Supplementary Material for "Ecological opportunity shapes a large Arctic charr species radiation"

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Appendix S1. Methods: Sampling details.
Benthic multi-mesh gillnets of 30m length x 1.5m height with mesh sizes (knot-to-knot) of 5.0, 6.25, 8.0, 10.0, 12.5, 15.5, 19.5, 24.0, 29.0, 35.0, 43.0 and 55mm (Central European standard protocol for gillnet fishing) were set at various depths in all lakes. In lakes deeper than 15m and larger than 15ha, also pelagic multi-mesh gillnets of the same dimensions and mesh like the benthic nets were set. All nets were set overnight for about 15 hours.

Appendix S2. Methods: Diversity of charr ecomorphs.
In many cases, charr forms of polymorphic lake populations can be assigned to one of five specialized ecomorphs (ecomorph sensu Williams, 1972), which are characterized by their ecology, morphology, and behaviour, that we summarize below.

Anadromous ecomorph

A major dichotomy among charr populations is the distinction between sea-migratory (anadromous) and freshwater resident populations (Klemetsen et al., 2003). Unlike salmon that stay several years at sea and only return to freshwater for spawning, anadromous charr perform seasonal migration and yearly return to freshwater for overwintering (Klemetsen et al., 2003). Anadromous charr exhibit extremely fast growth at sea (Klemetsen et al., 2003), larger size, later maturation, smaller eyes and shorter pectoral and pelvic fins than the resident populations in lakes (Loewen et al., 2009). During migration and at sea, they are of silvery coloration (Fraser, 1998). Belly colouration of anadromous charr around spawning time can range from yellow to red, which might be related to individual dietary specialization (Krähenbühl et al., unpublished data).

Littoral benthic ecomorph

Littoral benthic charr are specialized to feed on benthic invertebrates in the shallow areas of lakes (Jonsson and Jonsson, 2001). They are mostly deep-bodied with long fins, and have solid head and mouth structures, a blunt snout and often a subterminal mouth (Fraser et al., 1998, Snorrason et al., 1994, Alekseyev et al., 2014). Some lakes harbour multiple forms of the benthic ecomorph that differ in body size, habitat and age at maturity (e.g. Lake Thingvallavatn, Sandlund et al., 1992) or head shape (Markevich et al., 2017). Littoral benthic forms tend to show dull coloration (e.g. Loch Rannoch, Adams et al., 1998; Thingvallavatn, Sandlund et al., 1992), and might display bright orange belly coloration during spawning time (e.g. Thingvallavatn, Sandlund et al., 1992; Lake Kamkanda, Alekseyev et al., 2014).

Planktivorous ecomorph

Planktivorous charr are characterized by slender body form, short fins, terminal mouth and thin and long gill rakers (Jonsson and Jonsson, 2001). Body color of planktivores varies among systems from very colorful (e.g. Loch Ericht, Fraser et al., 1998; Rannoch, Adams et al., 1998, Lake Kamkanda, Alekseyev et al., 2014) to pale silvery (e.g. Thingvallavatn, Sandlund et al., 1992). Planktivorous charr are usually smaller than piscivorous, and sometimes larger than littoral benthic ecomorphs (Alekseyev et al., 2002; Sandlund et al., 1992).

Piscivorous ecomorph

Piscivorous charr have big, deep heads with long and wide jaws (Adams et al., 1998; Sandlund et al., 1992; Skoglund et al., 2015), and often show bright belly coloration during spawning time (Sandlund et al., 1992; Alekseyev et al., 2014; Fraser et al., 1998). Like the anadromous ecomorph, piscivores can grow to very large size (Klemetsen et al., 2003). Cannibalism is common in charr (Klemetsen et al., 2003) and seems to increase with latitude (Griffiths, 1994). A few lakes are known to harbour two species of piscivorous Arctic charr feeding on different fish species (e.g. in Lama-Melkoy'e, Pichugin and Chebotareva, 2011). The highest diversity of piscivorous charr forms is found in a relative of Arctic charr, in *Salvelinus namaycush*, where up to four morphs differing in morphology, life history, physiology (e.g. buoyancy), diet and depth occur in sympatry (Muir et al., 2015).

Profundal dwarf ecomorphs

Dwarf charr living in the profundal zone of deep lakes are the smallest charr ecomorphs found so far (Klemetsen, 2010). An extreme case represents the blind and tiny charr species discovered at greatest depth (400-450m) of Lake Tinnsjøen, Norway (Søreide et al., 2006). Profundal dwarf ecomorphs of charr usually have pale body coloration, very large eyes which are thought to be adaptive in the dark profundal environment, a subterminal mouth (Klemetsen, 2010) and a round snout (Skoglund et al., 2015; Kottelat and Freyhof, 2007). Their diet mainly consists of soft bottom benthos (Hindar and Jonsson, 1982; Knudsen et al., 2016).

Generalist charr populations:

Charr are often considered as undifferentiated habitat generalists (Klemetsen et al., 2003). The term generalist has been used both for solitary charr populations that show strong seasonal variation in resource use (e.g. Eloranta et al., 2010) and charr species co-occurring with other specialized charr ecomorphs (e.g. Skoglund et al., 2015). Here, we use the term generalist for populations that do not show the specialized trait combinations outlined for the ecomorphs above, but are morphologically intermediate to all of them.

Appendix S3. Methods: Stable isotope analysis

A total of 556 samples of muscle tissue were dried at 55°C for 48h in a drying oven (WTC Binder). Dried tissue samples were grinded in a Tissuelizer (Qiagen, Tissue Lyser 2). 0.25 to 0.35mg (for equipment 1) or 0.9 mg (for equipment 2) of tissue powder were weighted into tin capsules (Säntis analytical, capsules for solids 5x9mm) and packed tightly. Measurements of carbon $\delta^{13}C$ and nitrogen $\delta^{15}N$ stable-isotope ratios were made using two sets of equipments: an EA Flash 2000 in combination with a IRMS Delta V Advantage (both Thermo Scientific); and an EA Vario Pyro Cube from Elementar in combination with an IRMS Isoprime100. Acetanilide containing 10.36% nitrogen and a $\delta^{15}N$ value of 1.18 as well as 71,09% carbon and a $\delta^{13}C$ value of -29.53 was used as a standard. Standards were put after every 10th sample. $\delta^{13}C$ values were lipid corrected following Post et al. (2007). The average standard deviation of the standard was <0.18‰ for $\delta^{13}C$ (0.02-0.54) and <0.17‰ for $\delta^{15}N$ (0.05-0.5).

Appendix S4. Methods: DNA extraction, PCR and genotyping

DNA was extracted from fin clips using 150 µl Chelex ® 100 (Bio-RAD) in 5% concentration diluted in ultra-pure water with 10 µl of TE buffer and 5 µl of proteinase K

(10mg/ml) added. Nine microsatellite markers were amplified using a PCR multiplex containing 2.5 µl Qiagen PCR Multiplex Kit (Qiagen, Basel, Switzerland), 1.75 µl DNAse-free water, 0.135 µl of primer mix, and 0.75 µl of extracted DNA per reaction. Markers were OMM1228, OMM5151, OMM1329, OMM1236, OMM1211, OMM5146, OMM1302 and BX890355 (Küttner et al., 2011, Palti et al., 2002, Rexroad and Palti, 2003; Rexroad et al., 2008) and Ssa100 (alias BHMS321) (Hoyheim, B. unpublished, primer sequences in Quéméré et al., 2016).
PCR consisted of an initial denaturation step of 15min at 94°C followed by 30 cycles of 30s at 94°C, 90s at 55°C and 90s at 72°C, with a final elongation step of 30min at 60°C. The PCR product was diluted with 25 µl ultra-pure water and a denaturation step at 92°C was applied for 2min. Denaturized product was loaded on the Applied System 3130xl Genetic Analyzer for fragment analysis with 0.15 µl LIZ600 size standard and 18 µl ABI-HiDi per sample. Peaks were scored with the software Genemapper v. 4.0 by two researchers. Both researchers independently scored the same 288 individuals to assess differences in scoring. The number of differences for each of the nine markers for every individual divided by the total number of comparisons (2592 comparisons), were less than 2%. Differences were independent of marker identity.

Appendix S5. Methods: Construction of population tree.
We generated a population tree using ecomorphs from all six large lakes as well as samples from different river stretches as populations. Because individuals of the piscivorous ecomorph within Lakes Tasersuaq and Saqqaata Tasia belonged to two different genetic clusters, we used the two genetic clusters as separate populations in each of these lakes. For river sites, we grouped unassigned individuals by genetic assignment of the Structure analysis to avoid artificial pooling of distinct species that may exist within a river site. We only included groups with at least five individuals. We used the program Neighbor in the software package Phylip 3.69 (Felsenstein, 2009) to build a Neighbour-joining tree. We calculated allele frequencies using the function “char2genet” from the R-package “adegenet” and calculated Cavalli-Sforza genetic distances (Cavalli-Sforza & Edwards, 1967) using the program gendist in Phylip 3.67. We performed 1000 boostrap replicates across all loci using Seqboot and obtained bootstrap values using the program Consense in Phylip.

Appendix S6. Methods: Estimating the extent of ecomorphological specialization for the four major directions of ecomorphological diversification and relating it to ecological opportunity.
To measure the degree of morphological specialization for the planktivorous, littoral benthic and profundal dwarf ecomorphs, we performed a PCA using all 24 size-corrected traits combining all individuals from all lakes (N=271). Size correction of traits was performed in a pooled regression of log10(trait) against log10(SL) across all lakes. All populations of the planktivorous ecomorph had high PC1 (short, narrow and shallow head, short jaws) and those of the littoral benthic ecomorph had low PC1 values (long, wide and deep head, long jaws), whereas populations of the profundal dwarf ecomorph were associated with high PC2 values (large eyes, shallow caudal peduncle) (Figure S2). Because separation between planktivorous and littoral benthic ecomorph was not fully parallel to PC1, and neither was that of the profundal dwarf to PC2, we rotated the first two PC axes by an angle of 60° to obtain parallel alignment to the axis of littoral benthic - planktivorous divergence (Figure S2). For each lake, we used the 90%- and 10%-quantile of the rotated PC1, and the 90%-quantile of the rotated PC2 as a measure of morphological specialization towards planktivorous, littoral benthic and profundal ecomorphology, respectively. Piscivory in charr is usually associated with large body size (Riget et al., 2000) and we therefore used the 90%-quantile of SL as a measure of specialization towards piscivory. We related each of these measures of specialization to three log-transformed proxies of ecological opportunity (maximum lake depth, lake area and lake

volume) in separate linear models.

Appendix S7. Methods: Morphospace calculation per lake.
We estimated per-lake morphospace using a resampling approach. To consider the full extent of ecomorphological variation and to prevent sample size effects, we performed a random stratified sampling across ecomorphs within lakes to obtain a subsample of 12 (six per ecomorph for Normu 1'ip Saqqata Tasia, four for Timerliit Lake2, three for Saqqaata Tasia) or 10 individuals per lake (two per ecomorph for Tasersuaq). The two anadromous from Saqqata Tasia, the single littoral benthic from Amikitap Tasia, and all unassigned individuals were excluded. With this subsample, we performed a PCA on the 24 size-corrected traits (centering and scaling the data), and estimated morphospace by calculating convex hull area per lake in PC1-2 space using the R-function chull. We repeated the resampling 1000 times and determined the lake with the larger morphospace in each permutation for each pairwise combination of lakes. Size correction was performed in a pooled regression of log10(trait) against log10(SL) across all lakes.

Appendix S8. Results: Ecological and morphological differentiation of five sympatric charr ecomorphs in Tasersuaq and Saqqaata Tasia.
We found littoral habitat, high $\delta^{13}C$ values, deep-body with large, wide, and deep head, long jaws and rather small eyes for the littoral benthic ecomorph; frequent pelagic habitat relative to other ecomorphs, low $\delta^{13}C$ and $\delta^{15}N$ values, small size, slender body form, small, narrow head with short jaws and large eyes for the planktivorous ecomorph; profundal habitat, low $\delta^{13}C$ and intermediate $\delta^{15}N$ values, very small size, narrow caudal peduncle, large, wide and deep head, large jaws and eyes for the profundal dwarf ecomorph; mainly littoral habitat, high $\delta^{15}N$ values, large size, large, wide and deep head and large jaws and eyes for the piscivorous ecomorph; littoral habitat, stable isotopic values outside the space of lake residents, large size, deep-body with a small, narrow head, short jaws and small eyes for the anadromous ecomorph. These ecological and morphological characteristics are consistent with descriptions of these charr ecomorphs elsewhere in the world (e.g. Sandlund et al., 1992; Knudsen et al., 2016; Loewen et al., 2009)

Appendix S9. Methods and results: Differentiation in body shape between profundal dwarfs and piscivores in Tasersuaq and Saqqaata Tasia based on trait ratios.
We assessed morphological differentiation among ecomorphs of Tasersuaq and Saqqaata Tasia based on ratios of body measurements using the method by Baur and Leuenberger (2017). Specifically, we performed "shapePCAs" using all 24 linear measurements for each lake separately. In both lakes, all ecomorphs were differentiated from each other in PC1-2 space of this ratio-PCA (Figure S5). Unlike in the morphological PCA based on residuals from regressions between traits and SL, the piscivorous and profundal dwarf ecomorphs were clearly differentiated in shapePCA along the first axis.

Appendix S10. Results: Subtle ecological and morphological differentiation between sympatric piscivore species.
In Tasersuaq, the locally rare, but across the drainage widespread piscivore had smaller eyes and head (significant after multiple testing correction), shorter upper jaws and a narrower anal fin (not significant after multiple testing correction) than the locally common piscivore. There were no significant differences in morphometric distances between the two genetic clusters of piscivores in Saqqaata Tasia. Concomitantly, DAPC on the same 24 measurements (retaining 8 and 4 PC axes in Tasersuaq and Saqqaata Tasia, respectively) distinguished the two piscivores in Tasersuaq, but not in Saqqaata Tasia (Figure S8). There were no significant differences in $\delta^{13}C$ or size (Tasersuaq: $\delta^{13}C$ $p=0.46$, size $p=0.46$; Saqqaata Tasia: $\delta^{13}C$ $p=0.47$,

size p=0.71). The more widespread piscivore had slightly lower $\delta^{15}N$ than the other piscivore in Saqqaata Tasia, (p=0.027), but not in Tasersuaq (p=0.14).

Appendix S11. Assessing different methods to identify charr diversity
The degree of divergence among sympatric charr ecomorphs varies greatly both between and within systems. Ecomorphological divergence ranges from very clearly distinct forms (e.g. profundal vs. littoral species in Fjellfrøsvatn (Norway), Klemetsen et al., 1997; four species in Thingvallavatn (Iceland), Sandlund et al., 1992; Gíslason et al., 1999) to intra-population variation in morphology correlated with dietary variation (e.g. pelagic-littoral divergence within the "littoral" form in Fjellfrøsvatn (Norway), Knudsen et al., 2007). Hence, a single criterion for species identification likely fails to live up to the species diversity found in rich charr communities and a combination of approaches may be required for uncovering the actual diversity of large charr assemblages. In the following, we use our dataset of lakes harbouring from a single monomorphic population up to six species to compare different approaches to identifying charr species.

For each lake, we compared the number of charr groups suggested by modality in size distribution, modality in two-dimensional stable isotope space, genetic clustering analysis, and visual assignment of individuals to ecomorphs. The two latter analyses were performed as described in the main text. Modality in size distribution, and isotopic space was assessed using Gaussian mixture modelling as implemented in the R package mclust (Fraley and Raftery, 2002; Fraley et al., 2012). For size, we assessed the most likely number of modes using the function mclustBootstrapLRT for the models assuming equal or variable variance. Because Gaussian mixture models assume normal distributions, they can be sensitive to outliers. We therefore excluded a very small outlier individual from Tasersuaq. To determine the number of clusters in isotopic space, we excluded individual outliers of the lake-wide distribution for each lake (Tasersuaq (n=5), Saqqaata Tasia (n=2), Timerliit Lake2 (n=1), Tasilikulooq Upper Lake2 (n=1)), and then assessed the best supported number of mixture components for the model assuming ellipsoidal clusters of varying volume, shape and orientation (VVV model) using mclustBootstrapLRT.

We found evidence for a trimodal size distribution in Tasersuaq and Normu 1'ip Saqqaata Tasia, a bimodal distribution in Saqqaata Tasia and Amikitap Tasia, and a unimodal distribution in Timerliit Lake2 and Tasilikulooq Upper Lake2 (Table S13, Figure S9). Gaussian mixture models applied to stable isotopic space suggested four clusters in Tasersuaq, three in Saqqaata Tasia, and two in all other lakes except Taslilikulooq Upper Lake with only one cluster (Table S13, Figure S9).
Whereas different size modes did mostly not correspond to ecomorphs, clusters in stable isotope space roughly corresponded to different ecomorphs, except in Saqqaata Tasia where one cluster comprised two ecomorphs (Figure S10). Neither the assessment using size distributions nor that using stable isotopes was able to identify all five ecomorphs present in the two largest lakes (Table S13). This occurred because the littoral benthic ecomorph showed a large range of sizes, whereas the anadromous ecomorph's size range was similar to that of the piscivore (Figure S11). Furthermore, the anadromous ecomorph was rare in both lakes and was an outlier in isotopic signatures, so that these individuals had to be excluded for the Gaussian mixture model analysis based on isotopes. Note that also the genetic cluster analysis alone underestimated charr species richness, as the profundal dwarf and planktivorous ecomorphs showed weak genetic differentiation so that Structure could not differentiate them based on our nine microsatellite loci.

The ability to determine species richness of a community is affected by the abundance, spatial distribution and distinctness of species, and by sampling design and methods for species recognition. Rare, weakly distinct and highly localized species are especially difficult to detect, and we cannot exclude that we have missed such. In our study, we aimed to alleviate this problem by combining genetic data of charr from the entire drainage, which allowed us to recognize locally rare, but overall common species (e.g. two anadromous individuals in Saqqaata Tasia). Furthermore, by sampling all major lake habitats we also caught habitat specialists, such as the profundal dwarf species. Finally, we combined genetic, morphological and ecological data to identify charr species. For the largest charr community (six, maybe seven, species in Lakes Tasersuaq and Saqqaata Tasia), any of the clustering methods (size, stable isotopes, or genetic data) alone as well as visual assignment to ecomorphs underestimated species diversity, suggesting that a combination of methods is crucial to recognize high species diversity in charr.

Identification of sympatric charr forms based on size has a long tradition (e.g. Gessner, 1575) and is still used (e.g. Alekseyev et al., 2002; Riget et al., 2000). However, detectability of sympatric charr morphs based on size alone seems to reach a maximum at three, a limit that may also apply to growth curve models (Woods et al., 2012). That this method often does not account for the actual diversity present is exemplified in our study and in Gordeeva et al. (2014) who found in many Transbaikalian lakes more genetic clusters than size morphs were present. On the one hand, this might be explained by increasing species numbers leading to more overlap and fewer gaps in the size distribution, thereby erasing modality. On the other hand, as communities grow, convergence in niche and associated traits is expected based on theory (Scheffer and van Nes, 2006) and empirical studies on large adaptive radiations (e.g. Muschick et al., 2012; Mahler et al., 2013), and hence may also occur in speciose communities of young postglacial fish.

Supplementary tables

Table S1. Lake characteristics and number of charr used for different analyses.

Lake	Area (ha)*	Max. depth (m)†	Altitude (m) ‡	#charr caught	#morphology	#stable isotopes	#genetics
Tasersuaq	893.4	87.9	15	252	102	207	244
Saqqaata Tasia	193.3	37.6	20	118	70	113	116
Normu 1'ip Saqqaata Tasia	150.6	20.3	25	77	28	74	77
Amikitap Tasia	70.84	15	90	46	20	38	46
Timerliit Lake2	20.7	22.7	65	54	35	54	54
Timerliit Lake1	9.1	1.1	50	6	0	0	5
Tasilikulooq Upper Lake2	11.5	23	115	83	17	70	82
River	-	0.5	-	66	-	-	62
Total				702	272	556	686

* Area was assessed using ACME planimeter area calculation which is based on Google Maps.
† Depth was determined for every lake by echo sounding.
‡ Elevation was taken from a hiking map (Photogrammetry, Harvey Map Services, Scotland, revised 2002).

Table S2. Sampling effort per lake.

Lake	Lake area (ha)	Max. depth (m)	# benthic nets	# pelagic nets
Tasersuaq	893.4	87.9	20	6
Saqqaata Tasia	193.3	37.6	9	5
Normu 1'ip Saqqaata Tasia	150.6	20.3	5	1
Amikitap Tasia	70.84	15	6	0
Timerliit Lake2	20.7	22.7	3	1
Tasilikulooq Upper Lake2	11.5	23	6	0
Timerliit Lake1	9.1	1.1	2	0

Table S3. Ecomorph characteristics used to assign individuals based on visual appearance (photos).

	Body size	Head shape	Mouth position	Eyes	Pectoral and pelvic fins	Body shape	Colouration back/belly/fins
Littoral benthic	small to large	robust head, rather long and high	terminal to subterminal	rather small	rather long	deep-bodied and deep caudal peduncle	green to brown/yellow to orange/with yellow to orange
Planktivore	small	small head, pointed snout	terminal	rather large	rather short	overall slender	silvery to brown/often orange/orange
Piscivore	large	rather large head	terminal	intermediate	variable length	fusiform, variable cross section	Gray to brown/orange to red/orange to red
Anadromous	large	rather small head	terminal	small	short	fusiform, mostly roundish cross section	darkgray to silvery/white, yellow or orange/ white, yellow or orange
Profundal dwarf	small	rather long head, roundish snout	terminal to subterminal	large	intermediate length	rather slender, but often blown up when caught	pale brownish/ white/ pale to yellowish
Generalist	intermediate	intermediate	terminal	intermediate	variable	variable	variable

Table S4. Number of individuals measured per ecomorph and lake for morphometric analyses.

	Littoral benthic	Piscivore	Planktivore	Profundal dwarf	Anadromous	Generalist	Unassigned
Tasersuaq	12	40	22	23	5	-	-
Saqqaata Tasia	14	30	15	9	2	-	-
Normu 1'ip Saqqaata Tasia	12	16	-	-	-	-	-
Amikitap Tasia	1	17	-	-	-	-	2
Timerliit Lake2	4	8	-	-	-	20	3
Tasilikulooq Upper Lake2	-	-	-	-	-	17	-

Table S5. F_{ST}s between ecomorphs within lakes. Below diagonal are multilocus F_{ST} values, above diagonal P-values. N indicates sample size.

Tasersuaq	N	Littoral benthic	Piscivore 1	Piscivore 2	Planktivore	Profundal dwarf
Littoral benthic	20	-	<0.001	<0.001	<0.001	<0.001
Piscivore 1	74	0.221	-	<0.001	<0.001	<0.001
Piscivore 2	9	0.157	0.119	-	<0.001	<0.001
Planktivore	39	0.140	0.191	0.145	-	<0.001
Profundal dwarf	82	0.098	0.145	0.126	0.025	-

Saqqaata Tasia		Littoral benthic	Piscivore 1	Piscivore 2	Planktivore	Profundal dwarf
Littoral benthic	18	-	<0.001	<0.001	<0.001	<0.001
Piscivore 1	18	0.197	-	<0.001	<0.001	<0.001
Piscivore 2	24	0.196	0.143	-	<0.001	<0.001
Planktivore	26	0.125	0.165	0.161	-	0.35
Profundal dwarf	9	0.166	0.164	0.167	0.003	-

Timerliit Lake2		Piscivore	Generalist
Piscivore	12	-	0.239
Generalist	27	0.016	-

Normu 1'ip Saqqaata Tasia		Littoral benthic	Piscivore
Littoral benthic	13	-	<0.001
Piscivore	48	0.194	-

Table S6. Results of locus-by-locus AMOVA between the planktivorous and profundal dwarf ecomorphs of Tasersuaq.

Marker	F_{ST}	p-value
BX890355	-0.0036	1
OMM1211	0.0066	0.231
OMM1228	0.0495	<0.001
OMM1236	0.0169	0.025
OMM1302	0.0859	<0.001
OMM1329	0.0062	0.314
OMM5146	0.0526	0.003
OMM5151	-0.0038	0.913
Ssa100	-0.00142	0.835
overall	0.023	<0.001

Table S7. Differentiation in morphology (lower left) and size (upper right) between ecomorphs within lakes. Morphological differentiation is measured as Bhattacharyya distance in the morphospace of PC1-2 of lake-specific PCA, and size differentiation as P_{ST}. Significance is indicated with asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Tasersuaq	Littoral benthic	Piscivore	Planktivore	Profundal dwarf	Anadromous
Littoral benthic	-	0.28**	0.01 ns	0.35 **	0.28*
Piscivore	1.36***	-	0.47***	0.71***	0 ns
Planktivore	2.90***	1.98***	-	0.46***	0.78***
Profundal dwarf	1.86***	0.17 ns	1.60***	-	0.95***
Anadromous	0.82 ns	1.49**	1.67***	2.82***	-

Saqqaata Tasia	Littoral benthic	Piscivore	Planktivore	Profundal dwarf
Littoral benthic	-	0.55***	0.03 ns	0.09 ns
Piscivore	0.78***	-	0.70***	0.68***
Planktivore	2.49***	1.43***	-	0.13 ns
Profundal dwarf	1.53***	0.23 ns	0.91***	-

Timerliit Lake2	Littoral benthic	Piscivore	Generalist
Littoral benthic	-	0.54***	0.14 ns
Piscivore	0.17 ns	-	0.52***
Generalist	0.28 ns	0.11 ns	-

Normu 1'ip Saqqaata Tasia	Littoral benthic	Piscivore
Littoral benthic	-	0.46***
Piscivore	0.27*	-

Table S8. Trait loadings for morphological PCAs of different lakes.

	Tasersuaq		Saqqaata Tasia		Normu 1'ip Saqqaata Tasia		Amikitap Tasia		Timerliit Lake2			Tasilikulooq Upper Lake2	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC3	PC1	PC2
Anterior distance to pelvic fin	-0.19	-0.04	-0.18	0.14	**-0.21**	-0.07	-0.06	0.11	0.09	0.16	0.12	0.03	**-0.26**
Anterior distance to dorsal fin	-0.16	-0.21	-0.2	0.15	-0.16	0.12	-0.09	0.15	0.06	0.13	**-0.45**	**-0.21**	**0.27**
Tail depth	-0.15	**-0.37**	-0.18	**0.26**	-0.1	**0.48**	-0.03	**-0.44**	0.03	**-0.35**	0.07	**-0.22**	**0.26**
Dorsal head length	**-0.24**	0.12	**-0.23**	-0.17	**-0.26**	-0.02	**0.3**	0.11	**0.28**	0.1	0.09	-0.2	-0.1
Head length	**-0.25**	0.11	**-0.25**	-0.11	**-0.28**	0.01	**0.28**	-0.07	**0.29**	0.07	0.17	**-0.27**	**-0.23**
Eye diameter	-0.14	**0.4**	-0.07	**-0.45**	-0.2	0.06	0.1	**0.27**	0.2	**0.22**	**0.29**	-0.19	**0.32**
Eye hight	-0.14	**0.23**	-0.07	**-0.38**	**-0.23**	0.12	**0.24**	**0.21**	0.16	**0.23**	**0.22**	-0.18	0.19
Distance eye-operculum	**-0.23**	0.01	**-0.23**	-0.01	**-0.24**	0.12	0.2	**-0.29**	**0.22**	0.06	**0.37**	-0.11	**-0.35**
Snout length	**-0.24**	-0.01	**-0.25**	0.02	**-0.23**	-0.2	**0.23**	**0.22**	**0.27**	0.03	-0.1	**-0.27**	-0.19
Interpreorbital distance	**-0.22**	-0.15	**-0.23**	0.02	**-0.24**	-0.13	**0.24**	0.06	**0.26**	-0.03	-0.15	**-0.21**	-0.03
Upper jaw length	**-0.25**	0.06	**-0.25**	-0.01	**-0.26**	-0.13	**0.3**	0.06	**0.29**	0.02	-0.08	**-0.3**	-0.13
Maxilla length	**-0.24**	0.09	**-0.24**	-0.04	**-0.25**	-0.15	**0.27**	0	**0.29**	0.05	-0.03	**-0.27**	-0.08
Maxilla width	-0.2	0.05	-0.19	0.16	-0.04	**-0.32**	0.18	**-0.23**	**0.21**	-0.05	**-0.3**	-0.15	**-0.25**
Lower jaw length	**-0.24**	0.06	**-0.24**	-0.1	**-0.26**	-0.09	**0.28**	-0.11	**0.29**	0.06	0.02	**-0.28**	-0.06
Interorbital distance	-0.18	**-0.22**	**-0.23**	-0.02	**-0.24**	**0.23**	0.17	0.01	0.11	**-0.23**	0.02	-0.15	0.2
Upper jaw width	**-0.23**	-0.14	**-0.23**	0.16	-0.2	0.18	**0.23**	**-0.22**	**0.21**	**-0.27**	-0.2	**-0.25**	0.1
Upper jaw width notch	**-0.24**	-0.02	**-0.23**	0.06	**-0.24**	-0.04	0.13	0.2	**0.24**	0.02	**-0.26**	-0.1	-0.19
Lower jaw width	-0.2	**-0.24**	-0.2	**0.22**	-0.12	**0.26**	0.04	**-0.25**	0.12	**-0.36**	-0.15	-0.18	**0.29**
Length of pectoral fin	-0.19	**0.27**	-0.18	**-0.32**	**-0.23**	-0.16	**0.29**	0.06	**0.21**	-0.16	0.08	**-0.24**	-0.06
Width of dorsal fin	-0.2	-0.16	-0.2	0.04	-0.09	-0.08	0.17	-0.12	-0.02	**-0.26**	**0.24**	**-0.25**	-0.1
Length of anal fin	-0.19	**0.24**	-0.18	**-0.29**	-0.1	**-0.38**	0.18	0.07	0.17	-0.16	**0.34**	-0.05	-0.09
Width of anal fin	-0.15	0.13	-0.12	-0.08	0.04	-0.07	0.08	-0.05	0.01	**-0.38**	0.14	-0.06	-0.13
Head depth	**-0.25**	0	**-0.24**	-0.05	**-0.26**	0.04	**0.28**	-0.11	**0.27**	-0.05	-0.1	**-0.23**	-0.12
Body depth at anal fin origin	-0.07	**-0.49**	-0.12	**0.43**	-0.06	**0.41**	-0.05	**-0.5**	-0.02	**-0.42**	0.07	-0.18	**0.36**

Table S9. Ecological differentiation between ecomorphs within lakes. Differentiation in stable isotope space ($\delta^{13}C$ and $\delta^{15}N$, lower left) is measured as Bhattacharyya distance, and depth differentiation for charr caught in benthic nets (upper right) as P_{ST}. Significance is indicated with asterisks (* P < 0.05, ** P < 0.01, *** P < 0.001)

Tasersuaq	Littoral benthic	Piscivore	Planktivore	Profundal dwarf	Anadromous
Littoral benthic	-	0 ns	0.05 ns	0.76***	0 ns
Piscivore	0.79***	-	0.03 ns	0.65***	0.03*
Planktivore	0.93***	2.67***	-	0.50***	0.09*
Profundal dwarf	1.00***	1.36***	1.35***	-	0.83***
Anadromous	1.91***	0.78**	2.30***	1.58***	-

Saqqaata Tasia	Littoral benthic	Piscivore	Planktivore	Profundal dwarf
Littoral benthic	-	0.16*	0.09 ns	0.81***
Piscivore	1.10***	-	0.32***	0.38***
Planktivore	1.12***	4.17***	-	0.96***
Profundal dwarf	1.30*	2.66***	1.37***	-

Timerliit Lake2	Littoral benthic	Piscivore	Generalist
Littoral benthic	-	0 ns	0 ns
Piscivore	1.65***	-	0 ns
Generalist	0.23 ns	0.5***	-

Normu 1'ip Saqqaata Tasia	Littoral benthic	Piscivore
Littoral benthic	-	0 ns
Piscivore	3.47***	-

Table S10. Habitat differentiation between ecomorphs within lakes. Results from Chi-Square tests of habitat categories (littoral benthic, pelagic, profundal) are given below the diagonal. Significance after sequential Bonferroni correction is indicated in bold.

Tasersuaq	Littoral benthic	Piscivore	Planktivore	Profundal dwarf	Anadromous
Littoral benthic	-				
Piscivore	0.747	-			
Planktivore	0.035	**0.001**	-		
Profundal dwarf	**<0.001**	**<0.001**	**<0.001**	-	
Anadromous	1	0.514	0.18	**<0.001**	-
Overall	**<0.001**				

Saqqaata Tasia	Littoral benthic	Piscivore	Planktivore	Profundal dwarf
Littoral benthic	-			
Piscivore	**0.016**	-		
Planktivore	**0.002**	**<0.001**	-	
Profundal dwarf	**<0.001**	**0.021**	**<0.001**	-
Overall	**<0.001**			

Timerliit Lake2	Littoral benthic	Piscivore	Generalist
Littoral benthic	-		
Piscivore	0.30	-	
Generalist	0.51	0.72	-
Overall	0.65		

Normu 1'ip Saqqaata Tasia	Littoral benthic	Piscivore
Littoral benthic	-	
Piscivore	*0.06*	-

Table S11. Individual trait comparison between Tasilikulooq Upper Lake2 and Timerliit Lake2. P-values are given for linear models of trait against standard length (SL), lake and their interaction, and for linear models of trait against standard length (SL) and lake. P-values were adjusted for multiple testing using Holm's method; significance is indicated in bold.

	Model with interaction		Model without interaction
Trait	Lake	Lake x SL	Lake
PFL	1	1	1
DFL	1	1	1
TD	0.775	1	**0.012**
DHL	**<0.001**	**<0.001**	1
HL	**0.002**	**0.003**	1
ED	**0.003**	**0.003**	0.691
EH	**<0.001**	**<0.001**	0.373
EOL	**0.008**	**0.011**	0.158
SNL	0.142	0.173	0.691
IPOD	0.437	0.444	1
UJL	0.093	0.114	0.694
MAXL	0.088	0.106	0.918
MAXW	0.299	0.368	0.348
LJL2	0.070	0.079	1
INB	0.059	0.067	1
UJW	0.130	0.156	0.802
UJWN	0.767	1	**0.007**
LJW	0.507	0.548	1
FpecL	0.480	0.530	1
FdorW	1	1	0.158
FanaL	0.557	0.637	0.918
FanaW	1	1	1
HD	0.049	0.059	1
BDA	0.888	1	0.348

Table S12. Average morphospace occupation and P-values (below diagonal) for the difference in morphospace occupation between lakes as determined with 1000 permutations.

	Morphospace per lake	**Tasersuaq**	**Saqqaata Tasia**	**Normu 1'ip Saqqaata Tasia**	**Amikitap Tasia**	**Timerliit Lake2**
Tasersuaq	52.3					
Saqqaata Tasia	48.6	0.424				
Normu 1'ip Saqqaata Tasia	39.7	0.248	0.292			
Amikitap Tasia	14.4	<0.001	<0.001	0.001		
Timerliit Lake2	22.0	0.009	0.010	0.032	0.080	
Tasilikulooq Upper Lake2	9.1	<0.001	<0.001	<0.001	0.049	0.010

Table S13. Number of ecomorphs or clusters found for different methods within each lake. For the cluster analysis using body size, both results assuming equal or unequal variance within groups are shown. For visual assigments to ecomorphs, number of ecomorphs including generalists are given in brackets. A genetic cluster was considered to be present in a lake if minimum three individuals with maximum assignment likelihood > 0.6 were found.

Method / Lake	**Tasersuaq**	**Saqqaata Tasia**	**Normu 1'ip Saqqaata Tasia**	**Amikitap Tasia**	**Timerliit Lake2**	**Tasilikulooq Upper Lake2**
Visual assignment to ecomorphs with photos	5	5	2	2	2 (3)	0 (1)
Size modes (mclust V/E, without individual outliers)	3/2	2/2	3/3	2/2	1/2	1/1
Stable isotope clusters (mclust VVV, without individual outliers)	4	3	2	2	2	1
Genetic clusters (hierarchical Structure analysis across Eqaluit)	5	5	3	2	1	1
Combined	6	6	3	2	2	1

Supplementary Figures

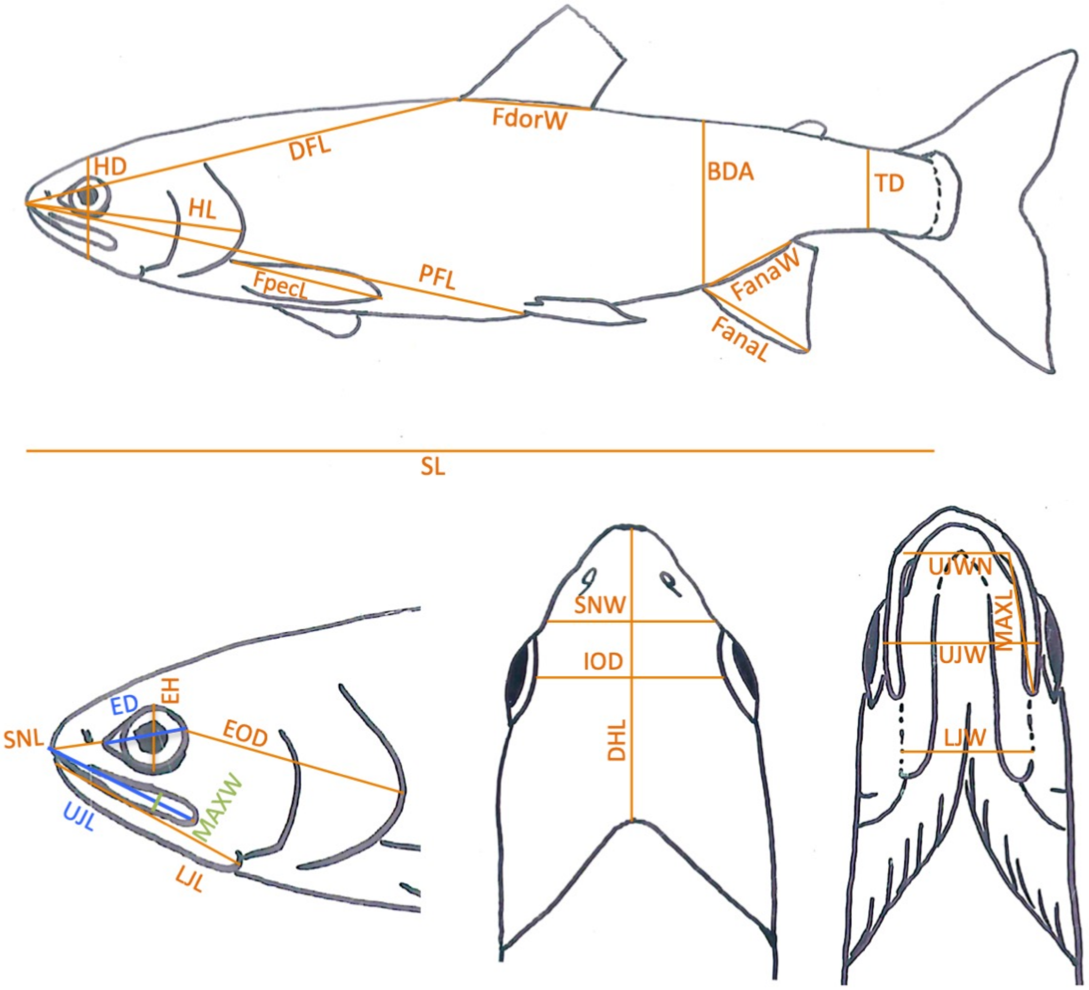


Figure S1. SL, standard length: from the tip of the mouth to the posterior end of the lateral muscle tissue. PFL, pelvic fin length: from the tip of the snout to the anterior part of the pelvic fin insertion. DFL, dorsal fin length: from the tip of the snout to the anterior insertion of the dorsal fin. TD, tail depth: smallest vertical body depth at the caudal peduncle DHL, dorsal head length: from the tip of the snout to the dorsal end of the cranial bone. HL, lateral head length: from the tip of the mouth to the lateral end of the operculum. ED, eye diameter: from the supraoccipital bone to the anterior end of the ligamentous triangle of the eye. EH, vertical eye height: from the ventral end of the infraorbital ring to the most dorsal transition of eye to frontal bone. EOL, eye-operculum length: from the supraoccipital to the posterior end of the operculum. SNL, snout length: from the anterior end of the ligamentous triangle of the eye to the tip of the mouth. SNW, snout width: distance between the anterior ends of the ligamentous triangle of the eyes. UJL, upper jaw length. MAXL, maxillary bone length. MAXW, maxillary bone width: maximal width of the maxillary bone without teeth. LJL, lower jaw length. IOD, interorbital distance: distance between the dorsal margins of the orbits of the eyes. UJW, upper jaw width: maximal width between the upper jaws. UJWN, upper jaw width notch: distance between the anterior ends of the maxillary bones. LJW, lower jaw width: width of the lower jaw at the posterior end. FPecL, pectoral fin length. FdorW, proximal width of the dorsal fin. FanaL, anal fin length. FanaW, proximal width of the anal fin. HD, head depth: vertical head depth at the center of the eye. BDA, body depth at the anterior end of the anal fin.

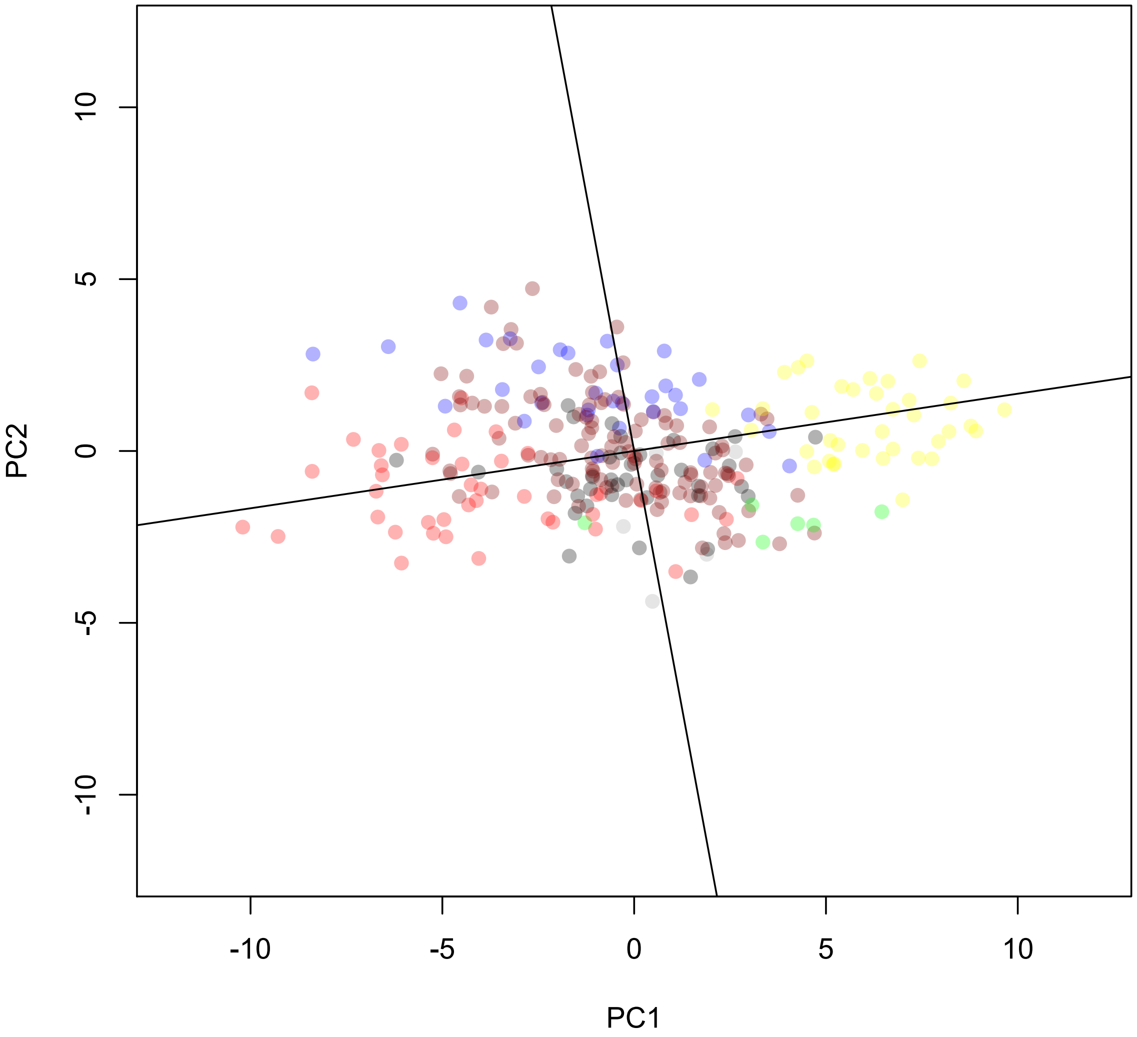
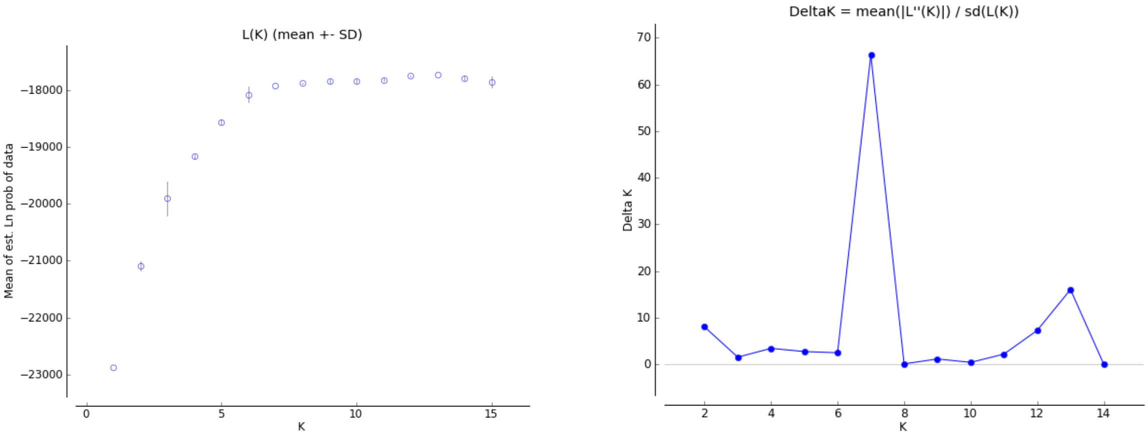


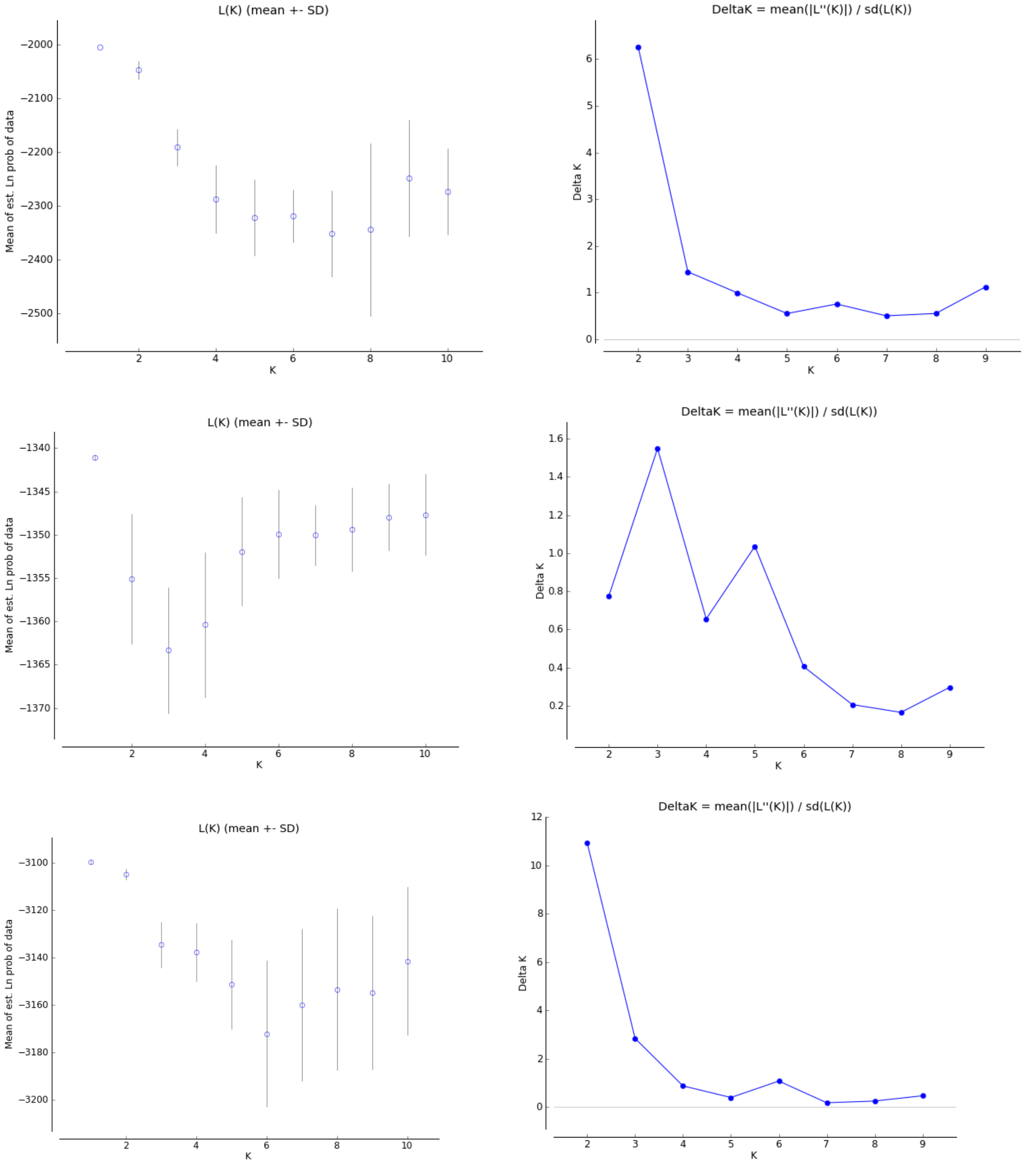
Figure S2. PCA combining all individuals from all lakes. Colors correspond to ecomorphs as in Figure 2 (blue: profundal dwarf; red: littoral benthic; orange: planktivore; darkred: piscivore; green: anadromous; black: generalist; gray: unassigned). Lines represent the new coordinate system to which this is rotated (-60°) to obtain parallel alignment to the major axis of littoral benthic-pelagic divergence.

a)



L(K) (mean +- SD)
Mean of est. Ln prob of data
−18000
−19000
−20000
−21000
−22000
−23000
0
5
10
15
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
K

b)



L(K) (mean +- SD)
Mean of est. Ln prob of data
−2000
−2100
−2200
−2300
−2400
−2500
2
4
6
8
10
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
6
5
4
3
2
1
0
2
3
4
5
6
7
8
9
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
−1340
−1345
−1350
−1355
−1360
−1365
−1370
2
4
6
8
10
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
2
3
4
5
6
7
8
9
K
L(K) (mean +- SD)
Mean of est. Ln prob of data
−3100
−3120
−3140
−3160
−3180
−3200
2
4
6
8
10
K
DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
12
10
8
6
4
2
0
2
3
4
5
6
7
8
9
K

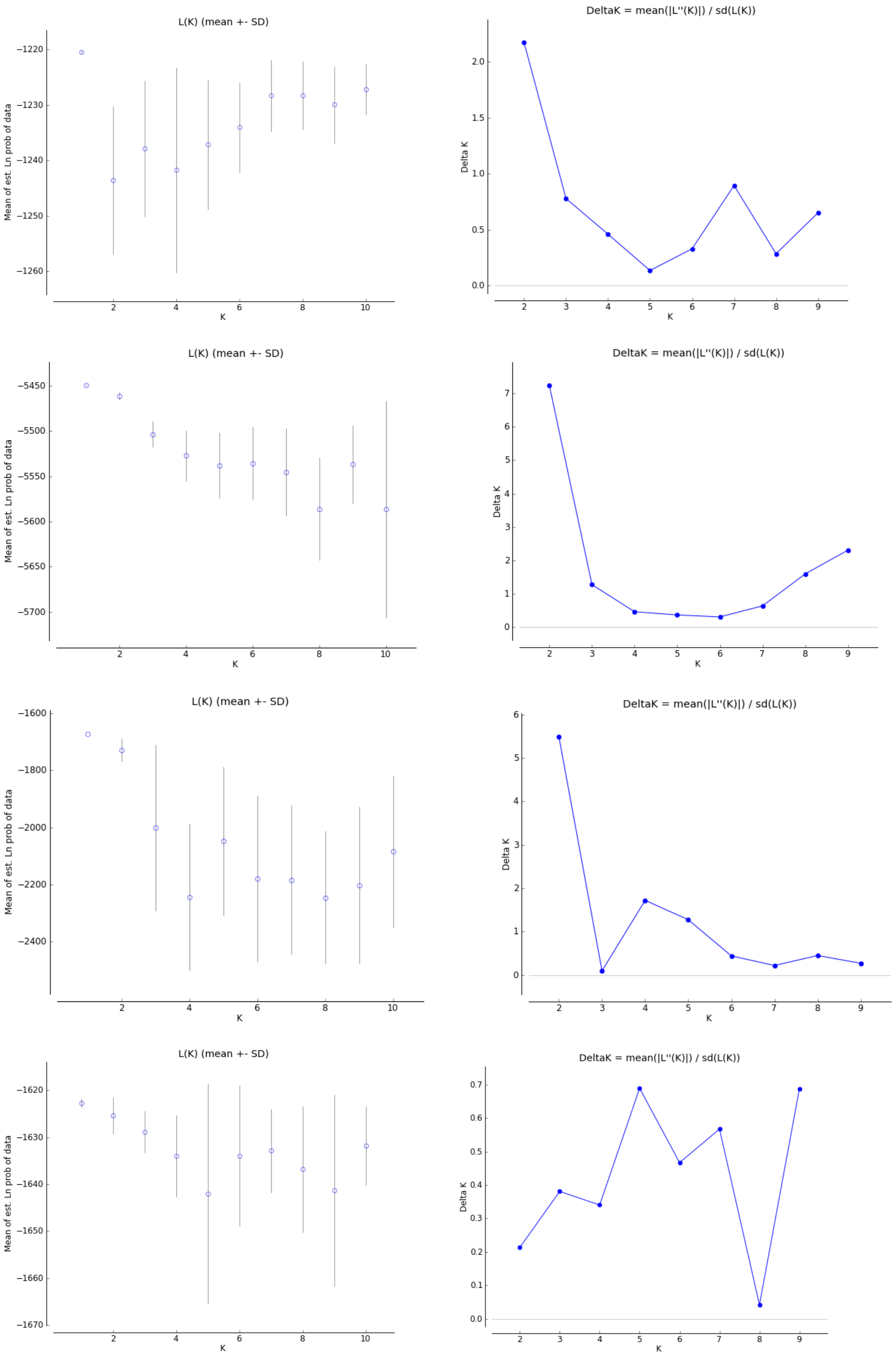


Figure S3. Mean ln probability ± standard deviations and delta K of 10 runs for K=1 to K=15 as obtained from Structure harvester for the different steps of the hierarchical Structure analysis. a) First step of the hierarchical structure approach using all individuals from all lakes and rivers. b) Second step with separate runs for each of the seven clusters found in (a).

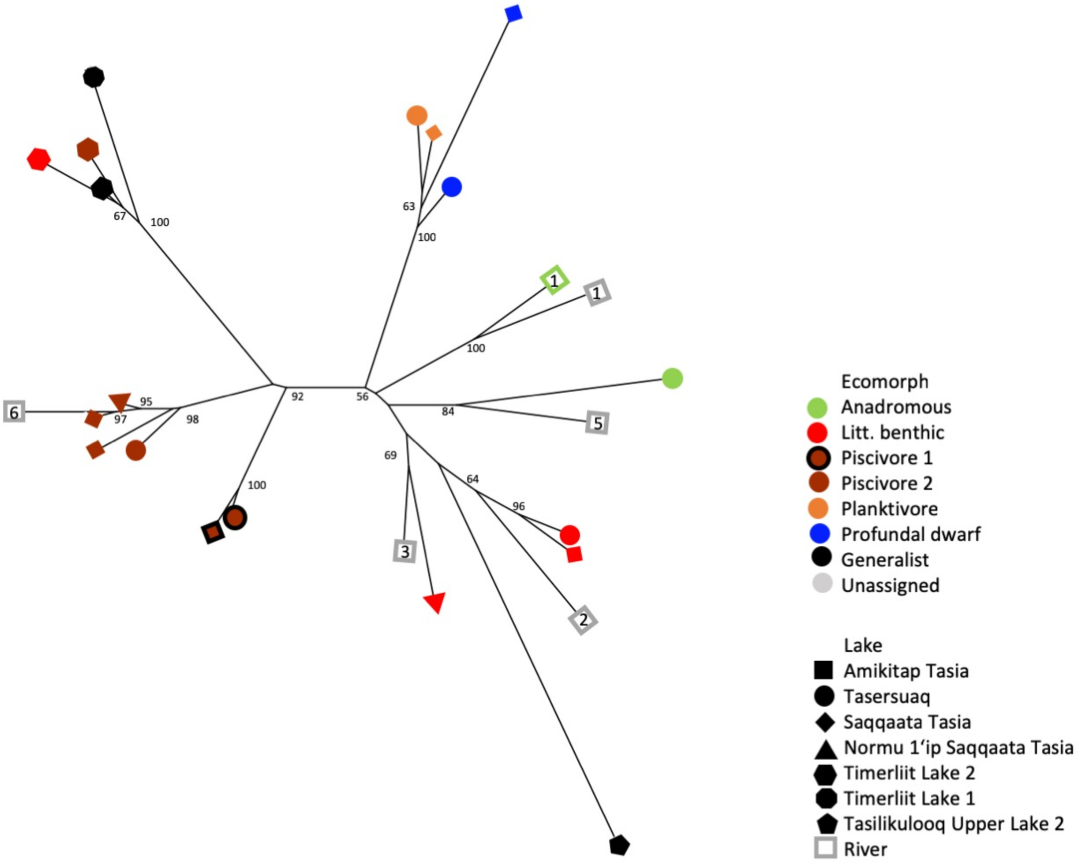


Figure S4. Neighbour-joining tree based on Cavalli-Sforza genetic distances including charr ecomorphs from all lakes and charr populations from river sites. Colors correspond to ecomorphs, symbols to lakes. For rivers, numbers correspond to river sites as indicated in Figure 1. Bootstrap values based on 1000 replicates are given when ≥ 50%.

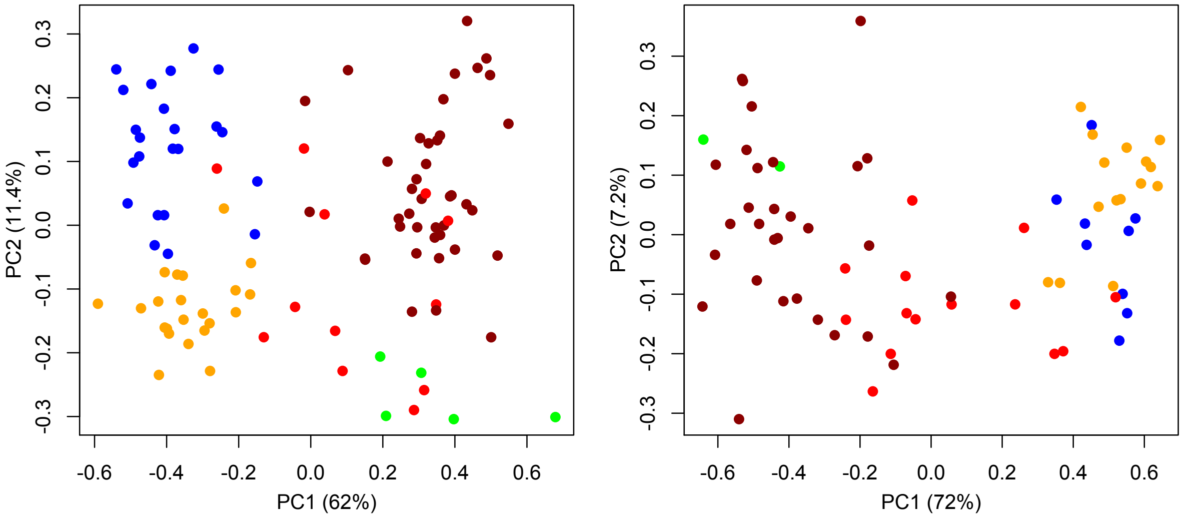


Figure S5. Ecomorph differentiation in shape PCA based on ratios of traits in Tasersuaq (left) and Saqqaata Tasia (right). Color codes of ecomorphs correspond to those of Figure 2.

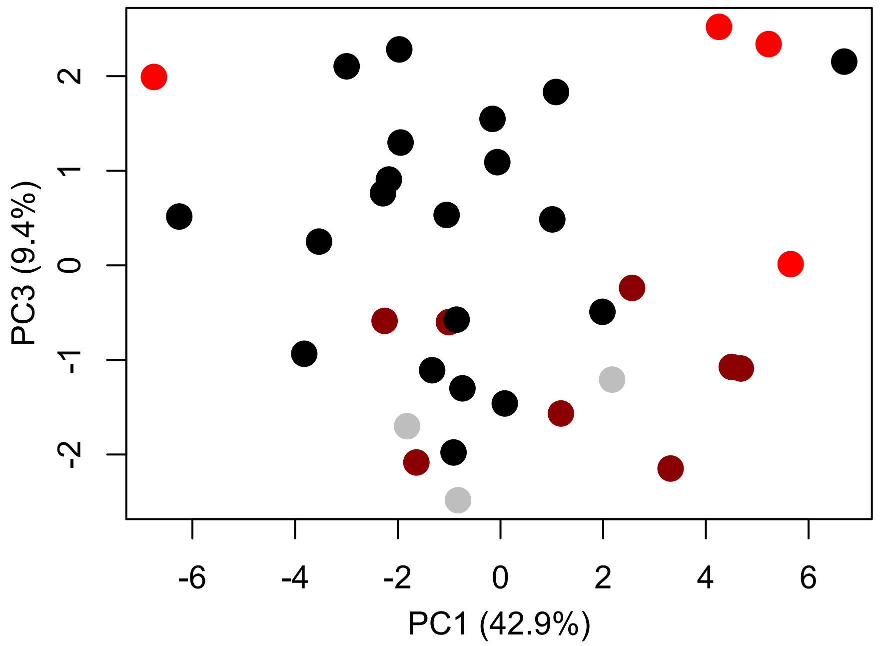


Figure S6. Morphological differentiation of ecomorphs in Timerliit Lake2. Littoral benthic (red) and piscivorous (darkred) ecomorphs are significantly differentiated from each other in morphospace of PC1-PC3, the generalist (black) is not (Bhattacharyya distance: piscivore-littoral benthic 1.39, p=0.002; piscivore-generalist 0.49, p=0.002; generalist-littoral benthic 0.39, p=0.077). PC3 was mainly driven by distance between snout and dorsal fin, distance between eye and operculum, eye size, anal fin length and maxilla width (Table S8)

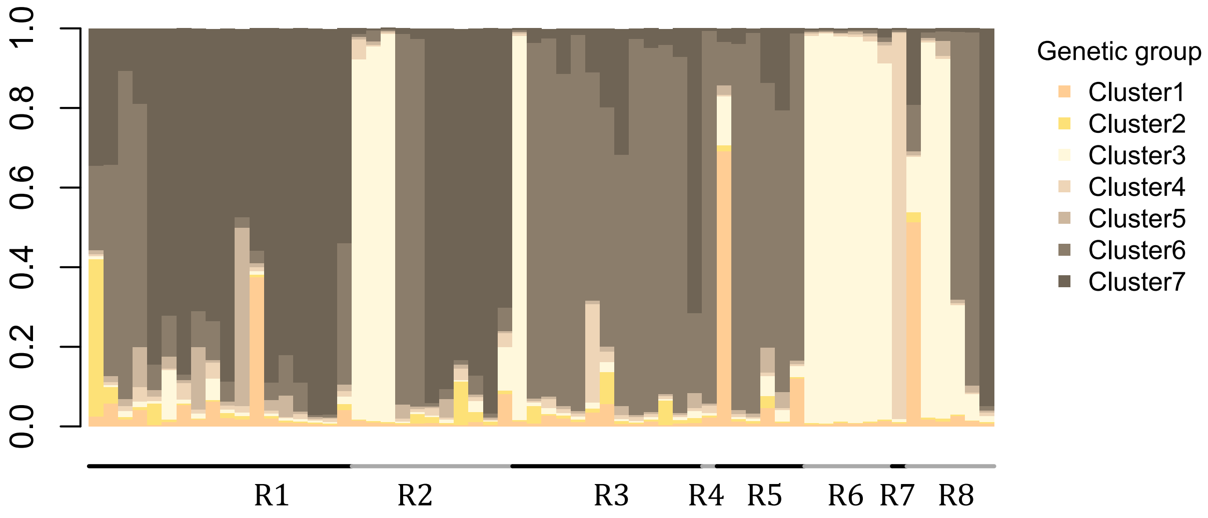


Figure S7. Structure results of the hierarchical Structure analysis on the entire Eqaluit drainage for river sampling sites. Color codes of genetic clusters correspond to those of Figure 2.

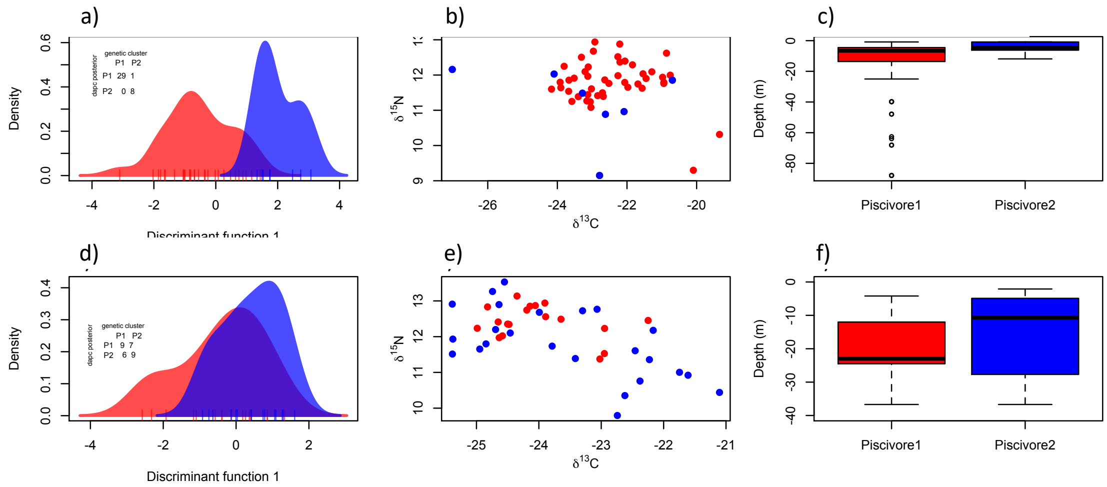


Figure S8. Weak phenotypic and ecological differentiation between two cryptic sympatric piscivore species (red: piscivore 1, blue: piscivore2). In Tasersuaq (a-c), the two genetic clusters of piscivores can be morphologically distinguished using DAPC on 24 linear traits (a), and they marginally differ in their depth occupation (c), but not in stable isotopic signature (b). In Saqqaata Tasia, the two genetic clusters of piscivores slightly differ in stable isotopic composition (e) and marginally in depth occupation (f), but not in morphology (d). Inset tables in a) and d) indicate assignment success with DAPC.

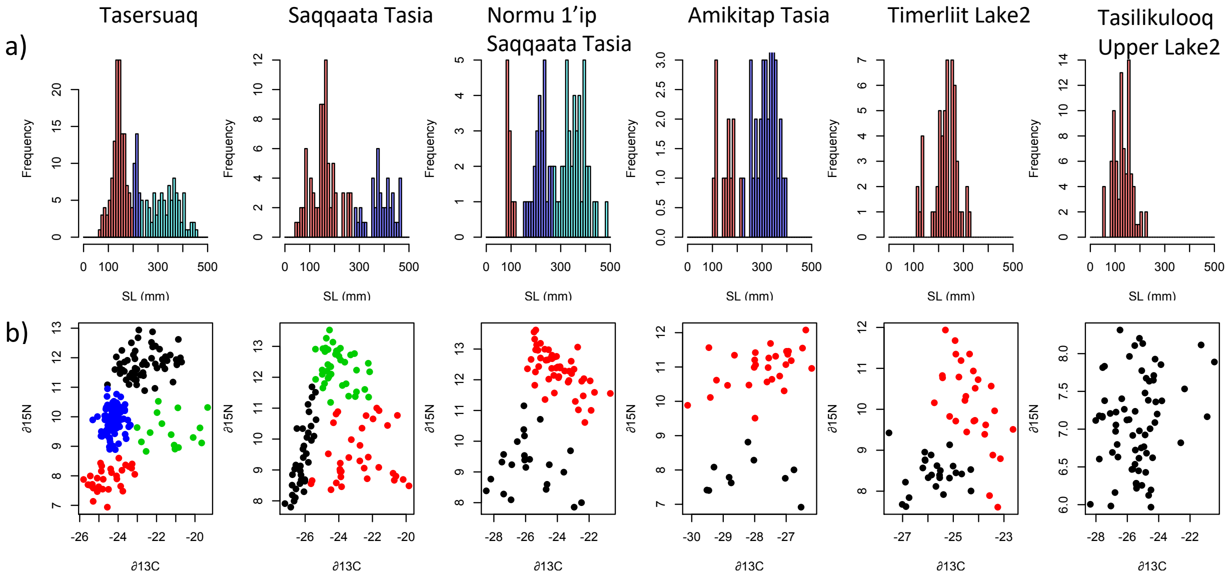


Figure S9. Groups inferred by mclust for body size (a) and stable isotopic space (b) within each lake. Individuals are colored by group assignment for the most likely number of clusters inferred with mclust.

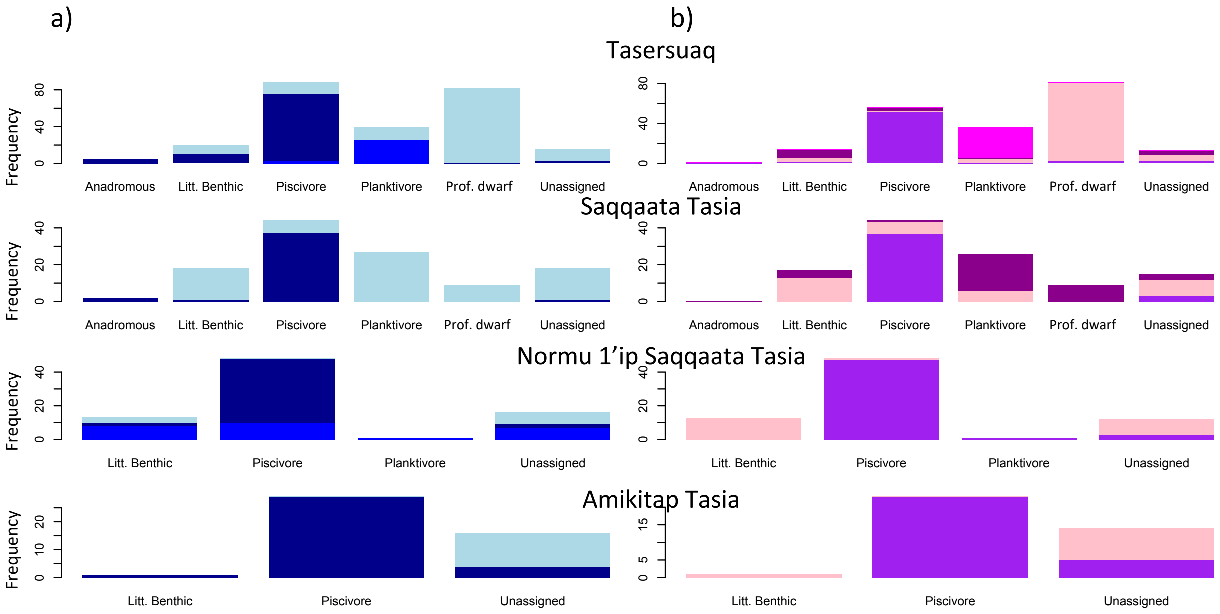


Figure S10. Comparison between ecomorph assignment and (a) size groups and (b) stable isotopic groups obtained from the mclust analysis for the four largest lakes of Eqaluit. X-axis indicates ecomorph groups, colors correspond to the groups obtained in the mclust analysis.

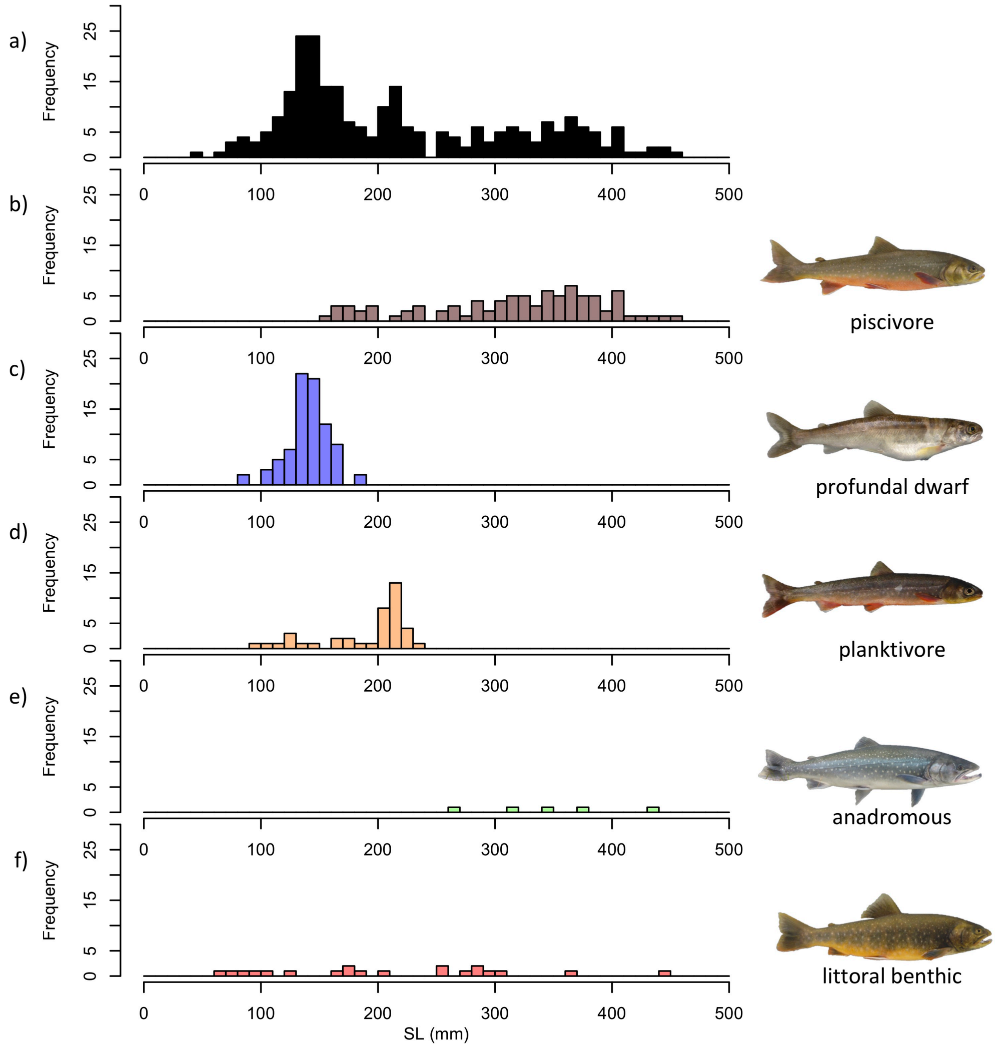


Figure S11. Histograms of body size for (a) all charr caught in Tasersuaq and (b-f) for different ecomorphs of that lake.

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