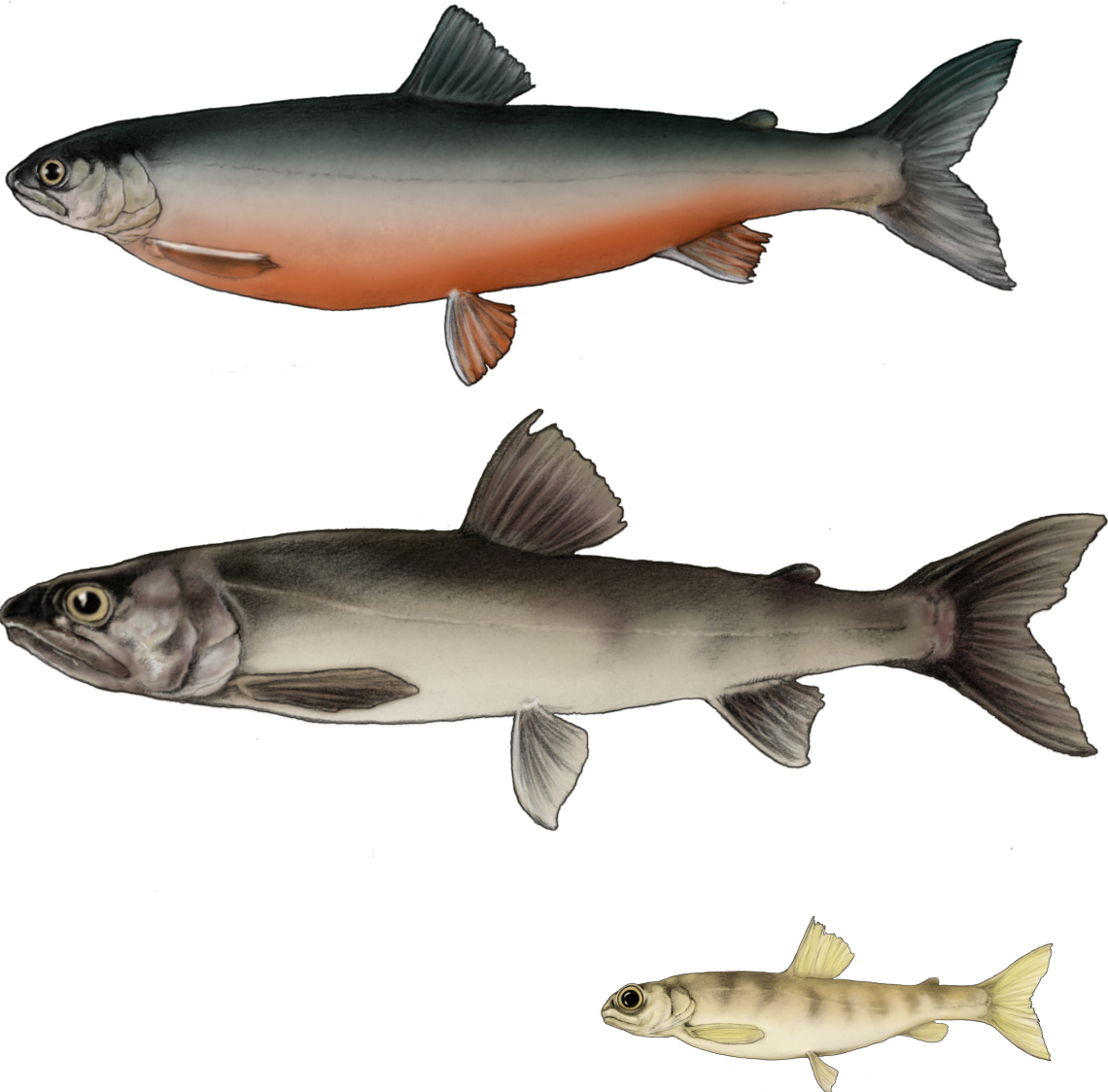


Morphological divergence in a trimorphic population of Arctic charr (*Salvelinus alpinus* (L.)) in Skogsfjordvatn, northern Norway.

Sigrid Skoglund

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“Stories of an ugly-looking Arctic charr living in Skogsfjordvatn have long been told amongst the local people on Ringvassøya. The specific charr was colloquially called Storskoiltrøya (Big-headed charr), because of its abnormal large head. In the old days when food were scarce, the fishermen made use of the charr by salting it and kept it as food throughout the coldest season.”

(Laina Dalsbø, pers. comm. 2011)

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Summary

Sympatric polymorphisms are found in many freshwater fish taxa, including the salmonid Arctic charr (*Salvelinus alpinus* (L.)). Polymorphism is often expressed as differences in morphology, behaviour and life-history strategies, and may be driven by alternative phenotypic adaptations to resource use such as habitat and prey preferences. Morphological divergence is usually linked to different functions of the morphological trait; body form is related to habitat preference and swimming efficiency, while head form is related to prey acquisition and foraging behaviour. Here, I study the correlations between morphology and ecological function in sympatric morphs of Arctic charr.

The oligotrophic lake Skogsfjordvatn (Norway) has been found to inhabit a trimorphic population of Arctic charr: a littoral spawning omnivore morph (LO morph), a profundal spawning benthivore morph (PB morph) and a profundal spawning piscivore morph (PP morph). The three charr morphs reveal highly variable morphologies regarding both body- and head morphology. They also diverge in resource use (i.e. diet and habitat), life-history strategies, and into three genetic groups.

The LO morph appears as a typical charr found in monomorphic populations. It predominantly utilizes the littoral-pelagic habitats, has a wide diet niche and express similar life-history traits found in monomorphic charr. The other two morphs reside in the profundal habitat throughout their lifetime, and were found to diverge in morphology, prey utilization and have highly contrasting life-history strategies. The small-sized PB morph is found to have a paedomorphic appearance with a body- and head shape adapted to live close to the soft profundal bottom and to utilize benthos submerged in the sediment. The PP morph has a large, robust head and an elongated body shape strongly related to its piscivorous behaviour, predominantly utilizing small charr and three-spined sticklebacks as prey. Both the profundal morphs have large eyes, suggested as an adaptation to survival in a darker environment. All the morphs reveal morphologies that clearly are adaptations to their environmental surroundings and their foraging ecology. Thus, the study provides empirical support for incipient ecological driven speciation to be in action.

Introduction

“... I look at varieties which are in any degree more distinct and permanent, as steps leading to more strongly marked and permanent varieties; and at these latter, as leading to sub-species, and to species...” (Darwin 1859)

Over 150 years have past since Charles Darwin wrote the famous book “On the Origin of Species” (1859) and we are still amazed and puzzled by the varieties of creatures living among us. The process of speciation may be described by three essential components; a source of divergent natural selection that separates different phenotypes, some sort of reproductive isolation between them and a genetic mechanism linking them (Rundle & Nosil 2005). The source of divergence is often related to ecological factors that enforce different selection pressures on alternative phenotypes, a process commonly known as ecological driven speciation (Schluter 2001, 2009; McKinnon *et al.* 2004; Rundle & Nosil 2005; Sobel *et al.* 2010). Ecological factors can be differences in the environment or interactions within the population related to resource acquisition, such as different habitat preference or prey selection (Schluter 2001). Divergent selection on traits may give rise to resource polymorphism, which is defined as *the occurrence of discrete morphs showing differential niche use, usually through discrete differences in feeding biology and habitat use* (Skúlason & Smith 1995). Polymorphic populations are found in many freshwater fish taxa, such as salmonids, cichlids and sticklebacks (Robinson & Parsons 2002). Polymorphism can be initiated in phenotypic plastic populations, where the individuals have the ability to alter different phenotypes in response to environmental changes (Skúlason & Smith 1995). As an effect of different selective pressures from the environment this may result in morphological varieties within a population (West-Eberhard 1989; Smith & Skúlason 1996). Here, I will explore whether differences in habitat and/or dietary resource acquisition is related to divergences in body- and trophic morphology found amongst three sympatric morphs of Arctic charr (*Salvelinus alpinus*).

Polymorphic fish populations often express different phenotypes that may reveal differences in morphology (e.g. trophic adaptive traits, body shape and coloration), behaviour and life-history strategies (e.g. maturation and growth), and they may also vary in more than one phenotypic characteristic (West-Eberhard 1989; Smith & Skúlason 1996; Parsons, Skúlason & Ferguson 2010). Sympatric polymorphism in freshwater fishes usually appears as

a divergence along a benthic-limnetic resource axis, with a littoral morph utilizing benthic prey and a pelagic morph utilizing limnetic prey (Schluter & McPhail 1992; Wootton 1998; Svanbäck & Eklöv 2003). In these situations, disruptive natural selection against intermediate phenotypes is suggested to promote divergence into different specialized phenotypes exploiting different niches. Specialized phenotypes may exhibit a higher fitness in their preferred resource habitats (e.g. littoral or pelagic niches), while intermediate phenotypes that are not adapted to either benthic or limnetic resources, will be selected against (Dieckmann & Doebeli 1999; Schluter 2001). Other mechanisms that may drive the speciation process to complete the reproductive isolation, is mating preference (e.g. colour patterns, body size, courtship behaviour) and different spawning time or place (Schluter 2001; Pianka 2011).

Important aspects of fishes ecological niche use and behaviour are expressed in their morphology, as form and function are highly related (Webb 1984; Wootton 1998). In general, the body shape of fish is closely connected to habitat complexity and swimming behaviour (Schoener 1971; Webb 1994) and the head shape relates to foraging and prey specializations by adapting different trophic morphologies (Snorrason *et al.* 1994; Adams *et al.* 1998). There are three main categories of body shape based on locomotion and prey acquisition (Webb 1984, 1994; Webb & Weihs 1986; Svanbäck 2004). First are the *cruisers* with a fusiform body shape, adapted to swim over long distances and to exploit prey that are dispersed, as e.g. typical for pelagic fish that hunt for zooplankton prey. Next are the *manoeuvres* that have a short and deep laterally compressed body. They often occur in more structurally complex habitats such as the littoral zone or close to the bottom relying on their manoeuvrability and balance. Lastly are the *accelerators* assumed to have a typical predator morphology with an elongated body to reduce drag and larger fin area in the posterior part of the body to boost acceleration (e.g. body shape of pike (*Esox lucius*)) (Webb 1984, 1994; Wootton 1998). Furthermore, resource polymorphisms are found to be highly related to foraging behaviour reflected in the head shape, mouth position and the morphology of the gill rakers (Skúlason, Noakes & Snorrason 1989; Smith & Skúlason 1996; Adams *et al.* 1998). In general, planktivore fish have a pointed head shape with a terminal positioned mouth and acquire long and dense gill rakers to filtrate zooplankton. Benthivore fish may have a more rounded head shape and a small sub-terminal positioned mouth to prey on benthic invertebrates. They also have shorter and less dense gill rakers compared with the planktivores. Piscivore fish have the most robust head with a pointy shape, and a large terminal mouth adapted to predate on fish prey in the water column (Skúlason *et al.* 1989).

In Arctic and subarctic areas there are numerous lake systems that were formed as the icecap retreated after the last glacial epoch (10 000 – 15 000 years ago). These postglacial lakes are excellent environments to study early speciation processes, as they are usually species poor with low resource competition and offer a diversity of underutilized habitat and food resources, which promotes processes such as character release and resource polymorphism (Hindar & Jonsson 1993; Robinson & Wilson 1994; Jonsson & Jonsson 2001). The salmonid Arctic charr is an excellent study organism for resource polymorphism as it is a well-documented polymorphic species existing both in resident (non-migratory) and anadromous populations (Klemetsen 2010). Arctic charr can appear as 2 - 4 sympatric morphs typically adapted to utilize different habitat and diet niches in the lake due to different ecologic selection pressures (Jonsson & Jonsson 2001). The morphs are found to diverge in morphology, habitat- and diet utilization, as well as life-history strategies (e.g. growth, reproduction, age and size at maturity) (Sandlund *et al.* 1992; Adams, Woltering & Alexander 2003; Klemetsen 2010).

The majority of studies of polymorphism in Arctic charr are based on landlocked populations that diverge in utilizing benthic vs. limnetic resources (Jonsson & Jonsson 2001 and references herein). A classic example is the four morphs in Thingvallavatn (Iceland) where two benthic specialists differing in body size (large and small morph) and utilizes different benthic prey resources, while in the limnetic habitat two other morphs diverge in feeding behaviour (one planktivore morph and one piscivore morph) (Malmquist & Snorrason 1992; Sandlund *et al.* 1992). Furthermore, in Loch Rannoch (Scotland) three morphs diverge in morphology and diet choice (Adams *et al.* 1998). Here, a brightly coloured morph preys on zooplankton in the pelagic zone, while two more cryptically coloured morphs diverge in prey choice in the littoral zone, specializing either on benthic invertebrates or fish. They all differ in head morphology related to their feeding behaviour (Snorrason *et al.* 1994; Adams *et al.* 1998). Few Arctic charr studies show divergence caused by niche utilization in different depths of the lake (Klemetsen 2010 and references herein). However, in lake Fjellfrøsvatn (Norway) there are two distinct morphs that segregate in habitat and prey choice between the littoral and the profundal habitat (Knudsen *et al.* 2006; Amundsen, Knudsen & Klemetsen 2008). They are spatially and temporally isolated in reproduction, have different life-history traits and different heritable morphologies (Klemetsen *et al.* 1997, 2002; Westgaard, Klemetsen & Knudsen 2004). One morph typically utilizes the littoral-pelagic resources and appears as a typical monomorphic charr in behaviour, life-history strategy and morphology. In contrast, a small-sized morph exploits the soft-bottom resources in the profundal zone and

resides there throughout its lifetime. It has a paedomorphic appearance and matures at a small size and young age (Klemetsen *et al.* 1997).

Recently, another lake (Skogsfjordvatn, Norway) with profundal morphs has been identified, where three morphs diverge in both habitat and prey resources (see Table 1 for details, R. Knudsen, unpublished), as well as in life-history strategies and spawning ecology (Smalås 2013). The morphs have been given names based on their observed spawning habitat and their main prey resource use: a littoral spawning omnivore morph (hereafter referred to as the LO morph), a profundal spawning benthivore morph (the PB morph) and a profundal spawning piscivore morph (the PP morph) (Fig. 1). The LO morph occurs predominantly in the upper water layers (i.e. littoral-pelagic habitats), utilizing a wide variety of pelagic zooplankton and near-shore prey resources, also reflected in a large range in $\delta^{13}\text{C}$ signals from muscle tissue as commonly seen in monomorphic charr in North Scandinavia (Eloranta, Knudsen & Amundsen 2013). The LO morph perform an ontogenetic niche shift (both habitat and dietary shifts) (Klemetsen *et al.* 2003), with juvenile charr in the profundal zone, medium sized charr in the pelagic and adult charr in the littoral zone (Smalås 2013). Smalås (2013) found that the LO morph matures at $\sim 21\text{cm}$ and spawns in early autumn (Sept/Oct) in the littoral zone.

In the profundal zone ($>20\text{m}$ depth), the other two morphs are found segregating in prey resource use and have contrasting life-history traits (Smalås 2013). The PB morph is a small-sized charr that matures at a young age (Smalås 2013) and is only caught in the profundal zone feeding on soft-bottom profundal benthic invertebrates (mussels, chironomids and oligochaetes). Stable isotope signals from muscle show high $\delta^{15}\text{N}$ values, typically seen in other profundal benthivore fish (Harrod, Mallela & Kahilainen 2010; Siwertsson *et al.* 2013). The final profundal spawning morph is called the PP morph. Local fishermen in Skogsfjordvatn refer to it as “Storskoiltrøya” (Eng: *Big-headed charr*) because it has a more robust and longer head compared to the LO morph. The PP morph has a piscivorous and cannibalistic behaviour, preying on small-sized individuals of all charr morphs and occasionally on three-spined sticklebacks. The piscivorous behaviour is also reflected in the highest $\delta^{15}\text{N}$ values from muscle tissue (Guiguer *et al.* 2002; McCarthy *et al.* 2004). Smalås (2013) found that the PP morph have a slow growth and matures at an average size of $\sim 26\text{cm}$.

Genetic analyses based on microsatellites have shown that all the three morphs are distinctly different genetically and thus are reproductively isolated (R. Knudsen, unpublished). The differences are larger than what has been found in other comparable sympatric charr systems (e.g. Westgaard *et al.* 2004). The two profundal morphs (PB and PP

morphs) are found to have the largest genetic distance (F_{st} value = 0.30, R. Knudsen, unpublished).

The aim of this study is to explore if the observed different resource utilizations in respect to habitat and prey resources among the three sympatric morphs have caused an adaptive morphological divergence in body and head morphology. The main questions addressed are firstly, whether the three Arctic charr morphs in Skogsfjordvatn are different in morphology in terms of body shape, head shape and in traditional linear measurements of morphological traits. Secondly I address whether the potential morphological differences could be a result of selection on adaptive traits in respect to different resource utilizations (i.e. habitat and diet) among the three morphs. Finally, I will discuss and propose suggestions on how two of the morphs may have developed and adapted to utilize the less profitable profundal zone of Skogsfjordvatn.

The LO morph seems to have a wide niche distribution in respect to habitat and diet use, while the trophic niche of the PB morph and PP morph appear to be more narrow as they are restricted to the profundal prey resources (Table 1). Thus, the LO morph is hypothesized to have morphological characteristics similar to typical omnivore charr found in monomorphic populations (Klemetsen *et al.* 2003; Knudsen *et al.* 2007).

The resource utilization of soft-bottom prey by the PB morph is hypothesized to result in a blunt head shape and a sub-terminal mouth position, as well as an epibenthic body shape related to a life close to the bottom substrate, supported by studies on similar profundal morphs (Knudsen *et al.* 2006).

In contrast to both the other morphs, the PP morph is hypothesized to have a large head relative to its body size, as well as having a large and terminal positioned mouth related to its piscivory behaviour (Adams *et al.* 1998; Wootton 1998). Furthermore, the body shape of the PP morph should be elongated to reduce drag when hunting fish prey (Webb 1984; Svanbäck 2004).

I also expect that living in a darker habitat such as the profundal zone, are likely to have an effect on eye size. With this in mind, both of the profundal morphs are hypothesized to have larger eyes than the LO morph who mainly lives under brighter light conditions in upper water layers.

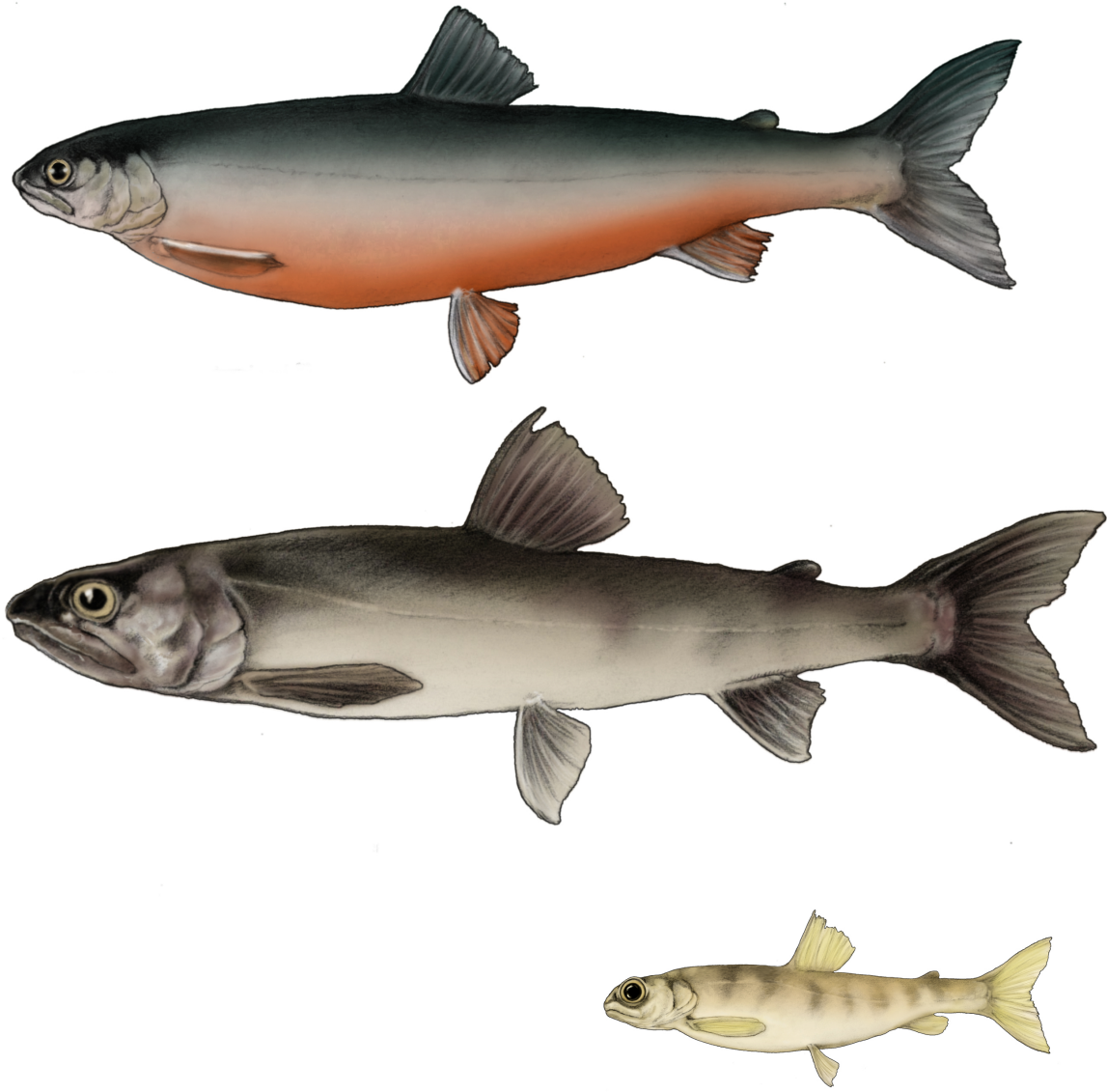


Fig 1: Graphical illustration of adults of the three Arctic charr morphs in Skogsfjordvatn, Norway. From top: LO morph, PP morph and PB morph (Ill.: Sigríð Skoglund).

Table 1: Diet data from the three morphs of Arctic charr in Skogsfjordvatn, based on stable isotope signals from muscle tissue (SIA mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with standard deviation) and stomach data (% prey abundance). Prey groups are explained in detail in table A1 in Appendix (R. Knudsen, unpublished)

	SIA FROM MUSCLE		STOMACH DATA (% PREY ABUNDANCE)				
	$\Delta^{13}\text{C}$ (SD)	$\Delta^{15}\text{N}$ (SD)	LITTORAL BENTHOS	ZOO- PLANKTON	ADULT DIPTERA	PROFUNDAL BENTHOS	FISH
LO morph	-27.1 (-30.1, -16.9)	5.4 (3.6, 6.2)	39	49	10	0	2
PB morph	-26.2 (-27.2, -24.7)	8.6 (8.2, 9.3)	15	1	2	82	0
PP morph	-24.5 (-26.6, -22.9)	9.7 (7.3, 11.3)	15	0	3	24	58

Study area

Skogsfjordvatn (69°56'24"N, 19°10'00"E) is an oligotrophic lake located on Ringvassøya in northern Norway (Fig. 2). It is situated 20 m above sea level and is closely connected to the marine fjord Skogsfjord by the one-kilometre long river Skogsfjordelva. The lake has a surface area of 13.6 km² and a maximum depth of about 100 meters with well defined littoral, pelagic and profundal zones. However, the main area of the lake has a depth of 50-60 meters. It is dimictic, with ice usually covering the lake from December to May. The drainage area of the watercourse consists of high alpine areas to lowlands dominated by birch forest, marches and heather. At the northeast side of the lake there are several farmlands that may provide the lake with some nutrient loadings.

FISH COMMUNITY OF SKOGSFJORDVATN

Following the glacial retreat (~12000 years ago), the landmasses rose due to relief of the pressure from thick ice. The rising of landmasses resulted in a drop in ocean level (30-35m) over the postglacial time period on Ringvassøya (Bratrein 1989). Skogsfjordvatn has an elongated shape (Fig. 2) and is situated at a low altitude, and known to originally have been a marine fjord (Bratrein 1989). This has resulted in the present fish community of both anadromous and resident populations of Arctic charr and brown trout (*Salmo trutta*), and anadromous Atlantic salmon (*Salmo salar*). The lake also inhabits a population of three-spined sticklebacks (*Gasterosteus aculeatus*) and occasionally catadromous European eel (*Anguilla anguilla*). Most of the fish species occupies the littoral zone, and Arctic charr occur as the dominant species in both the pelagic and profundal habitats.



Fig 2: Map overview of Skogsfjordvatn situated on Ringvassøya in northern parts of Troms county, Norway (Statens Kartverk 2013).

Materials and methods

Fish sampling and analyses in the field

Fish sampling in the lake was done in May, June and August 2011. Arctic charr were caught by using monofilament gillnets of different mesh sizes. We used three different types of gillnets: Two of them were multi-meshed gillnets with a range of mesh sizes (OG: 10-45mm, and NORDIC: 5-55mm) and the last type were standard sized gillnets (SG) with only one mesh size throughout the net. Gillnets were set at approximately the same locations each sampling months in all three habitats of the lake: the littoral zone (0-12m) and pelagic zone (floating 0-6m deep net at 30m depth), as well as at three different depths of the profundal zone (25m, 35m and 45m). Gillnets were set in the evening and collected the next morning, giving a fishing period of about 12 hours each time.

In the field, the charr were subjectively sorted in three different morph groups based on the general appearance of the charr. Identification was mainly associated with differences in head and body morphology and colouration (i.e. the LO and PP morphs) combined with sexual maturation in smaller individuals (i.e. the PB morph). Later, the sorting in field was confirmed by genetic analysis (R. Knudsen, unpublished).

The charr were photographed for morphological analyses (Fig. 3, see Morphological analysis) before further processing of the fish. Fork length (mm) and weight (g) were measured, and otoliths were collected for age analysis. Gill samples were taken and preserved in ethanol for genetic analysis, in order to check for genetic differentiation and possible reproductive isolation between the morphs. Stomach content was preserved in ethanol for diet analysis and muscle samples were frozen SIA for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (see Table 1 for details).



Fig 3: Photographing Arctic charr for morphometric analysis-

Morphological analysis

GENERAL METHODS

The charr were digitally photographed for shape analysis and linear measurements. The lateral left side of each fish was attached with pins to a neutral coloured styrofoam plate. Pins were used to stretch out the dorsal, pelvic and anal fins in order to reveal the accurate position of each fin. A red-coloured tread was used to highlight the posterior point of the upper maxilla bone, especially on smaller fish where this point is difficult to observe. All fish were photographed with a digital camera (Nikon Coolpix 5400) on highest resolution (5MP). The camera was fixed to a camera stand with a standardized distance of 75cm from the camera lens to the fish and two spotlights were used to enhance light conditions. Each photo included two scales (mm), identification number, sampling date and the capture habitat (littoral, pelagic or profundal).

Subsamples of the total catch of charr used in the morphological analyses varied from 47 to 61 individuals per morph (Table 2). For the LO morph, the sampled individuals were caught in both littoral and pelagic habitats and were randomly selected within the size-range from 18 – 32 cm. All individuals of the PB morphs were used, due to an overall small sample size. For the PP morph, individuals were first selected within the same size range as for the LO morph. To get a larger sample size, some individuals (n=7) outside the given size range were also included. All individuals were caught before the spawning season (Sept/Oct.) to reduce variability due to secondary sexual traits. However, significant differences were found between the sexes in body shape ($p = 0.005$, Table A2; Appendix) and head shape ($p = 0.008$, Table A3; Appendix) as well as between the maturity stages in both shape analysis (Table A2 and A3). The differences were minor compared to overall morphological differences and not clearly identified in any of the PCA graphical outputs. Thus, in further analyses the different sexes and maturity stages are not focused on.

Table 2: Size (in cm) and number (n) of Arctic charr included in the morphological analysis, divided into the three morph groups LO morph, PB morph and PP morph.

	Individuals (n)	Mean length (cm)	Size range (cm)
LO morph	61	24.8	(18.7 – 31.9)
PB morph	47	10.7	(7.8 – 13.7)
PP morph	51	25.4	(10.1 – 44.8)

MORPHOMETRICS

Shape variation among individuals can be measured quantitatively by using morphometric methods. Traditionally morphometric analyses have been related to the size (length, depth, width) of different morphological characters on specimens (wing length, beak depth etc.). This method is highly influenced by the *size* factor, especially for species with indeterminate growth (e.g. fish) (Bookstein *et al.* 1985). Therefore many of the linear measured traits are correlated with the individual body size, giving few independent variables to compare between individuals and little information about the shape of the individual. It is however useful when absolute or relative sizes are of interest, as when comparing length measurements in morphology studies (Adams, Rohlf & Slice 2004).

Another morphometric approach is landmark-based geometric morphometrics, which rely on landmark-coordinates to define and analyse *shape* variation between individuals (Rohlf & Marcus 1993). Landmarks are either two- or three-dimensional points, and are set to similar locations on the body of each specimen. Each landmark gives up to three coordinate values that in combination with all landmarks can be used to visualize the individual shape (in a xyz-graph). Thus, geometric morphometrics is a more graphical approach that has given the opportunity to illustrate, explain and quantify shape variation in a more expressive way (Bookstein 1997; Adams *et al.* 2004; Slice 2007).

In my study I have used both of these morphometric approaches. First, I looked at shape variation by using landmark-based morphometry in two-dimensional scale (Fig 5). Secondly, I used traditional morphometrics to compare linear measurements of eight selected morphological traits (Table 3).

LANDMARK-BASED GEOMETRIC MORPHOMETRICS (BODY AND HEAD SHAPE)

All the digital photographs were first piled together in a stacked tps-file using the software tpsUtil v.1.53(Rohlf 2010b) and further opened in the program tpsDig v.2.16 (Rohlf 2010a) to place landmarks for the geometric morphometric analysis. Twenty landmarks were set on standardized anatomic locations on the fish to determine body shape, while a total of 16 landmarks were used to explore variation related to head morphology (Fig. 5). A scaled distance was measured on each photo to give a scale factor that converts the pixels to millimetres, in order to set all individual fish to the same scale. Scale factors are particularly important when different focal lengths have been used during photographing (Viscosi & Cardini 2011).

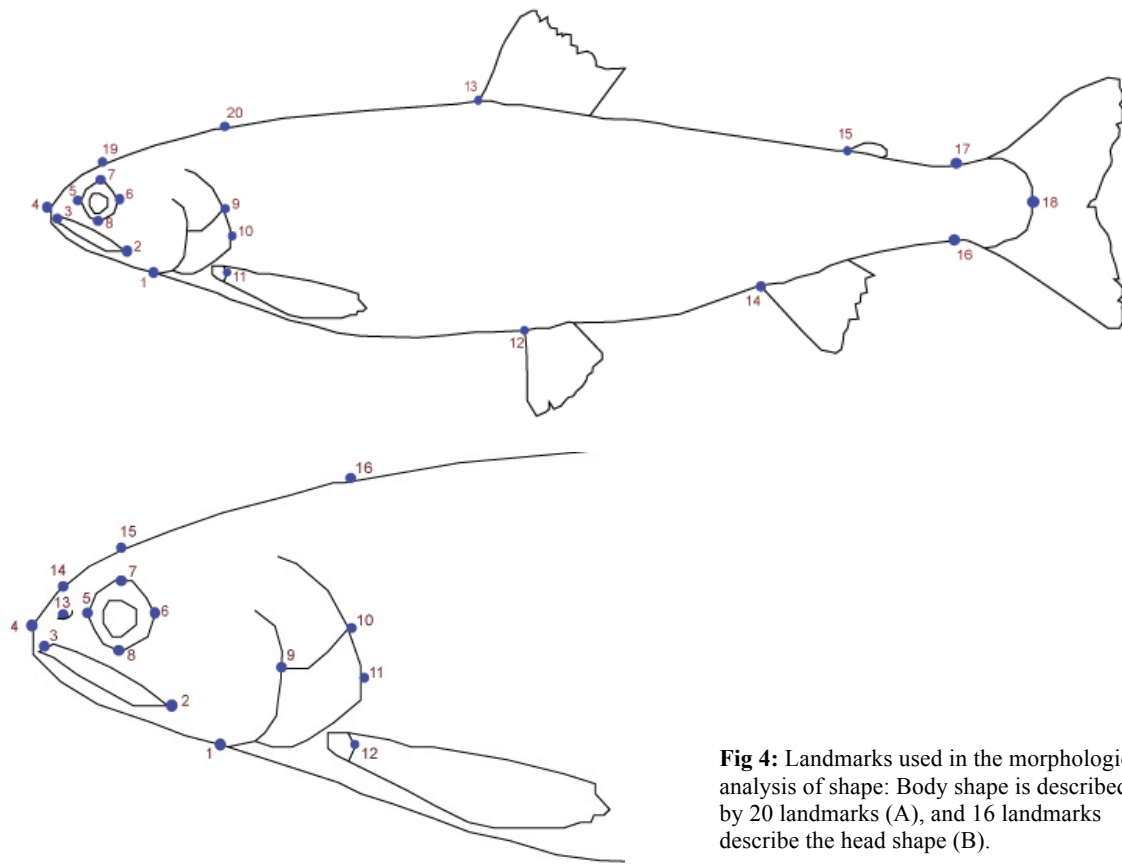


Fig 4: Landmarks used in the morphological analysis of shape: Body shape is described by 20 landmarks (A), and 16 landmarks describe the head shape (B).

The landmarks were optimally aligned using a General Procrustes Analysis (GPA) in the software MorphoJ v.1.05c (Klingenberg 2011). GPA is used to standardize the landmark coordinates, and removes the non-shape effects of size, position and orientation of each specimen (Bookstein 1997; Adams *et al.* 2004; Slice 2007). In other words, it centres all original landmarks data, scales them to the same size and rotates them into the same position and orientation. The GPA results in a new set of landmark coordinates called Procrustes Coordinates, which were used in statistical analysis to describe the shape variation. Statistical analyses of shape using the Procrustes coordinates were conducted in MorphoJ. To graphically illustrate the body and head shape of the different morphs, I created outlines of each “extreme” shape variation by using the wireframe outline-tool in MorphoJ. A common problem in geometric morphometrics of fish is the occurrence of bent individuals, an unwanted effect while photographing. To reduce the amount of outliers, the most extremely bent individuals ($n = 6$) were removed from the dataset using MorphoJ (the individuals are not included in table 2).

LINEAR MEASUREMENTS OF INDIVIDUAL MORPHOLOGICAL TRAITS

Eight morphological traits were studied by measuring the distance between specific landmark-pairs on each fish (Table 3, Fig. 5). These traits were selected based on previous studies identifying significant differences between littoral and profundal spawning morphs of Arctic charr, whitefish (*Coregonus lavaretus*) and lake charr (*S. namaycush*) (Klemetsen *et al.* 2002; Kahilainen & Østbye 2006; Zimmerman, Krueger & Eshenroder 2006; Siwertsson *et al.* 2013). Three of the traits (snout length, maxilla length and eye diameter) have been shown to have a genetic basis in littoral and profundal spawning Arctic charr morphs in Fjellfrøsvatn (Klemetsen *et al.* 2002). Calculations of distances between landmarks were made using an internet-accessible landmark measurement tool (Krieger 2006). This tool required input files with information about which landmark-pairs to include in the analysis, the individual scaling factor and the identification number of each specimen. All measurements were allometrically aligned to an average fish of 19.5 cm fork length. First, all morphological trait values were \log_{10} -transformed to reduce heterogeneity in variance. Second, the traits were size-adjusted by using the allometric growth formula (Senar, Lleonart & Metcalfe 1994):

$$\log_{10}Y_i = \log_{10}M_i + b (\log_{10}L_m - \log_{10}L_i)$$

where Y_i is the size-adjusted morphologic measurements of fish i , M_i is the original morphologic trait measurement of fish i , b is the linear regression coefficient (slope) of the measured trait ($\log_{10} M_i$) against body length ($\log_{10}L_i$), L_m is the average fork length of all fish and L_i is the total fork length of fish i . The size-correction method was validated by linear regressions of each size-adjusted trait against fork length, and no correlation was found (Table A4, Appendix), indicating that most of the size effects were removed.

Table 3: Eight morphological traits (incl. abbreviations applied in text/figures) selected for linear measurements based on distance between landmark-pairs (illustrated in Fig. 5).

Morphological traits		Landmark - pairs
Head length	HL	4 – 10
Maxilla length	ML	3 – 2
Snout length	SL	5 – 4
Eye width	EW	5 – 6
Body depth anterior	BDA	12 – 13
Body depth posterior	BDP	14 – 15
Caudal peduncle depth	CP	16 – 17
Post-pelvic fin length	PPF	12 – 18

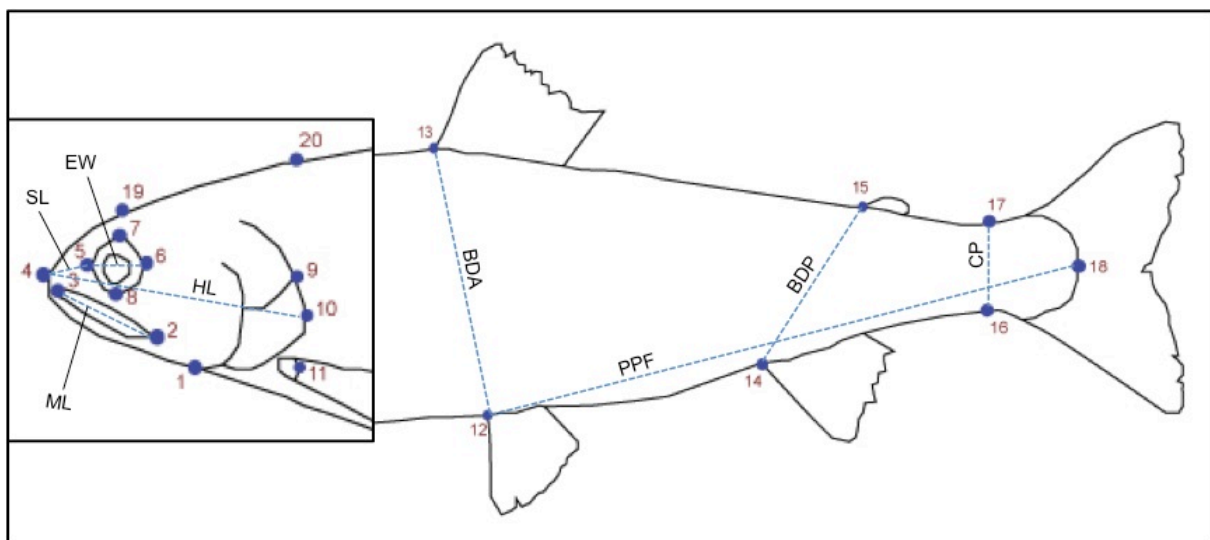


Fig 5: Linear distance (in dotted line) of eight morphological traits between existing landmarks on Arctic charr. See Fig 4 for details on landmarks and Table 3 for abbreviations of the morphological traits.

Statistical analysis

PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) is a method used to analyse the covariance between multiple variables or "components". These principal components are combinations of the original variables, and make it possible to present a multivariate data set in two dimensions with a minimal loss of information. The data is put in a coordinate system with axes that correspond to the most important principal components (PC axes). The axes describe the major overall morphological variation in the landmark data set or "linear measurements data set". The first PC is the axis that shows the largest variation in morphology, the second PC axis shows the next largest etc. Points on the PCA plot represent the morphology (configuration of landmarks or combination of linear measurements) of a single fish. The points that are close to each other, correspond to fish that have similar morphology, while distanced points are associated to different morphologies (Zelditch *et al.* 2012). The PCA of body and head shape was performed in the program MorphoJ (Klingenberg 2011), which uses the Procrustes coordinates to identify the covariance among all fish individuals. To study differences in linear measurements (morphological traits), the software SPSS (IBM Corp. 2010) was used to perform a PCA.

ANALYSIS OF VARIANCE (ANOVA) AND POST-HOC TESTS

ANOVA is a statistical technique that analyses the mean values (within and) between groups to test whether or not the groups are equal. In this study ANOVAs were used to test for differences between the charr morphs (LO, PB and PP morphs) in all PC axis that explained >5% of the morphological variation. Significant ANOVA tests were followed up by post-hoc tests (TukeyHSD) to identify which of all pairs of groups that were significantly different. The statistical tests ANOVA and TukeyHSD were performed in the program R (R Development Core Team 2011) for analysing body- and head shape, and linear measured morphological traits.

Results

Morphology

GENERAL OBSERVATIONS

According to general observations in field and while photographing the charr (Fig. 6), the LO morph showed the typical appearance of a charr found in a monomorphic population (Klemetsen *et al.* 2003). Immature individuals had a silvery color with light spots on the lateral sides and a darker dorsal side, while mature individuals had typical spawning coloration with a red-orange belly and white edges on the paired fins. The profundal PB morph was small sized, and had the appearance of a young charr with a pale yellow and cryptic coloration and pale brown parr marks. Furthermore, most PB morphs emerged at the lake surface with an inflated swim bladder. The PP morph had a generally large head and mouth, and an elongated body. It was less colourful compared to the similar sized LO morph, ranging from pale to completely dark with no clear spawning colours.

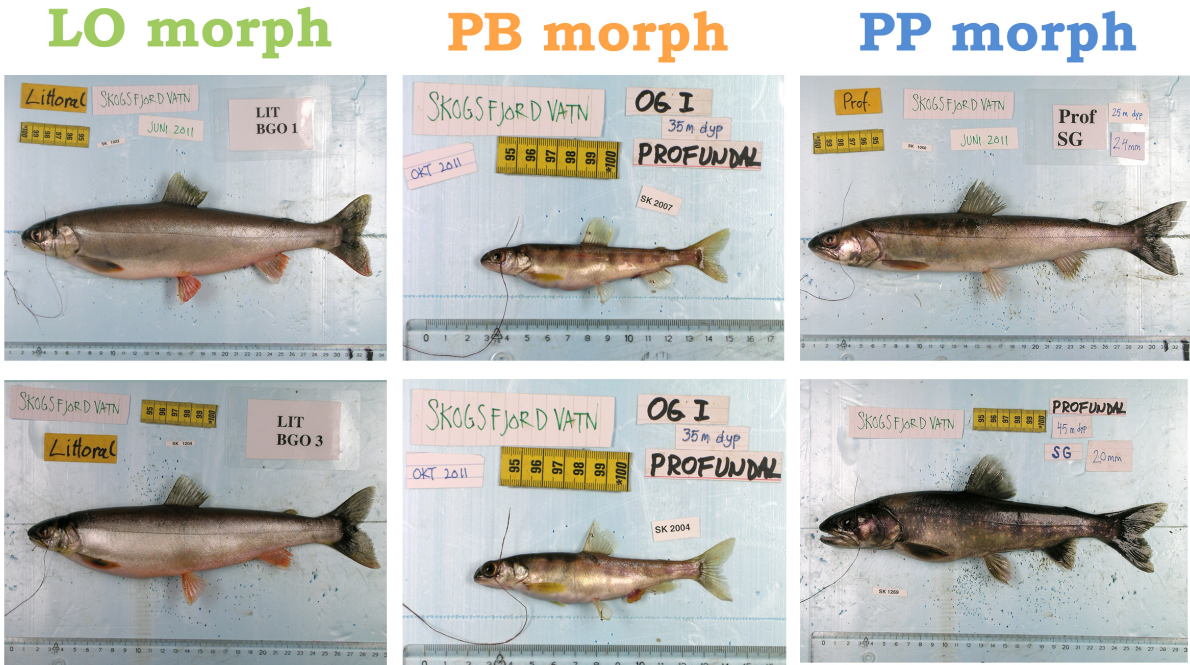


Fig 6: Qualitative morphological differences observed in field of the three morphs of Arctic charr, showed with two individuals of each morph, from left: the LO morph, the PB morph and the PP morph. Note the different scaling on the photos.

BODY SHAPE

In the PCA of the body shape, the first and fourth PC axis showed largest variation between the three morphs (Fig. 7, Table 4). The first PC axis (60.4% of the total variance) separated all three morphs ($p < 0.001$, Table 4), and showed significant difference between the littoral morph and the two profundal morphs. The LO morph clearly separated from the two other morphs (PP and PB morph) by having negative values associated to an overall deeper body form, a shorter head length compared to body length, and small eyes. Both PP and PB morphs had positive values related to a more elongated body form, as well as larger head length and larger eyes. The second PC axis (11 % of the total variance, $p < 0.01$) was mainly associated to the bending of the fish, an unfortunate method error that may occur when photographing fish (Fig A1; Appendix). The third PC axis (5.6 % of the total variance, $p = 0.39$) did not significantly separate the different morphs, but showed differences based on the individual variation of body depth (deeper vs. more elongated body form) (Fig A1). On the fourth PC axis (5% of the total variance, Fig. 7) all three morphs were significantly different from each other ($p < 0.001$, Table 4), including a clear separation between the two profundal morphs (PB and PP morph). PC 4 mainly explained variation in the pelvic fin position and in the head shape. The PP morph individuals showed negative values on PC 4, which indicate that the posterior pelvic fin is placed further back on the body than for the other two morphs (LO and PB morph).

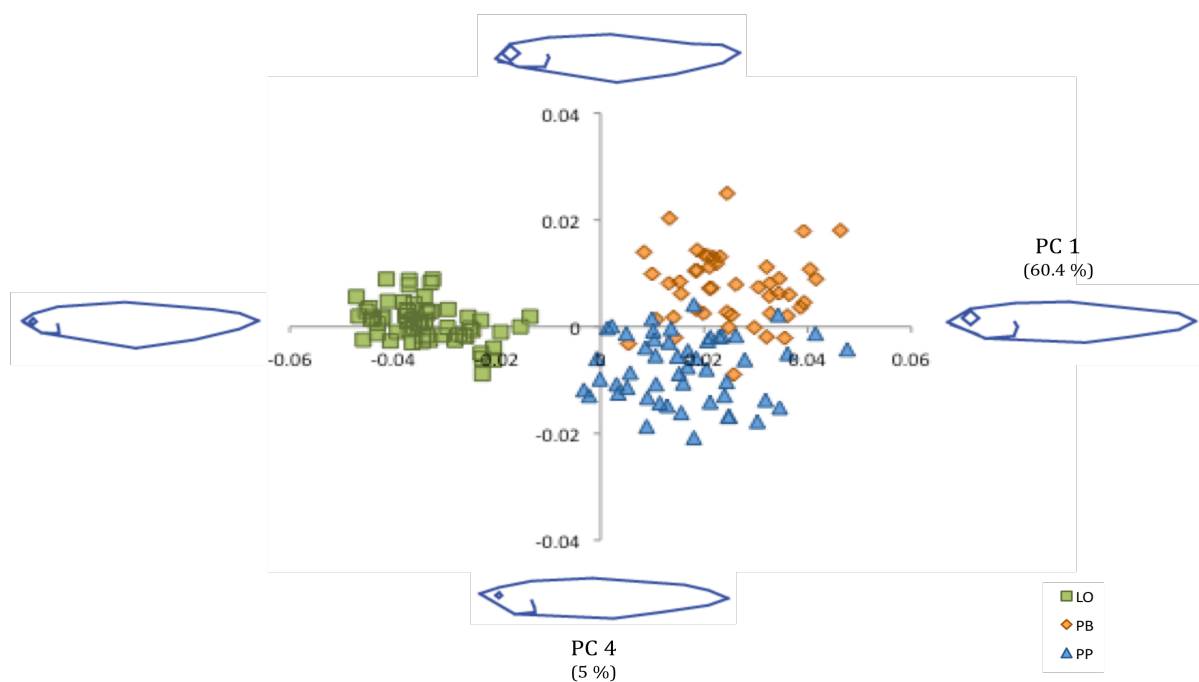


Fig 7: Principal component analysis of body shape (PC 1 and PC 4) in three morphs of Arctic charr: LO morph, PB morph and PP morph. Graphical illustrations show the body shape at each extreme value on both axes (PC1: 0.06 and -0.06, PC4: 0.04 and -0.04).

HEAD SHAPE

In the PCA of the head shape, the first and second PC axis accounted for 52 % of the total morphological variation. The first axis (35.9 %, Fig. 8) was strongly associated with the height and length of the head and the eye size, and showed a significant separation of all three morphs ($p < 0.001$, Table 4). Individuals of the LO morph were located at high values and related to a shorter, yet deeper head shape and a smaller eye size, while individuals of the two profundal morphs were positioned at lower values, having a longer and more narrow head shape, including larger eye size. At the second PC axis (16.1 %, Fig. 8) individuals of the PP morph separated significantly from individuals of the PB and the LO morphs ($p < 0.001$, Table 4). The second PC axis mostly described the differences in head curvature/shape, the upper maxilla bone (mouth) size and position. The high values observed for the PP morph on PC 2 were related to a more pointed head shape, a terminal mouth position and longer upper maxilla bone. The PB and LO morphs were both found at low values on the PC 2 axis, related to a more blunt head shape, a sub-terminal mouth position and a smaller maxilla. The eye size were slightly larger for individuals with low values at both PC 1 and PC 2, which was particularly expressed in the PB morph individuals (Fig 8). The PB and LO morphs did not differ significantly on the PC 2 ($p = 0.74$, Table 4). The third PC axis explained 9.7% of total variation, but there were no significant differences among the morphs ($p = 0.56$) (Table 4, and Fig A2; Appendix).

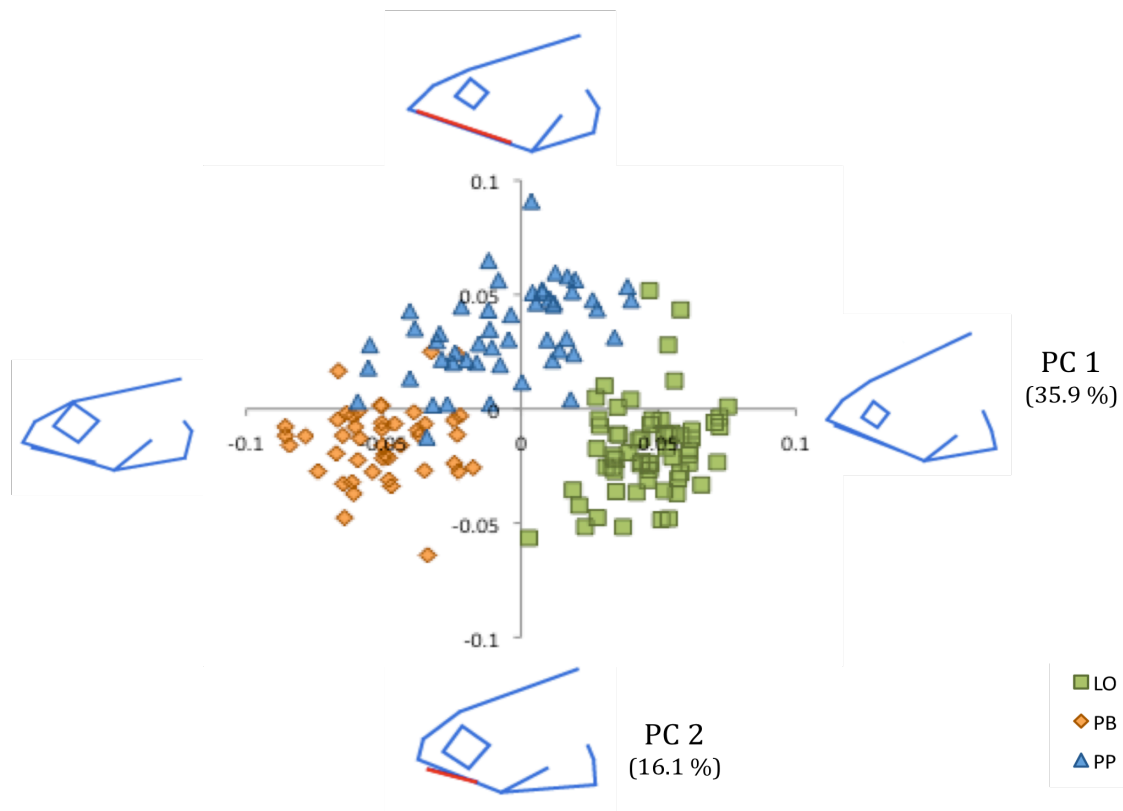


Fig 8: Principal component analysis of head shape (PC 1 and PC 2) in three morphs of Arctic charr: LO morph, PB morph and PP morph. Graphical illustrations show the head shape of the extreme values (0.1 and -0.1) on PC1 and PC2 axes.

Table 4: Statistical analysis of the PCA of body- and head shape, based on one-way ANOVA showing F-value (F), degrees of freedom (df) and *p*-value. Post-hoc tests (Tukey HSD) show differences between the morphs: LO, PB and PP morph (in *p*-value). Significant *p*-values are shown in bold.

		ANOVA : POP			Tukey HSD (<i>p</i> -value)		
		F	df	<i>p</i> value	LO-PB	LO-PP	PB-PP
Body shape	PC 1 (60.4 %)	588.9	2;156	<0.001	<0.001	<0.001	<0.001
	PC 2 (11.0 %)	4.84	2;156	<0.01	0.09	0.007	0.52
	PC 3 (5.6 %)	0.94	2;156	0.39	0.38	0.95	0.59
	PC 4 (5 %)	92.08	2;156	<0.001	<0.001	<0.001	<0.001
Head shape	PC 1 (35.9 %)	327.3	2;156	<0.001	<0.001	<0.001	<0.001
	PC 2 (16.1 %)	118.1	2;156	<0.001	0.74	<0.001	<0.001
	PC 3 (9.7 %)	0.58	2;156	0.56	0.87	0.53	0.86

Linear morphological traits

INDIVIDUAL MORPHOLOGICAL TRAITS

Almost all the linear morphological traits showed significant differences among all the three morphs ($p < 0.01$, Table 5). All individual morphological traits were allometrically size-corrected to the mean size (= 19.5cm) of all analysed charr. Generally, the average values of morphological traits in the PP and LO morphs were at opposite extremes, while the PB morph expressed intermediate values (Fig. 9). The PP morph showed an overall significantly longer head size compared to body size ($p < 0.001$), and therefore many other head measurements such as maxilla length, eye width and snout length, were all significantly larger than found for the two other morphs (Fig. 9). The LO morph had a significantly shorter head length compared to the two profundal morphs (PB and PP morph), also resulting in shorter head measurements (maxilla length, eye width and snout length; Fig. 9). The PB morph was found to have intermediate values in all the head measurements. All body depth values (BDA, BDP and CP) were significantly smaller for the PP morph relative to both of the other morphs. The LO and PB morph did not differ significantly in two of the body-depth measurements (BDA: $p = 0.35$ and CP: $p = 0.69$; Table 9). The distance between the pelvic fins and the caudal fin base (PPF) was found to be significantly shorter for the PP morphs compared to the other two morphs ($p < 0.001$). The LO morph had the longest PPF distance (Fig. 9).

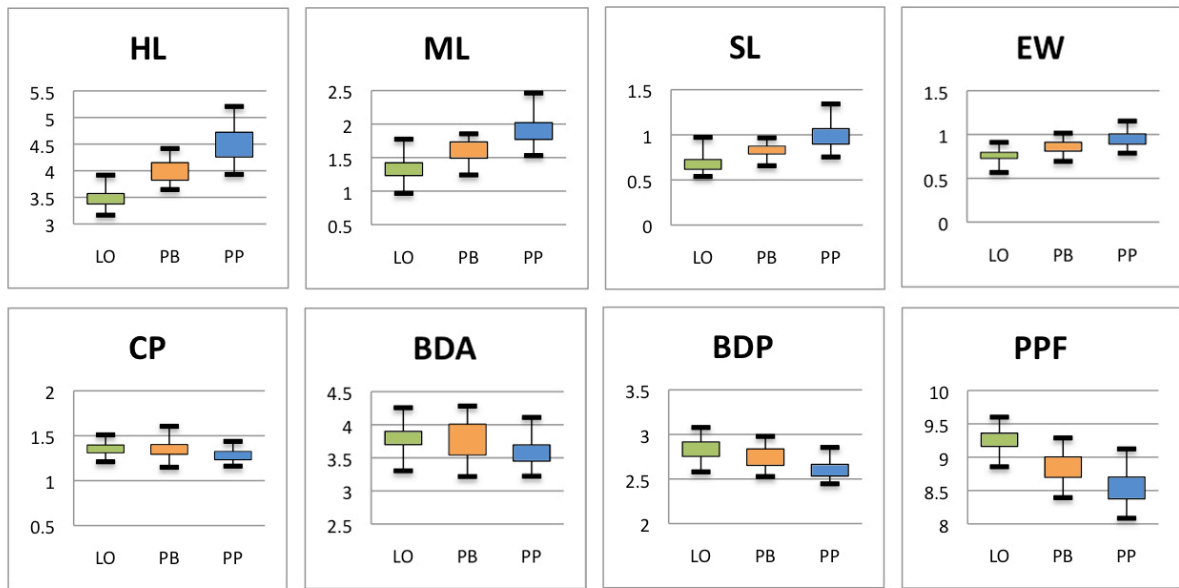


Fig 9: Box plot of 8 measured morphological traits in the three morphs of Arctic charr: LO morph (green), PB morph (orange) and PP morph (blue). The coloured boxes represent 1st to 3rd quartile, while whiskers are max- and min values. Measurements are based on the linear distance between landmark-pairs (abbreviations in Table 3 and 5). All measured traits were size-adjusted to fit an ideal fish with fork length 19.5cm. Note the different values on the y-axis (in cm).

Table 5: Statistical analysis of linear measurements of 8 morphological traits among three morphs of Arctic charr: LO morph, PB morph and PP morph, including one-way ANOVA (F-value, degrees of freedom (df) and p-value) and post-hoc test Tukey HSD (in p-value). Significant p-values are shown in bold.

Measured morphological trait	Landmark pairs	ANOVA: POP			Tukey HSD (<i>p</i> -value)		
		F	df	<i>p</i> -value	LO-PB	PP-PB	PP-LO
Head length	HL 4 – 10	276.8	2;156	<0.001	<0.001	<0.001	<0.001
Eye width	EW 5 – 6	101.4	2;156	<0.001	<0.001	<0.001	<0.001
Maxilla length	ML 2 – 3	155.8	2;156	<0.001	<0.001	<0.001	<0.001
Snout length	SL 4 – 5	159.3	2;156	<0.001	<0.001	<0.001	<0.001
Body depth anterior	BDA 12 – 13	15.2	2;156	<0.01	0.35	<0.001	<0.001
Body depth posterior	BDP 14 – 15	55.76	2;156	<0.001	<0.001	<0.001	<0.001
Posterior pelvic fin	PPF 14 – 18	162.9	2;156	<0.001	<0.001	<0.001	<0.001
Caudal peduncle depth	CP 16 – 17	15.2	2;156	<0.01	0.69	<0.001	<0.001

PCA OF MORPHOLOGICAL TRAITS

In the PCA of all the linear morphological traits, the first and second PC axis accounted for 81.5 % of the total variation. The first PC axis (60.5 %) showed a complete separation among all the three morphs ($p < 0.001$, Table 7). The LO morph was found at one extreme (lowest values) and the two profundal morphs separated at higher values, with the PP morph at the highest values (Fig. 10). Positive values of PC 1 explained measurements related to having a larger head compared to body length (increased head depth and length, snout length, eye width and maxilla length) (Table 6). Thus, the PP morph have a larger head, longer snout and maxilla length than the LO morph. PC 1 also show that the PP morph have a shorter PPF distance than the LO morph. The second PC axis explained 21% of the total variation (Fig. 10), and was associated to the caudal peduncle depth and body depth posterior to the dorsal fin. PC 2 showed that the PP morph had significantly smaller caudal peduncle depth and posterior body depth than both the LO morph ($p < 0.001$) and the PB morph ($p < 0.001$) (Table 7). The PB morph and LO morphs were not significantly different from each other on PC 2 ($p = 0.24$).

Table 6: Loadings of eigenvectors on three principal components for 8 measured morphological traits among morphs of Arctic charr in Skogsfjordvatn. High values (>0.8 , both positive and negative) indicate importance of the morphological traits on specific PC axis (shown as bold values).

Morphological trait	PC 1	PC 2	PC 3
Head length (HL)	.979	-.283	-.220
Maxilla length (ML)	.953	-.221	-.201
Snout length (SL)	.942	-.277	-.260
Post pelvic fin length (PPF)	-.903	.241	.204
Eye width (EW)	.842	-.333	.071
Caudal peduncle depth (CP)	-.176	.947	.386
Body depth posterior (BDP)	-.519	.820	.543
Body depth anterior (BDA)	-.258	.531	.955

Table 7: Statistical analysis of PCA of morphological traits among three morphs of Arctic charr: LO morph, PB morph and PP morph, including one-way ANOVA (F-value, degrees of freedom (df) and p-value) and post-hoc test Tukey HSD (in p-value). Significant p -values are shown in bold.

	ANOVA : POP			Tukey HSD (p -value)		
	F	df	p value	LO-PB	LO-PP	PB-PP
PC 1 (60.5 %)	281.9	2;156	<0.001	<0.001	<0.001	<0.001
PC 2 (21.0 %)	26.3	2;156	<0.001	0.24	<0.001	<0.001
PC 3 (5.9 %)	8.3	2;156	<0.001	0.68	<0.001	<0.05

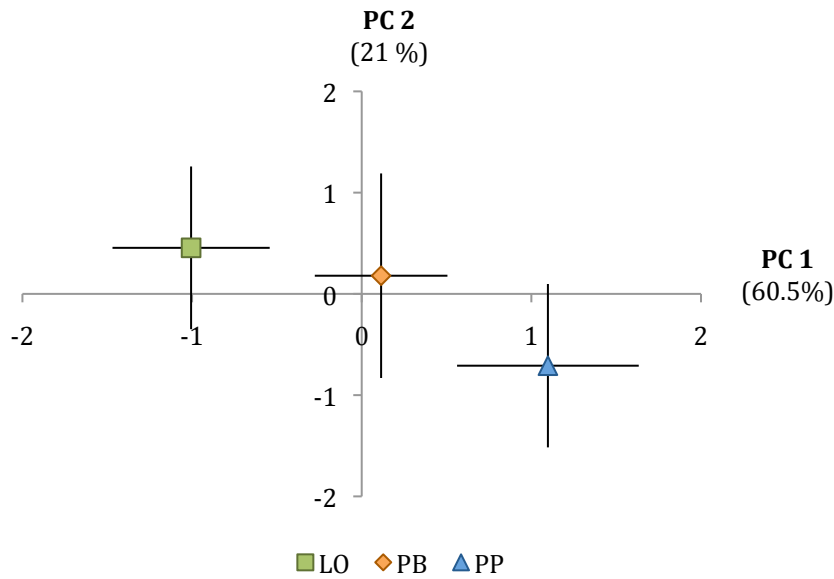


Fig 10: Principal component analysis of morphological traits (PC 1 and PC 2) among three morphs of Arctic charr: LO morph, PB morph and PP morph. See Table 6 for loadings of eigenvectors for each component.

The third PC axis (5.9 %) (Fig. 11) was mainly associated with a single variable, the body depth anterior to dorsal fin (BDA) (Table 6). The PP morph had significantly smaller anterior body depth than both the LO ($p < 0.001$) and PB morphs ($p < 0.01$). The PB and LO morphs did not vary from each other on PC 3 ($p = 0.68$) (Table 7).

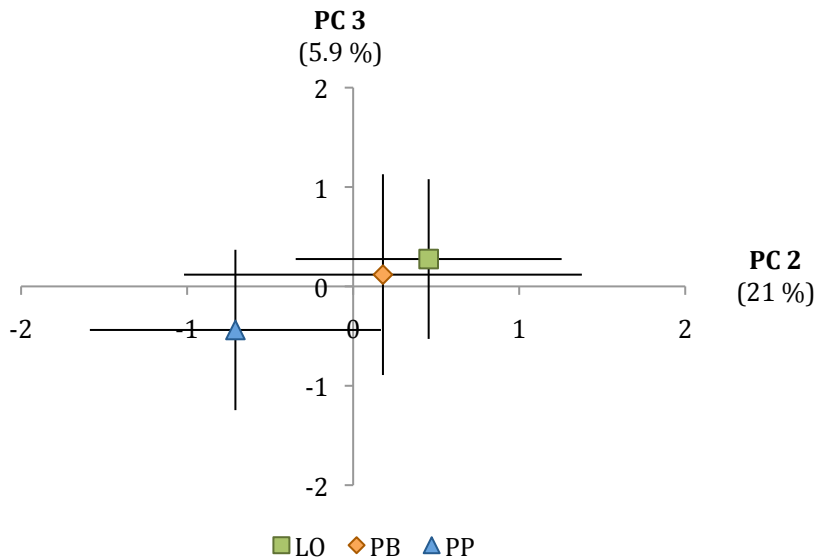


Fig 11: Principal component analysis of morphological traits (PC 2 and PC 3) among three morphs of Arctic charr: LO morph, PB morph and PP morph. See Table 6 for loadings of eigenvectors for each component.

Discussion

The present study revealed clear significant differences among the three Arctic charr morphs in Skogsfjordvatn regarding body- and head shape, as well as in 6 of 8 linear measured morphological traits. Hence, the overall morphological analysis confirmed the hypothesis of the existence of three morphological forms within the charr population. The littoral spawning omnivore (LO) morph was found to have a deep, yet fusiform body shape with a small head length compared to body length. Other head measurements, such as maxilla length, snout length and eye width, were significantly smaller for the LO morph compared to the two profundal morphs. The profundal spawning benthivore (PB) morph resembled the LO morph in body depth, but differed by having a larger head length compared to body length, as well as generally larger head measurements. In contrast to both the LO morph and the PB morph, the profundal spawning piscivore (PP) morph differed by having a more elongated body shape and a longer and more robust head, including the overall largest head measurements of all morphs. Thus, there were large variations between all three morphs, with the LO and PP morph allegedly in great contrast to one another.

The three morphs in Skogsfjordvatn have been found to diverge in morphology, foraging behaviour, in time and place of spawning, and in other life-history traits (Smalås 2013; R. Knudsen, unpublished). Environmental differences are suggested to be important factors for promoting morphological divergence in a population, and may evidently initiate speciation (Schluter 2000). In freshwater fish populations, sympatric polymorphism normally occur as a divergence between littoral and pelagic resources, typically related to different prey utilizations and/or ecological interactions among the morphs (Skúlason & Smith 1995). The present case is quite special compared to previous studies, as the three charr morphs in Skogsfjordvatn are segregated along the depth gradient between the upper water layers (i.e. the littoral and pelagic zone) and the profundal habitats; a type of segregation that has seldom been described in the literature (but see Klemetsen et al. 2010, and among European whitefish, *Coregonus lavaretus* (Kahilainen & Østbye 2006; Harrod *et al.* 2010; Siwertsson *et al.* 2013)). However, most interesting from the present study is the discovery of a highly morphologically specialized piscivore morph (the PP morph) living in the deep profundal zone (>20m). There they have adapted to exploit the prey resource of small fish including the PB morph, juveniles of the LO morph and in some cases three-spined sticklebacks. Such a deep-water piscivore morph of Arctic charr, with very specific life-history traits (Smalås

2013) and specialized trophic morphology, has to my knowledge not been described elsewhere.

The LO morph was caught in all the lake habitats, was found to include numerous of different prey groups in its diet (R. Knudsen, unpublished) and had the appearance of typical Arctic charr forms as reported from landlocked monomorphic populations and anadromous populations in northern Norway (Klemetsen *et al.* 2003). The body shape of the LO morph may resemble a mix between the morphological dichotomy of typical pelagic *versus* littoral/benthic fish (Webb 1984; Robinson & Parsons 2002), also found in other Arctic charr populations (Bjørn & Sandlund 1995). In general, fish morphology often mirrors their ecology related to habitat preference, locomotion and trophic niche (Webb 1984; Svanbäck 2004). The relative fusiform body shape of the LO morph is well-suited to enhance swimming efficiency over longer distances, as the LO morph performs ontogenetic niche shifts between different lake habitats and dietary groups during its lifetime (e.g. Klemetsen *et al.* 1989). Observations of anadromous charr in Skogsfjordvatn suggest that part of the LO morph population may undergo smoltification and migrate longer distances in the marine habitat (R. Knudsen pers. comm.). The LO morph also has a high body depth and caudal peduncle depth, which is considered to be more typical for littoral fish to maintain high manoeuvrability and balance. However, due to the overall fusiform body shape and small head measurements, the LO morph seems to be closer to Webb's (1984) description of a *cruiser* (i.e. a pelagic morph). This is also supported by the fact that a large part of the LO morph individuals were caught in the pelagic habitat (Smalås 2013), selectively feeding upon zooplankton prey (Skoglund, Knudsen & Amundsen, in press).

In contrast to the LO morph, the PB morph has a generally deeper and clumpy body shape and are more related to the *manoeuvre* body form (Webb, 1984) aimed to enhance balance and manoeuvrability. This may imply that the PB morph is more adapted to a benthic life close to the bottom and to utilize benthic prey resources. A deeper caudal peduncle area may also help in burst motion to flee from predators (Webb, 1984). Similar body shapes have been described earlier for profundal benthivore morphs of Arctic charr (Hesthagen, Hindar & Jonsson 1995; Alekseyev *et al.* 2002; Klemetsen *et al.* 2002) and from deep-water European whitefish morphs (Kahilainen & Østbye 2006; Siwertsson *et al.* 2013). The typical epibenthic body shape observed in the PB morph may also be related to different behaviours in sympatric profundal vs. littoral morphs as revealed from experimental studies (Klemetsen *et al.* 2002, 2006). In these studies, the profundal morph was less active and more associated to the bottom substrate, and also less effective to feed on zooplankton prey compared to the

littoral morph. The PB morph also exhibits a cryptic coloration of light yellow-brown that may give decent camouflage against the sandy bottom, presumably to avoid the predacious PP morph. Thus, the body shape of the PB morph seem to be highly related to its ecological performance with a probably lower activity level than the other charr morphs, as well as a life close to the sandy bottom and to avoid predators.

Dissimilar from both the LO and PB morphs, the piscivore PP morph has a body shape closer to the *accelerator* form (Webb, 1984). The body depth measurements revealed that the PP morph had a more elongated body than the LO morph, and the PP morph was also verified to have the longest head among the three morphs. The elongated body shape helps to reduce drag and strengthen flow, which are good adaptations when hunting evasive prey such as small fish (Svanbäck 2004). Furthermore, the pelvic fins of the PP morph also seem to be positioned further back on the body compared to the LO morph, and in the field the PP morph were observed to have larger fin area of all median fins (dorsal, anal and caudal fin). All these traits increase the surface area of the posterior part of body that may help to increase the acceleration and improve thrust motion when hunting (Webb, 1984). The overall body shape differences found among the three morphs seem to be explained functionally to their respective preferred habitats and ecological behaviour.

The study also confirmed clear differences in the head shape among all the three morphs, including differences in the linear measured morphological traits with the head length, eye- and upper maxilla size as the most differentiated traits. Correlations between head morphology and trophic ecology have been found in several monomorphic and polymorphic populations of *Salvelinus* sp. (Snorrason *et al.* 1994; Adams *et al.* 1998; Jonsson & Jonsson 2001; Knudsen *et al.* 2007; Ostberg, Pavlov & Hauser 2009; Woods *et al.* 2013). Relating head morphology to the observed diet differences (Table 1) makes it reasonable to suggest that the observed polymorphism in Skogsfjordvatn has a functional trophic basis. The LO morph was found to have a short and deep triangular head shape with small eyes and a short upper maxilla, while the two profundal morphs had more elongated head shapes and larger eyes. The LO and the PB morphs both have a blunt snout shape and sub-terminal mouth position, typically adapted to prey on benthic invertebrates. For the LO morph these characters were less pronounced; some individuals had a fairly sub-terminal positioned mouth adapted to feed on littoral benthos, while other individuals had a terminal mouth more adapted to prey on zooplankton. This could be a phenotypic plastic response, as the LO morph seems to be a typical generalist in feeding behaviour, migrating between the pelagic and littoral habitats to feed on both limnetic zooplankton and benthic prey resources

(Skoglund *et al.*, in press; R. Knudsen, unpublished). Additionally, due to strong interspecific resource competition with brown trout and three-spined sticklebacks in the littoral zone (Klemetsen *et al.* 1989; Jørgensen & Klemetsen 1995; Eloranta *et al.* 2013), parts of the LO morph population is probably pushed out in the pelagic habitat to exploit zooplankton. Furthermore, some individuals of the LO morph may even turn anadromous utilizing marine prey (R. Knudsen, unpublished). Overall, the LO morph appears to have a similar trophic morphology and diet niche as the littoral spawning morph in Fjellfrøsvatn (Klemetsen *et al.* 2002; Knudsen *et al.* 2007, 2010) and charr from typical monomorphic populations (Bjørn & Sandlund 1995; Klemetsen *et al.* 2003).

The two profundal spawning morphs seem to have a more narrow diet niche compared to the LO morph, which may indicate that they are more specialized on their preferred prey resource, subsequently leading to specialized trophic morphological traits. In contrast to the LO morph, the sub-terminal mouth and blunt head shape were more pronounced in the PB morph. This may reflect that the PB morph is more specialized to feed efficiently on benthos submerged in the bottom substrate such as mussels (*Pisidium* sp.), oligochaeta and chironomid larvae compared to the LO morph. A similar trophic morphology has been observed for other profundal charr morphs with comparable trophic ecology (Jonsson & Jonsson 2001; Klemetsen *et al.* 2002; Klemetsen 2010). The largest difference among the morphs was nevertheless the contrasting head morphology of the PP morph, with a more elongated head, a pointed snout shape and a more terminal mouth position. Generally all the head measurements (head length, snout length, upper maxilla- and eye size) were significantly longer for the PP morph compared to the other two morphs. The long, robust head and the large mouth implies highly specialized adaptation to predate on fish prey (Nilsson & Brönmark 2000), and similar observations have also been done for other *Salvelinus* sp. morphs (Adams & Huntingford 2002; Ostberg *et al.* 2009).

As hypothesized, both the profundal morphs had large eyes that likely represent adaptations to locate prey (i.e. benthos and small fish) and to avoid predators (for the PB morph) in a dark environment. Foraging on small prey in low-light environments such as the profundal zone in Skogsfjordvatn, may lead to a modification toward larger eye size (Huber *et al.* 1997; Schliewen *et al.* 2001). This has also been observed among other profundal morphs of Arctic charr (Klemetsen 2010 and references herein) and in profundal morphs of whitefish (Kahilainen & Østbye 2006; Siwertsson *et al.* 2010). The PP morph had significantly larger eyes than the PB morph, a trait that primarily may be related to its predacious behaviour to locate small evasive fish in low light environments (see e.g. Gartner, Crabtree & Sulak 1997).

The LO morph had the smallest eyes, likely a result of foraging in better light conditions in the upper water layers. Many of the classic polymorphic charr systems (Sandlund *et al.* 1992; Adams *et al.* 1998; Klemetsen 2010) show similar trophic morphologies as the morphs in Skogsfjordvatn, but none of these lakes have a deep-water adapted piscivore morph. In essence, the divergence observed in head morphology for the three morphs in Skogsfjordvatn seems to be highly correlated to their diverged trophic niches, with the LO morph as a generalist feeder compared to the two more specialized, but differentiated profundal morphs.

The working assumption is that the three morphs in Skogsfjordvatn have evolved from anadromous Arctic charr that invaded the lake following the last glacial retreat (~10 000 years ago). This ancestral charr population assumedly segregated into different morphs in sympatry or allopatrically through multiple invasions to the lake (Rune Knudsen, pers. com.). Arctic charr is known to be a highly phenotypic plastic species, able to exploit a wide potential diet niche and to adapt to different environments, and it has also recently claimed the title as the most variable fish species (Klemetsen, in press). Sympatric divergence may be promoted in phenotypic plastic populations, where individuals express alternative adaptive traits by specializing on alternative resources (e.g. prey and/or habitat) and manage to maintain these specializations over multiple generations through genetic fixation (Schluter & Rambaut 1996; Skúlason, Snorrason & Jónsson 1999; Schluter 2001). The Arctic charr in Skogsfjordvatn have been exposed to different ecological conditions which may have induced disruptive selection in a way that alternative phenotypes have been favoured within the population (West-Eberhard 1989; Doebeli & Dieckmann 2000). This is supported by the clear correlation between diet niches and specialized trophic morphology. Early in the diverging process alternative behavioural phenotypes are more likely to be expressed compared to alternative morphologies, particularly for functional behaviours like those related to foraging (West-Eberhard 1989). Alternative foraging behaviour has also been seen for the similar littoral-profundal morph pairs of Arctic charr in Fjellfrøsvatn (Klemetsen *et al.* 2002, 2006) and also in their trophic morphologies (Knudsen *et al.* 2006). It is important to be able to feed efficiently (while at the same time avoiding predators), and behavioural foraging specializations may subsequently lead to modifications of trophic morphological traits (Skúlason *et al.* 1999). Selection for profitable functional morphological traits is evident among the three morphs in Skogsfjordvatn.

In Skogsfjordvatn, the present morphological divergence is believed to be a result of specializations on alternative prey resources (i.e. littoral-pelagic prey, soft-bottom prey and fish) and adaptations to specific habitats (i.e. littoral-pelagic vs. profundal), as well as

ongoing ecological interactions among the morphs. Polymorphism among fishes are suggested to be related to the ability to compete for resources, resulting in more specialized feeding behaviours and subsequently in adaptive morphologies (Bolnick *et al.* 2003; McKinnon *et al.* 2004). Individuals that are able to specialize on specific prey resources and handle them more efficiently than their competitors, will gain higher fitness (Skúlason *et al.* 1999). Intermediate phenotypes that are less specialized on either prey resource will gain lower fitness and subsequently be selected against (Jonsson & Jonsson 2001). As a result, a reproductive barrier may rise between the specialized phenotypes and consequently lead to genetic fixation within groups (Schluter 2001; Rundle & Nosil 2005). In Skogsfjordvatn, there is most likely already a reproductive isolation between the three morphs as they are totally separated in different genetic assemblages and show microsatellite-based F_{ST} values ranging from 0.17 to 0.30 (R. Knudsen, unpublished). Other promoting isolation mechanisms could also be related to the morphology of the morphs as individuals may select their mates based on similar trophic related morphology, coloration or body size (i.e. assortative mating) (Schluter 2001, 2009; McKinnon *et al.* 2004; Rundle & Nosil 2005; Sobel *et al.* 2010). The ongoing reproductive isolation between the morphs found in Skogsfjordvatn is probably a result of assortative mating and segregation in spawning habitat (between LO morph and profundal morphs) and spawning time (among all morphs) (Smalås 2013).

Living in contrasting habitats such as in the littoral and the profundal zones should result in strong divergent selection due to different environmental factors (Schluter 2001, 2009). The profundal habitat in Skogsfjordvatn offers an environment that have low light conditions, minimal variations in temperatures throughout the year, and a soft-bottom sediment with few vertical obstacles such as plants or stones and with low prey diversity (Klemetsen 2010). This habitat is often used as a refuge for juvenile Arctic charr from piscivore bird and fish predators (Klemetsen *et al.* 1989; Sandlund *et al.* 1992). Thus, survival in such a deficient environment depends on adaptations through natural selection to specialize on the restricted prey resources with prey species that often hide in the soft sediment (Klemetsen 2010). Juvenile charr typically exhibit morphological adaptations and colorations of an epibenthic feeder with dark dorsal sides, light yellow and cryptic coloration on lateral sides with darker parr marks, a blunt snout shape and a sub-terminal mouth position adapted to feed on benthic or submerged prey species (Skúlason *et al.* 1989; Klemetsen *et al.* 2003). The PB morph resides in the profundal habitat throughout their lifetime and seems to retain these juvenile/embryonic traits into adulthood, as these traits are likely to be beneficial in such an environment. These developmental restrictions are defined as paedomorphism and is

well known from many fish taxa (Winterbottom 1990; Hastings 2002), including several deep-sea fishes (Marshall 1984) and also in a few cases of small-sized Arctic charr morphs (Balon 1980; Jonsson *et al.* 1988; Skúlason *et al.* 1989; Klemetsen *et al.* 1997).

Paedomorphism is suggested to be an important factor in the local diverging process that seems to occur for these profundal small-sized morphs (Klemetsen *et al.* 1997), and their morphological divergence may also be linked to their life-history strategy (Nordeng 1983; Jonsson & Jonsson 2001).

Smalås (2013) found distinct differences in the life-history strategies of the three morphs in Skogsfjordvatn, including very contrasting differences in growth rate and size/age at maturity between the two profundal morphs. As seen in other small-sized charr (Jonsson *et al.* 1988; Woods *et al.* 2013), the PB morph seem to gain a higher fitness by maturing early at a small size and young age, a strategy that retards their somatic growth as energy is invested in gonad growth. The delay in somatic growth and adaptations in trophic morphology may thus results in the paedomorphic appearance (Balon 1980), which was clearly seen in the PB morph in Skogsfjordvatn. Paedomorphic individuals of charr are often denoted “dwarf morphs” (Hesthagen *et al.* 1995; Klemetsen *et al.* 1997; Alekseyev *et al.* 2002) because of their embryonic look. However, the paedomorphic appearance is apparently not inherited to the next generation. When offspring of the profundal morph in Fjellfrøsvatn were given better foraging conditions, they doubled their growth rate and appeared as a typical charr (Klemetsen *et al.* 2002). Therefore the restricted ecological conditions of the profundal habitat (e.g. reduced nutrients, low prey diversity and low temperatures) are likely to be important for the morphological adaptations observed in the PB morph, as well as in other profundal charr morphs (Klemetsen 2010) and paedomorphic fishes (Moore 1994). The profundal environment seems to promote paedomorphism in the PB morph, representing a great example of ecological driven speciation where the environment plays an important factor in the morphological divergence (Schluter 2009).

The piscivore profundal morph seems to be most morphological differentiated among the three morphs, and is clearly separated from the LO morph in all analyses. In Arctic charr, piscivore behaviour is often a result of ontogenetic transformations and is commonly found in allopatric populations and only in a few polymorphic populations (Amundsen 1994; Snorrason & Skúlason 1994; Adams *et al.* 1998; Mittelbach & Persson 1998; Klemetsen 2010 and references herein). The PP morph has adapted typical piscivore traits (i.e. robust heads and large mouth) that are shown to be genetically based and distinguish them from their sympatric morphs (Adams & Huntingford 2002). Usually, sympatric piscivore morphs are

located mainly in the upper water layer preying on smaller fish in the pelagic habitat as a result of an ontogenetic diet shift toward larger prey (Sandlund *et al.* 1992). However in Skogsfjordvatn, the piscivore morph apparently resides in the profundal habitat throughout its lifetime, as it seldom was caught at shallow water above 20m. Development of piscivory behaviour in Arctic charr normally occurs in lakes with suitable density of prey species and low interspecific competition from other piscivore fish species (Jonsson & Jonsson 2001). In Skogsfjordvatn, the piscivore niche in the pelagic and littoral zone is predominantly occupied by brown trout (*S. trutta*). Only Arctic charr has been observed to occur in the profundal zone. With this in mind, it is reasonable to assume that the occurrence of the small-sized PB morph and the juveniles of the LO morph in the profundal habitat, may have opened up an ecological opportunity of a new prey resource for the ancestors of the present PP morph. The PB morph resides in the profundal habitat, and is thus available as a prey resource at all seasons. Additionally, the PP morph may utilize juveniles of the LO morph at deep-water during the ice-free season. Hence, the PP morph may have originated from individuals of the LO morph (or from the anadromous ancestral invader) that have become piscivorous as a result of niche expansion by specializing on underutilized fish prey in the profundal zone (Schluter 2000; Knudsen *et al.* 2006). Analogous, the evolving profundal charr morph in Fjellfrøsvatn is suggested to be a result of niche expansion by specializing on novel soft-bottom prey (Knudsen *et al.* 2006) parallel to the PB morph in Skogsfjordvatn. Alternatively, the PP morph may have originated from a secondary invasion of anadromous charr that subsequently have developed piscivorous traits through reinforcement processes (Howard 1993; Rundle & Schluter 1998), by adapting to predate on the underutilized fish prey resource (the PB morph) in the profundal zone. However, the suggestions for the origin of the observed polymorphism in Skogsfjordvatn are indirect and only relying on observations. Thus, further studies need to be done to conclude about the origin through diet niche expansions or divergence through reinforcement, as the latter requires tests of strict criteria that must be met before concluding speciation by reinforcement (Howard 1993; Rundle & Schluter 1998).

Conclusion

The present study confirmed the hypothesis of three sympatric morphs within the resident Arctic charr population in Skogsfjordvatn, with clear differences in body- and head shape, and other morphological traits. The morphological divergence seems to be highly correlated to the different resource utilizations of the morphs, including ecological performance and/or dietary use in their respective habitats. Living in contrasting habitats (e.g. littoral – profundal zone) seems to promote divergence among the morphs through different ecological selection pressures from the environment. The development of the morphs may have occurred as alternative ontogenetic paths due to development constraints (e.g. paedomorphism in the PB morph) and/or niche expansion to novel resources such as profundal fish prey (the PP morph). The observed polymorphism support other findings of a speciation mechanism strongly driven by ecological factors (Schluter 2001, 2009), including expansion of diet niche by adapting to underutilized prey resources (Knudsen *et al.* 2006; Gillespie 2009). It is also reasonable to suggest that reproductive isolation is under strong selection among the morphs in Skogsfjordvatn, as they have strongly contrasting life-history strategies, have adapted distinct morphologies related to foraging specializations and constitute significantly different genetic groups. Nevertheless, the Arctic charr in Skogsfjordvatn still holds many unsolved mysteries, which calls for further morphological investigations by e.g. looking at the morphs at different life stages and through ontogeny, as well as doing rearing experiments to investigate the inheritance of morphological adaptive traits.

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