growth and breeding status in individual fish genetically classified to either of the two populations by employing stomach content analysis, stable C and N isotopes, geometric morphometrics and an extensive analysis of gill raker morphology.

2 | MATERIALS AND METHODS

The focal cryptic, sympatric brown trout populations of this study are located in the closely interconnected Lakes Östra Trollsvattnet (ÖT; area of 0.10 km^2) and Västra Trollsvattnet (VT; 0.17 km^2) collectively referred to as Lakes Trollsvattnen. These lakes are located at an elevation of 698 m in a remote protected area (the Hotagen Nature Reserve) in the uppermost part of the River Indalsälven catchment area in central Sweden (Fig. S1), with a day-long hike from the nearest motorable road. The sympatric populations are hereafter referred to as populations A and B (cf. Palmé et al., 2013).

The Lakes Trollsvattnen brown trout have been genetically monitored for several decades as have several other populations in nearby waters included in the Lakes Bävervattnen Project (Charlier et al., 2011, 2012; Jorde & Ryman, 1996; Laikre et al., 1998; Palm & Ryman, 1999; Palm et al., 2003; Palmé et al., 2013). The monitoring effort is conducted to address questions relating to microevolutionary processes, and all sampling is conducted annually in late August.

We used several data sets comprising hundreds to thousands of fish for this study (Table 1). All fish were genotyped at a standard set of 14 polymorphic allozyme loci that have been used for monitoring brown trout populations within the Lakes Bävervattnen Project since the 1980s (Charlier et al., 2012; Jorde & Ryman, 1996; Palm et al., 2003; Palmé et al., 2013), and previous analyses indicate that they behave as selectively neutral ones (Jorde & Ryman, 1996; Charlier et al., 2012). Locus information as well as population genetic data for the two sympatric clusters of these lakes are provided in Palmé et al. (2013). We classified fish to either of the populations A and B using the GENECLASS2 software (Piry et al., 2004) and the STRUCTURE software (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000). As baseline for assignment in GENECLASS2, we used the classification of Palmé et al. (2013) based on fish sampled in Lakes Trollsvattnen during 1987–2005 (N = 4,140). In STRUCTURE, which was run without a baseline, we used the default model allowing for population admixture and correlated allele frequencies, with burn-in length of 250,000 steps and the number of Monte Carlo Markov chains (MCMC) of 500,000 replicates. For all fish caught within the Lakes Bävervattnen Project, in addition to genetic data, data on length, weight, sex, reproductive status (close to breeding or not at the time of collection) and age through otolith readings are recorded (cf. Palmé et al., 2013).

The morphological characterisation was conducted on 192 trout (104 and 88 genetically classified to populations A and B respectively) collected in 2011 (Table 1). We photographed the fish in the field, on the left side using a Nikon D50 digital camera with a 35-mm 1:2.5

TABLE 1 Data sets from brown trout for morphological and dietary analyses used in this study

Lake	Sampling year	N (population A)	N (population B)	N (total)	Analyses
Västra Trollsvattnet (VT)	2011	52	44	96	Body shape, gill rakers
Östra Trollsvattnet (ÖT)	2011	52	44	96	Body shape, gill rakers
Västra Trollsvattnet (VT)	2014	46	46	92	Stomach content
Östra Trollsvattnet (ÖT)	2014	57	47	104	Stomach content
Västra Trollsvattnet (VT)	1988, 1992, 1997, 2004	40	39	79	Stable isotopes
Östra Trollsvattnet (ÖT)	1988, 1992, 1997, 2004	40	40	80	Stable isotopes
Västra Trollsvattnet (VT)	1987–2014	1,493	1,550	3,043	Growth, breeding status
Östra Trollsvattnet (ÖT)	1987–2014	1,633	1,469	3,102	Growth, breeding status

See Supporting Information Fig. S1 for map of sampling sites.

Nikon lens fixed on a camera stand. From these fish, we also collected skulls for gill raker measures.

Stomach content analysis was performed on 196 fish collected in 2014 ($N = 103$ and 93 classified to A and B respectively; Table 1). These fish were caught by gillnets, which were checked every hour to reduce stress and thus increased digestion rate, and stomachs were then collected. In addition to these 196 fish analysed for stomach content, we used 159 temporally separated samples for stable isotope analysis of carbon and nitrogen (Table 1). These fish were collected in 1988, 1992, 1997 and 2004 and stored at −80°C until analysis (with $N = 10$ individuals representing each population and lake in the respective year, except for 2004 where population B from VT was represented by $N = 9$ individuals). The fish used for stable isotope analysis were selected on the basis of their assignment probability (Q-value obtained from STRUCTURE) so that both populations were equally represented in the material. In addition, the body length and age of the stable isotope fish were restricted to 23–30 cm and 5–7 years respectively, as isotopic signature may correlate with body size.

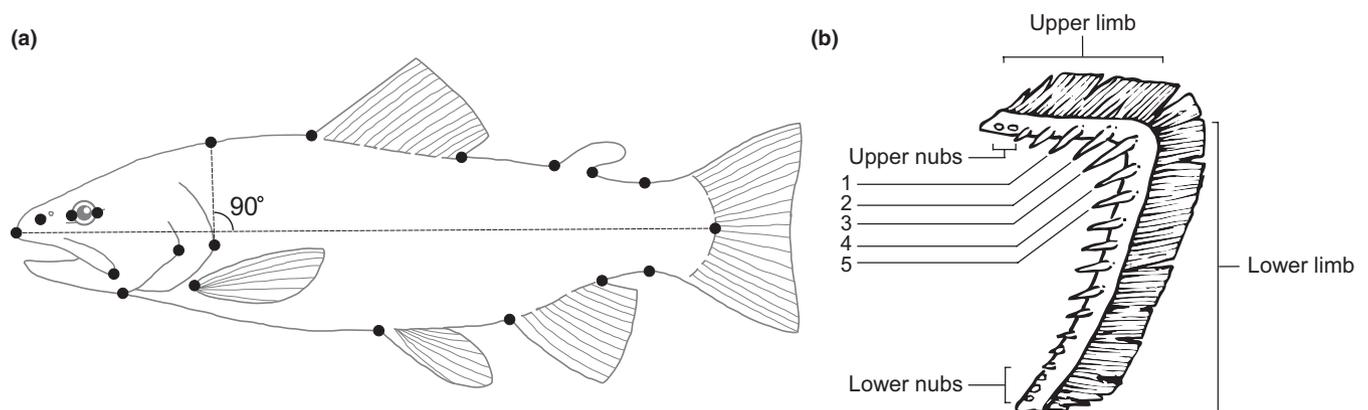## 2.1 | Analysis of body shape and gill rakers

Body shape was analysed using geometric morphometrics (Parsons, Robinson, & Hrbek, 2003; Rohlf & Marcus, 1993). We derived 20 landmarks from the photographs of each individual fish (Fig. 1a) using the tpsDig2 software (Rohlf, 2013a). All fish were superimposed by generalised procrusters' superimposition, a procedure resulting in all individuals being scaled and rotated so that they align as closely as possible (Rüber & Adams, 2001). Thereafter, we generated shape variables (expressed as relative warps) for each individual using the tpsRelw software (Rohlf, 2013b). Finally, we produced deformation grids using the tpsSplin software (Rohlf, 2004) to visualise the effects of population, lake, sex and maturity on body shape.

Gill raker metrics were estimated by counting the gill rakers and yet undeveloped rakers (nubs) on the first right and left gill arches and measuring the length and distance between the five rakers closest to the elbow of the gill arch (Fig. 1b). All gill raker-related measures used in the statistical analyses were calculated as the average between the first right and left gill arches.

2.2 | Stomach content and stable isotope analysis

To estimate diet, we performed a stomach content analysis using a stereo microscope and classified the food items to the lowest taxonomic level possible. We also noted the developmental stage of the insects ingested, that is "adult," "emerger," "pupa" or "larva." Stomach fullness, that is volume (ranging from 0% to 100%) and the proportion

FIGURE 1 The 20 landmarks (black dots) used for the geometric morphometric shape analysis (a) and gill structure (b). We measured the number of nubs and gill rakers, as well as the length of, and distance between, rakers 1–5. Gill raker 3 was classified as middle gill raker as its position was right in the middle of the gill arch elbow

of each food item (ranging from 0% to 100%), was estimated by eye. Food items were classified to either of eight broader categories: benthic prey, limnetic prey, fish, mammal, surface insects, nonfood, plant material and unidentified (Table S1). For each fish, "food category contribution" was quantified as the proportion of each food category found in the stomach in relation to stomach fullness. This implies that for each fish, food category contribution sums up to the degree of stomach fullness.

Long-term feeding was examined by measuring the ratio of the stable nitrogen isotope ^{15}N to the more common ^{14}N, as well as the carbon isotope ^{13}C to ^{12}C. The N isotope ratio gives information about an individual's average trophic position, while the C isotope ratio is strongly influenced by the primary carbon sources (Clarke, Vidergar, & Bennett, 2005). Additionally, we investigated the C:N ratio in muscle tissue, a proxy for lipid content (Fagan, Koops, Arts, & Power, 2011). Approximately 1,200 µg of freeze-dried muscle tissue from each individual was weighed into tin capsules and sent to the Stable Isotope Facility, UC Davis, California, for analysis using a PDZ Europa ANCA-GSL elemental analyser coupled to a PDZ Europa 20–20 isotope ratio mass spectrometer. The C:N mass ratio was calculated from the total amounts of C and N determined by the elemental analyser. The stable isotope signatures are reported as δ values, that is the ‰ deviation of the isotopic ratio of the sample from that of a standard (atmospheric nitrogen or Pee Dee Belemnite carbon).

2.3 | Growth and breeding condition

A subtle, but statistically significant dichotomy with respect to body length has previously been noted between the two sympatric populations in material collected until 2005, as well as a difference in proportion of breeders, that is fish mature to breed at the time of collection, with sampling consistently conducted during the last 2 weeks of August (Palmé et al., 2013). In the present study, we examined whether these differences are present in the total material covering the monitoring period 1987–2014 (N = 6,145) by comparing body length and breeding state of separate age classes between the two populations.

2.4 | Statistical treatment

We used the STATISTICA software (StatSoft Inc., 2005) for statistical analysis. In a first step of the body shape analysis, we performed an overall MANCOVA to test for effects of population (A and B), sampling lake, sex and breeding condition (maturity), with length as a covariate. However, after fitting the model, the interactions length × lake and length × population were significant (results not shown), which posed a problem evaluating potential effects of population and lake. Therefore, we used residuals obtained from linear regressions between length and each of the shape variables expressed as relative warps and conducted three MANOVAs on the residuals from this regression: one for the overall data (lakes and populations) and one within each lake.

In the analysis of gill raker measures, we first conducted regression analyses to examine the relationship between body length and each gill raker measure. Measures associated with gill raker length and inter-raker distance were correlated with body length, and we therefore used residuals from these regressions as variables for all gill raker measures. We performed a MANOVA to investigate potential effects of population, lake, sex and maturity, as well as *t* tests to examine whether gill raker measures differed between populations or lakes.

Chi-square tests were conducted to examine whether fish from the two lakes and populations differed with respect to the degree of how full their stomachs were and to investigate whether the number of fish that had consumed each of the food categories varied between the populations. We also performed a MANCOVA using the eight food categories to examine whether lake and population affiliation had an effect on food preference, taking lake, sex and breeding condition into account. For the comparison of consumed food categories and for the MANCOVA, we excluded stomachs that were empty (N = 3), or contained only unidentified matter (N = 1), thus using 192 of the 196 stomachs. Finally, we performed *t* tests on each food category to compare diet between populations and lakes. For stable isotopes and C:N ratio, three separate ANCOVAs were performed to investigate whether population and lake had any effect on δ^{13}C, δ^{15}N and the C:N ratio respectively, with length set as a covariate.

3 | RESULTS

3.1 | Genetic divergence

We measured genetic divergence between populations A and B as well as between the lakes for all separate sample sets used, as well as for the total material, and we consistently find statistically significant divergence between clusters of $F_{ST} \approx 0.1$ (F_{ST} calculated over 14 loci and ranging from 0.06 to 0.15; Table S2) and no statistically significant divergence between lakes ($F_{ST} \approx 0.0$). Fish used for the stable isotope analysis were selected based on a high assignment probability to either of the populations (Q-values obtained from STRUCTURE) and thus show a higher differentiation between populations than the other sample sets (F_{ST} ranging from 0.22 to 0.31; Table S2).

3.2 | Body shape

We did not observe any differences between the two sympatric populations with respect to body shape in the overall analysis, but there was an effect of lake, sex, maturity and the interaction sex × maturity (all four p < .001; Table 2). Fish inhabiting Lake Östra Trollsvattnet had a more shallow body and a shorter upper jaw than fish from Lake Västra Trollsvattnet (Fig. 2). In addition, there was a tendency for a population × lake interaction (p = .054; Table 2) suggesting a shape difference between populations A and B in one of the lakes.

When we analysed body shape of fish from each separate lake, we found a significant difference between the two populations within one of the lakes (Östra Trollsvattnet; p < .05; Table 2). The populations differed in head and tail orientation; fish from population B had an

TABLE 2 Effect of population, lake, sex and sexual maturity on body shape (MANOVA; shape variables are expressed as residuals obtained from a linear regression of body length vs. each relative warp) in both lakes combined and within each lake respectively

		Wilks λ	F	Effect *df*	Error *df*	*p*	Effect size[a]
Both lakes	Intercept	0.95	0.20	36	148	1.000	0.05
	Lake	0.52	3.86	36	148	**.000**	0.48
	Population	0.75	1.39	36	148	.090	0.25
	Sex	0.30	9.48	36	148	**.000**	0.70
	Maturity	0.54	3.49	36	148	**.000**	0.46
	Population × Lake	0.73	1.48	36	148	.054	0.27
	Sex × Maturity	0.58	2.92	36	148	**.000**	0.42
	Population × Lake × Sex	0.75	1.35	36	148	.111	0.25
Östra Trollsvattnet	Intercept	0.34	2.99	36	56	**.000**	0.66
	Population	0.47	1.74	36	56	**.031**	0.53
	Sex	0.21	5.85	36	56	**.000**	0.79
	Maturity	0.32	3.25	36	56	**.000**	0.68
	Sex × Maturity	0.41	2.24	36	56	**.003**	0.59
Västra Trollsvattnet	Intercept	0.46	1.77	36	55	**.027**	0.54
	Sex	0.22	5.36	36	55	**.000**	0.78
	Maturity	0.36	2.66	36	55	**.001**	0.64
	Population × Sex	0.51	1.46	36	55	.102	0.49
	Sex × Maturity	0.47	1.72	36	55	**.034**	0.53

The fish were collected in 2011 in Lakes Östra and Västra Trollsvattnet respectively (cf. Table 1). Probability values (*p*) below .05 are in bold.
[a]Effect size is expressed as partial eta-squared, which is a proportion of variability in the dependent variables (shape variables) explained by the explanatory variables.

FIGURE 2 Deformation grids showing shape differences between populations A and B in Lakes Östra (*N* = 52 and *N* = 44 respectively) and Västra Trollsvattnet (*N* = 52 and *N* = 44 respectively). Each grid is the consensus shape based on all individuals from each of the separate groups (population/lake combination). The head is to the left; deformations are scaled up five times
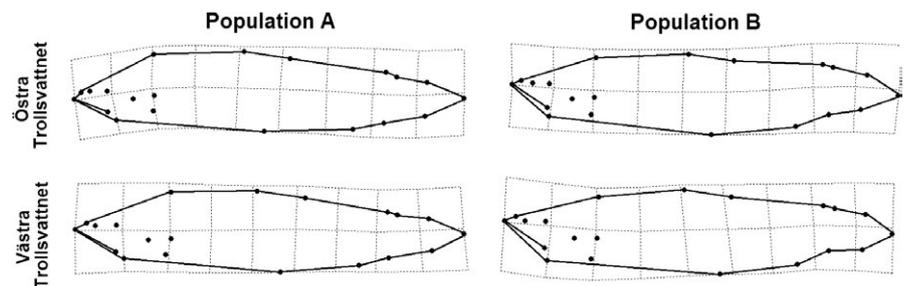
upward oriented head and a downward oriented tail region, while fish from population A showed the opposite (Fig. 2). No effect of population was detected in Lake Västra Trollsvattnet, however. Sex, maturity and the interaction sex × maturity had a significant effect on body shape within each lake (Table 2). Males had larger heads relative to their bodies than females, and mature fish were deeper-bodied than immature ones (Fig. S2).

3.3 | Gill rakers

There was an effect of lake, but no effect of population or the interaction population × lake on the gill raker measures (MANOVA; Table 3). We observed no effect of sex or maturity, so these variables were dropped from the final analysis. The *t* tests showed that the number of gill rakers on the upper limb of the gill arch and the distances between gill rakers 1–2, 2–3 and 3–4 explained the difference in gill raker measures between lakes (data not shown). Fish from Lake Östra Trollsvattnet had slightly more gill rakers on the upper limb of the gill arch and a somewhat longer distance between gill rakers 3 and 4 than fish from Lake Västra Trollsvattnet. Fish from VT had a slightly longer distance between gill rakers 1 and 2 as well as between rakers 2 and 3 (Table S3). Within the separate lakes, we did not find any differences between the two populations in gill raker measures (data not shown).

3.4 | Diet

The proportion of the different food categories in the diet of the brown trout in different lakes and populations is illustrated in Fig. 3. Neither the chi-square tests (data not shown) nor the MANCOVA

TABLE 3 Effect of population and lake on gill morphology (MANOVA; gill raker-related measures, length as covariate) in fish collected in 2011 in Lakes Östra and Västra Trollsvattnet (cf. Table 1)

	Wilks λ	F	Effect df	Error df	p	Effect size[a]
Intercept	0.02	581.67	13	176	**.000**	0.98
Population	0.92	1.19	13	176	.293	0.08
Lake	0.80	3.47	13	176	**.000**	0.20
Population × Lake	0.91	1.40	13	176	.162	0.09

The variables are residuals obtained from a linear regression of body length versus each separate gill raker measure. Probability values (*p*) below .05 are in bold.

[a]Effect size is expressed as partial eta-squared, which is a proportion of variability in the dependent variables (gill-raker related measures) explained by the explanatory variables.
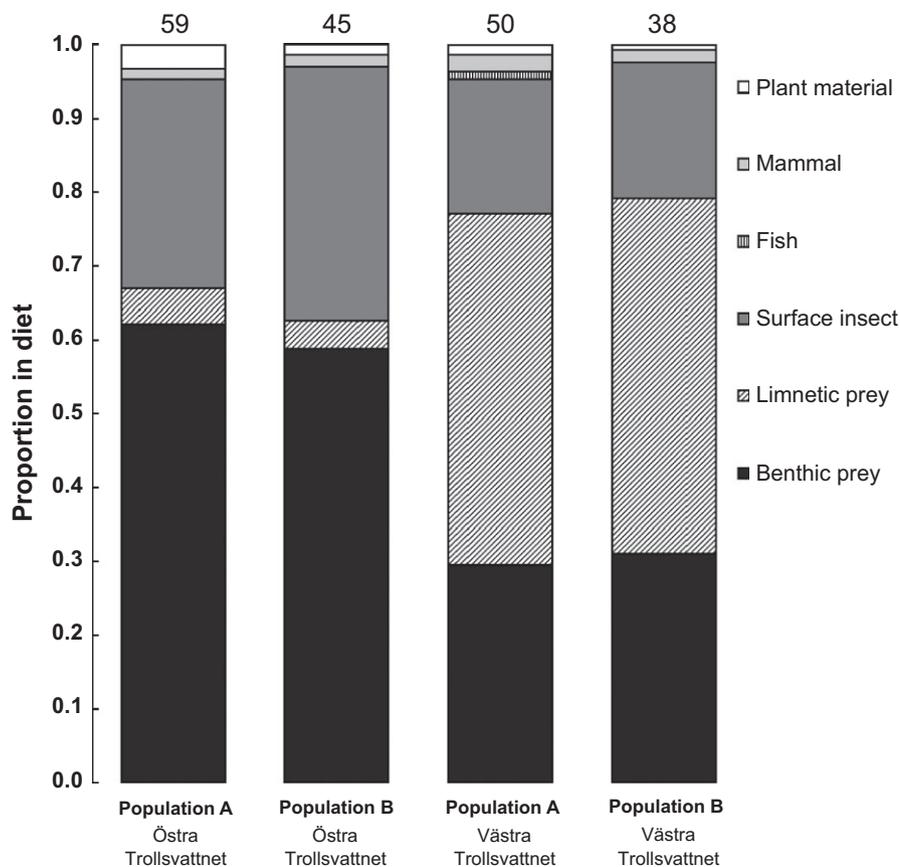
FIGURE 3 Food category contribution to diet of populations A and B in Lakes Östra and Västra Trollsvattnet. The food categories nonfood and unidentified are not included. Numbers on top of the bars indicate the number of individuals in each group

indicated that populations A and B differed in food preferences (Table 4). In contrast, the MANCOVA showed a significant effect of lake as well as of the covariates such as body length and stomach fullness (Table 4). The chi-square tests did not indicate that the populations differed with respect to stomach fullness, however. Sex and maturity did not affect the diet, and therefore, these variables were excluded from the final analysis and are not presented. Further, we used *t* tests and chi-square tests to investigate which food categories explained the divergence in diet between the lakes. The *t* tests showed that trout from Lake Östra Trollsvattnet (ÖT) had significantly more benthic prey in their stomachs (*t* = −5.12, *p* < .001) than did trout from Lake Västra Trollsvattnet (VT; Table 5, Fig. 3). In contrast, trout from VT had eaten more limnetic prey than trout from ÖT

(*t* = 7.68, *p* < .001; Table 5, Fig. 3). Additionally, trout from VT had a significantly larger proportion of unidentified matter in their stomachs than trout from ÖT (*t* = −7.78, *p* < .001). These significances were retained after Bonferroni correction. The exact contingency tests supported these results; benthic prey was present in significantly more stomachs from ÖT (*p* = .0005), while significantly more stomachs from VT contained limnetic prey and unidentified matter (both *p* ≪ .001).

Population did not affect neither nitrogen nor carbon isotope signatures, but there was an effect of lake on both of these variables; for nitrogen, there was also an effect of length (Table S4). We did not find any indication that population, lake or length affected intramuscular lipid content as measured by the carbon:nitrogen ratio.

TABLE 4 Effect of population and lake on diet (MANCOVA; food categories benthic and limnetic prey, surface insect, fish, mammal, plant material, nonfood and unidentified, length and stomach fullness as covariates) of fish collected in Lakes Östra and Västra Trollsvattnet in 2014 (cf. Table 1)

	Wilks λ	F	Effect *df*	Error *df*	*p*	Effect size[a]
Intercept	0.86	4.27	7	180	**.000**	0.14
Body length	0.86	4.14	7	180	**.000**	0.14
Stomach fullness	0.20	101.58	7	180	**.000**	0.80
Population	0.98	0.52	7	180	.818	0.02
Lake	0.62	15.47	7	180	**.000**	0.38
Population × Lake	0.98	0.41	7	180	.895	0.02

Probability values (*p*) below .05 are in bold.
[a]Effect size is expressed as partial eta-squared, which is a proportion of variability in the dependent variables (food category contribution) explained by the explanatory variables.

TABLE 5 Food category contribution to diet between fish collected in Lakes Östra (ÖT; *N* = 104) and Västra Trollsvattnet (VT; *N* = 88) in 2014 (*t* tests)

Nr	Food category	Food category contribution ÖT	Food category contribution VT	*t*	*df*	*p*
1	Benthic prey	0.17	0.07	−5.12	179	**.000**
2	Fish	0.00	0.01	1.09	190	.278
3	Mammal	0.01	0.01	−0.46	152	.648
4	Nonfood	0.00	0.00	1.57	89	.120
5	Limnetic prey	0.01	0.14	7.68	94	**.000**
6	Plant material	0.00	0.01	0.47	98	.640
7	Surface insect	0.07	0.05	−1.69	190	.094
8	Unidentified	0.16	0.04	−7.78	152	**.000**

Probability values (*p*) below .05 are in bold.
For food categories with unequal variances, the Welch *t* test was used to estimate the *t*, *df* and *p*; these values are in italics. All significances were retained after Bonferroni correction.

3.5 | Growth and maturation

Fish in population A were on average somewhat larger than those in population B, and this difference was observed in all seven age classes for both sexes. When considering all fish collected in 1987-2014, we found larger individuals in population A than in B in all the 14 within-lake comparisons, with 11 being statistically significant (*p* ranging from .000 to .038; Table S5, Fig. S3). This trend of fish from population A being slightly larger appears consistent over time, and this was also previously reported by Palmé et al. (2013) for the period 1987–2005.

Further, Palmé et al. (2013) report a higher proportion of mature fish in population B than in A during 1987–2005, and the pattern remains for the extended period 1987–2014. We found significant differences in both sexes within each of the lakes when all age classes were pooled (Table S6). The overall proportion of sexually mature fish was 0.33 in population A and 0.51 in population B.

4 | DISCUSSION

We found no indication of trophic divergence or separate food niche use in the two, genetically divergent, sympatric brown trout

populations analysed in this study. The populations did not differ with respect to diet (short term or long term) or gill raker metrics, but they appear to show a subtle difference in body shape within one of the lakes, Lake Östra Trollsvattnet. Fish from population A have a slightly downward oriented head and upward oriented tail region, while fish from population B show the opposite, although this cannot be seen when the total material from both lakes is considered. Also, this slight morphological divergence is not reflected in a difference in diet between the two populations in Lake Östra Trollsvattnet.

There is a weak but statistically significant difference in body length, with trout from population A being consistently larger than those from B. Also, fish from population B show a higher frequency of individuals ready to breed at the time of collection than population A. This distinction occurs in both lakes and is most pronounced for males. Data on body length and spawning frequency are available over a 25-year period of monitoring, and the differences between the populations appear consistent over this time.

In contrast to the results from the present study, trophic polymorphism, including coupling of body shape and gill raker morphology to dietary divergence (Wimberger, 1994), is often reported in salmonids (Ferguson & Mason, 1981; Gíslason, Ferguson, Skúlason, & Snorrason, 1999; Power et al., 2009; Præbel et al., 2013; Woods et al., 2013)

and other fish species (Andersson, Johansson, & Söderlund, 2006; Hjelm, Svanbäck, Byström, Persson, & Wahlström, 2001; Svanbäck & Eklöv, 2003). We observe differences between fish from the two lakes—fish from Lakes Östra and Västra Trollsvattnet differ regarding diet, body shape and gill rakers; however, these differences are not consistent with previous findings with respect to coupling morphology to diet. Generally, zooplankton (limnetic prey) feeders tend to have a more slender body with an upward oriented head as well as longer and more numerous gill rakers, while those primarily feeding on benthic prey have a deep body, a downward oriented head and fewer shorter gill rakers (Schluter, 1996). Benthic prey dominated the diet of fish from Lake Östra Trollsvattnet; yet, these individuals were more shallow-bodied and had slightly more gill rakers than fish from Lake Västra Trollsvattnet, who consumed more limnetic prey. A few studies report similar inconsistencies regarding body shape, gill rakers and diet (Andersson, 2003; Langeland & Nøst, 1995). Langeland and Nøst (1995) examined gill raker structure and food size in several salmonids and other fish species, and despite differing with respect to gill raker spacing, the fish species consumed prey of similar size. Andersson (2003) investigated body shape in young Arctic char raised on a benthic and a zooplankton diet respectively, but did not observe the commonly reported slender shape of plankton eaters. Such findings indicate that diet alone may not be enough to induce morphological differences such as those reported for fish populations in their natural habitats (Andersson, 2003).

The main dichotomy between the sympatric populations in Lakes Trollsvattnen appears to be the frequency of reproductively mature fish at time of collection (late August) and growth. Population B shows a higher proportion of mature fish than population A. There can be several explanations for this: (i) population B fish become mature at an earlier age than A, (ii) there is a temporal lag between the two populations with respect to spawning or (iii) fish from population B spawn more frequently than fish from A (brown trout are iteroparous and can reproduce several times in life). Our data set does not allow us to distinguish between these three scenarios, but we argue that explanation (ii) is unlikely because the time available for spawning is very short at the altitude of the study area. Winter conditions start in late October–early November, and spawning typically occurs during early September–early October. It does not appear likely that fish with undeveloped gonads in late August would spawn in 1–2 months, as the development of gonads from immature to mature for reproduction would take at least 4–6 months (Caputo, Giovannotti, & Splendiani, 2010; Crim & Idler, 1978; Hajirezaee et al., 2012). An observation that supports explanation (i) is that sexually mature brown trout from population A are on average slightly older than those from B (5.94 vs. 5.76 years respectively; $p < .01$). This difference is most pronounced in Lake Västra Trollsvattnet (6.14 vs. 5.86 years; $p < .001$), whereas in Lake Östra Trollsvattnet there was no statistically significant difference in age of mature individuals between the populations (Table S7).

Both explanations (i) and (iii) are in line with the observation that fish from population A are consistently larger than fish from B (Fig. S3), because if fish from population A mature later they would be larger at the first time of reproduction. Also, if fish from population A

reproduce less frequently than those from B, they can use resources to grow larger and thereby increase their reproductive success during years when they spawn.

The divergence in length and breeding condition are consistent on both spatial and temporal scales. However, the overall phenotypic difference between populations A and B is small and not discernible for the bare eye. Many studies examining both morphology and diet have reported trophic polymorphism coupled to genetic differentiation in salmonids (e.g. Ferguson & Mason, 1981; Power et al., 2009; Præbel et al., 2013; Sandlund et al., 1992). In these cases, genetic differentiation between the populations was detected on the basis of clear morphological differences that were obvious and gave a reason to suspect differential trophic resource use. In addition, the majority of these studies are on Arctic char and the whitefish species complex, in which occurrence of sympatric, morphologically differentiated forms is quite common.

Despite the fact that we compared two clearly genetically divergent, sympatric brown trout populations, we found no indication of trophic divergence or separate food niche use in the two. In contrast, fish in the two lakes differed in body shape, diet and stable isotopes despite no genetic difference between lakes. These genetically divergent populations apparently coexist using the same food resources and show the same adaptive plasticity to the local food niches of the two separate lakes. These patterns are different from the classical pattern of sympatric speciation in lakes, which states that adaptation to different food sources may give rise to divergent selection that creates and maintains genetic differentiation (Rundle & Nosil, 2005; Schluter, 1996).

It is possible that the genetic structure is created and maintained by different spawning sites even over the present very restricted geographic area as there are several small creeks potentially suitable for spawning flowing into and out of Lakes Trollsvattnen (cf. Fig. S1). Both anadromous and lake resident salmonids are known to show strong homing behaviour when returning to their natal streams to spawn (Dittman & Quinn, 1996; Elliott, 1994; Neville, Isaak, Dunham, Thurow, & Rieman, 2006; Nordgren, 2009), and several studies have suggested differences in spawning location as a possible mechanism maintaining sympatry (e.g. Duguid, Ferguson, & Prodöhl, 2006; Ferguson & Taggart, 1991; Walker et al., 1988), but not in waters of such restricted size as the present ones. On the other hand, genetic structure due to restricted dispersal of related individuals over a limited geographic area has been reported for brown trout in Mediterranean streams (Sanz, Fernández-Cebrián, Casals, Araguas, & García-Marín, 2011). This explanation, however, does not appear likely in the case of Lakes Trollsvattnen, as the two populations are physically admixed within both lakes. Instead, we suspect that the populations may segregate with respect to inflow and outflow spawning. Ferguson and Taggart (1991) found that the sympatrically occurring and genetically, morphologically and ecologically distinct morphotypes of brown trout—ferox, gillaroo and sonaghen in Loch Melvin, Ireland—differ with respect to water flow direction at their spawning sites. The gillaroo use the outflow for spawning, while the ferox and sonaghen use different inflows, and similar results are reported for ferox in other lakes (Duguid

et al., 2006). Thus, it is possible that one of the populations in Lakes Trollsvattnen spawns in inflowing creeks while the other one uses the outflow. We intend to investigate this potential isolating mechanism for the Lakes Trollsvattnen populations in a forthcoming study.

Similar observations to those that we document here have not been reported previously. However, it is possible that cryptic, sympatric genetic structures are more common than realised in landlocked salmonids, because such genetic structures are quite difficult to detect. Relatively extensive sampling is required, and rather strong genetic divergence might be necessary to provide good statistical power (cf. Palmé et al., 2013). This issue of potential for detecting cryptic structures also needs further scientific attention, and we intend to return to this issue in forthcoming work.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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