Title: *Diversity in the internal functional feeding elements of sympatric morphs of Arctic charr (Salvelinus alpinus)*

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Abstract

The diversity of functional feeding anatomy is particularly impressive in fishes and correlates with various interspecific ecological specializations. Intraspecific polymorphism can manifest in divergent feeding morphology and ecology, often along a benthic–pelagic axis. Arctic charr (*Salvelinus alpinus*) is a freshwater salmonid known for morphological variation and sympatric polymorphism. In Lake Þingvallavatn, Iceland, four morphs of arctic charr coexist that differ in preferred prey, behaviour, habitat use, and external feeding morphology. We studied variation in six upper and lower jaw bones in adults of the four morphs using geometric morphometrics and univariate statistics. We tested for allometric differences in bone size and shape among morphs, morph effects on bone size and shape, and divergence along the benthic-pelagic axis. We also examined the degree of integration between bone pairs. We found differences in bone size between pelagic and benthic morphs for two bones (dentary and premaxilla). We found clear bone shape divergence along a benthic–pelagic axis in four bones (dentary, articular-angular, premaxilla and maxilla), as well as allometric differences between morphs in the dentary. Most dramatic was the dentary, where morph explained more shape variation than bone size. Comparatively, benthic morphs possess a compact and taller dentary, with shorter dentary palate, consistent with visible (but less prominent) differences in external morphology. As these morphs emerged in the last 10,000 years, these results indicate rapid functional evolution of specific feeding structures in arctic charr. This sets the stage for studies of the genetics and development of rapid and parallel craniofacial evolution.

## Introduction

The origins and maintenance of biodiversity are strongly influenced by natural selection acting upon intraspecific variation with fitness consequences (Albert & Schluter, 2005; Pigliucci, 2008; Schluter & Conte, 2009), with many traits directly affecting survivorship and reproduction exhibiting extensive diversification. Adaptive evolution in vertebrates entails a substantial degree of variation in feeding behaviour and biomechanics of prey capture and processing that may lead to speciation (Bels et al., 1994; Gidmark et al., 2019; Schwenk, 2000). Morphological adaptations in functional feeding elements are associated with rapid diversification of ecology in many vertebrate groups, sometimes resulting in specialization on diverse prey types within lineages (Gidmark et al., 2019; Grant & Grant, 2003). The development of these feeding elements is influenced by an interplay of genes and environmental factors during ontogeny (e.g., Karagic et al., 2020). Additionally, developmental remodelling can accommodate ontogenetic niche shifts, for instance within fishes (Gidmark et al., 2019; Karagic et al., 2020), and may contribute to intraspecific and interspecific variation in adult morphology.

Extensive complexity and diversification of skull anatomy among fishes correlates with highly diverse feeding ecologies (Gidmark et al., 2019; Huby & Parmentier, 2019; Liem, 1993; Martinez et al., 2018). Evolution of several functional units (Huby & Parmentier, 2019) has given rise to a diversity of feeding methods (e.g., ram, suction, biting) for optimal foraging on specific prey types, varying widely within and among species (Huby & Parmentier, 2019; Liem, 1993). Independent evolution of oral jaws, hyoid “jaws” and pharyngeal jaws for prey capture and food processing likely contributed to trophic and morphological diversification (Gidmark et al., 2019). Radiations of ecomorphology and associated prey have occurred widely in fishes, including Pomacentridae (Cooper & Westneat, 2009), Labridae (Westneat et al., 2005), and Cichlidae (Mabuchi et al., 2007). While most studies investigate morphological variation among species or higher taxa (Huby & Parmentier, 2019; Konings et al., 2021; Liem, 1993; Martinez et al., 2018; Pauers & McMillan, 2014; Ronco et al., 2021), extensive variation in cranial morphology also exists within species (Kimmel et al., 2015; Pauers & McMillan, 2014).

Sympatric polymorphism, where two or more morphs within a species inhabit the same geographic area, has been found in many lacustrine fish species and seems to be promoted by vacant niches, habitat variance, differences in spawning preferences, and relaxation of interspecific competition (Markevich & Esin, 2018; Smith & Skúlason, 1996). Resulting morphological divergence is often due to differences in resource use, mainly in benthic vs. pelagic habitats, leading to specializations in traits related to foraging, prey capture and processing (Klemetsen et al., 2003). The occurrence of intraspecific morphs that utilize different resources (e.g., prey, habitats) has been widely documented in northern freshwater fishes inhabiting recently de-glaciated systems (Klemetsen et al., 2003; Skulason & Smith, 1995). Salmonid cranial morphology is less derived compared to more divergent groups (Goodrich, 1931; Kimmel et al., 2015), yet within this group many cases of sympatric morphological polymorphism have been described (Esin et al., 2018; Jacobs et al., 2019; Markevich & Esin, 2018; Muir et al., 2014). Arctic charr (*Salvelinus alpinus*) shows extensive phenotypic variation throughout its geographic distribution (Klemetsen, 2010) and sympatric polymorphism of lake populations is for instance found in Norway (e.g., Østbye et al., 2020; Skoglund et al., 2015), Siberia (e.g., Alekseyev et al., 2009), Greenland (e.g., Doenz et al., 2019) and Iceland

(Gíslason et al., 1999; Woods et al., 2013). In Iceland, the best example of sympatric polymorphism is Lake Þingvallavatn, Iceland's largest natural lake (Adalsteinsson et al., 1992). The lake formed ~10,000 years ago in a rift zone, and is currently a deep lake with lava rocks on many of its banks and shallows (Saemundsson, 1992). It was colonized by anadromous charr (Brachmann et al., 2021; Kapralova et al., 2011) that subsequently became isolated. The lake now hosts four morphs of arctic charr (Figure 1A and 1B), two benthic: large benthivorous (LB) and small benthivorous (SB) charr and two pelagic: planktivorous (PL) and piscivorous (PI) charr (Malmquist et al., 1985; Sandlund et al., 1992). The LB-, SB- and PL-charr are genetically distinct (Guðbrandsson et al., 2019; Kapralova et al., 2011), while the PI-charr is more heterogeneous genetically, with similarity to either LB- or PL-charr (Guðbrandsson et al., 2019). The morphs differ in adult size, diet, habitat use, life history, spawning times, and external and fin morphology (Jonsson et al., 1988; Malmquist, 1992; Malmquist et al., 1985; Sandlund et al., 1987; Skúlason, Snorrason, et al., 1989; Snorrason et al., 1989). Two are large (LB and PI) and two small (SB and PL), hereafter referred to as the morph size gradient. Benthic morphs live along the stony littoral bottom and feed mainly on snails (Malmquist, 1992), and have subterminal mouths (i.e., short lower jaw) and blunt snouts (Snorrason et al., 1994; Snorrason et al., 1989). Pelagic morphs more resemble anadromous charr, being more fusiform with a terminal mouth (i.e., long lower jaw) and pointed snout (Snorrason et al., 1994; Snorrason et al., 1989). PL-charr feed mostly on crustacean zooplankton, and the larger PI-charr feed mostly on three-spined stickleback (Malmquist, 1992; Snorrason et al., 1994). Most studies on arctic charr diversity have examined external morphology, except studies of gill raker counts in adults (Snorrason et al., 1989), fin ray variation in lab reared juveniles (Eiríksson et al., 1999) and an unpublished study of internal feeding elements in adults (Ingimarsson, 2002). Notably Ingimarsson (2002), using linear measures, found differences among morphs in maxilla and dentary bone shape, relative size of several skull elements, number of teeth on the dentary and palatine bones, as well as the angle of teeth on the maxilla, with divergence mainly along the benthic–pelagic axis. The specific adaptations in cranial morphology by morph and the degree of integration of variation in bone shape are understudied.

To investigate the functional morphology and impact of prey specialization during divergence of arctic charr along the benthic-pelagic axis (Noakes, 2008; Woods et al., 2013), we study cranial morphology in adults of the four sympatric charr morphs in Þingvallavatn. To test for morphological divergence and integration in internal feeding structures among the morphs, we examined variation in the shape of six cranial bones in the feeding apparatus: dentary, articular-angular, quadrate, premaxilla, maxilla and supramaxilla (Figure 1C). Specifically, we ask: 1) Is there an allometric component for size or shape of specific bones, and does this vary by morph? 2) Does the shape of any of those six bones differ among morphs? 3) Does morphological differentiation occur along a benthic-pelagic axis? 4) Does integration differ among pairs of bones, between the upper and lower jaw, and across morphs?

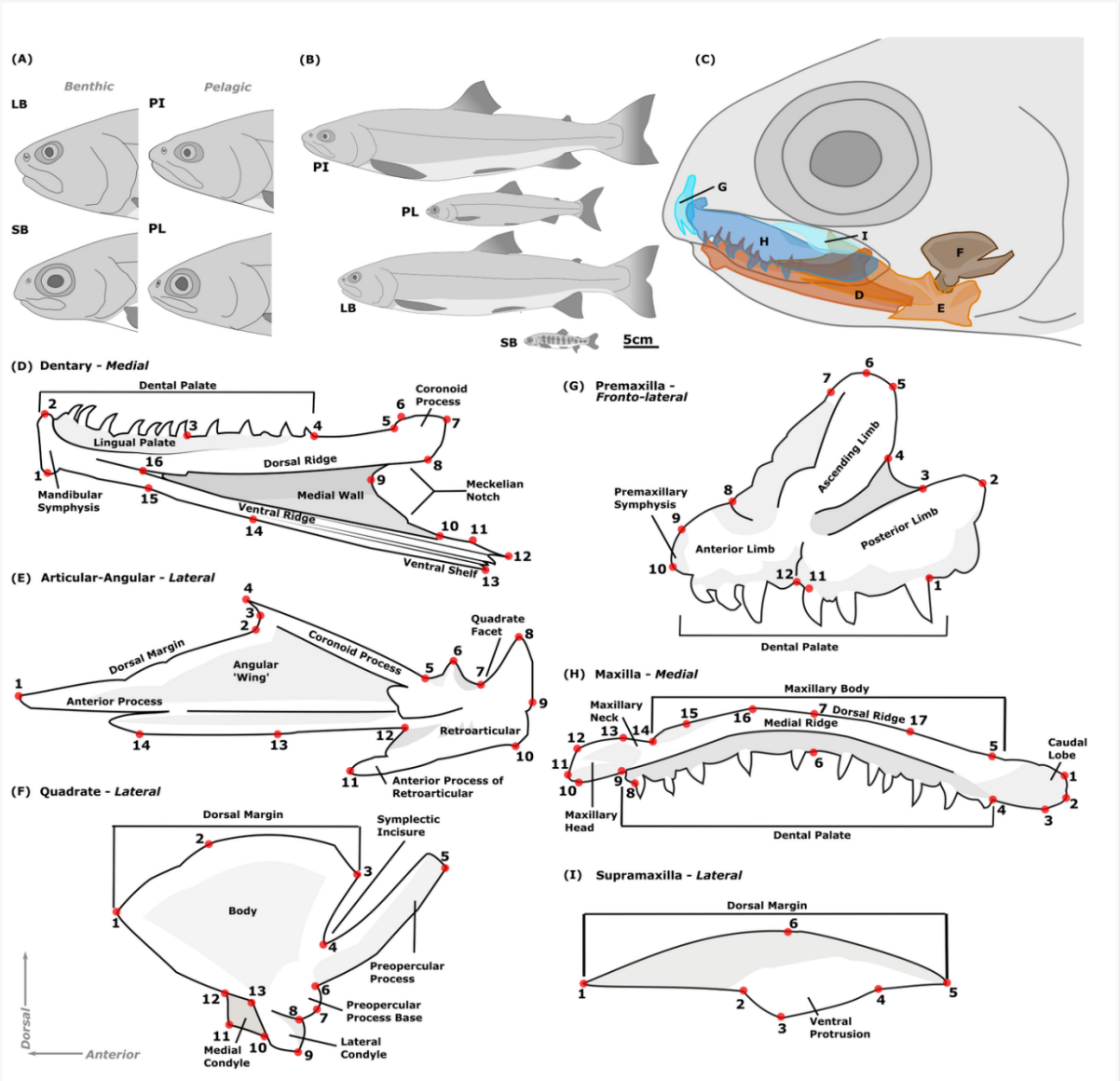


*Figure 1: External and internal morphology of Þingvallavatn arctic charr ecomorphs. A) Craniofacial variation; B) Postcranial shape and size variation; C) Primary upper and lower jaw bones studied; D – I) Anatomy of the upper and lower jaw bones with landmarks used to capture shape.*

## Methods

### *Sample and data collection*

Sexually mature arctic charr from Lake Þingvallavatn were collected (Fall 2020/21) using composite nets and seine nets (Online Resource 1). Fish were randomly sampled, except we aimed for similar representation of morphs and sexes (LB: 49 (21%), SB: 82 (34%), PL: 61 (25%) and PI-charr: 48 (20%)). Each fish was measured (weight, fork length (FL)), and photographed from left lateral view (Canon EOS 77D, EFS 18-135 mm lens), and sex, sexual maturity, and stomach contents were evaluated by dissection. Morph classification was based on sampling location

and date (morphs differ in spawning times and location), external morphology and stomach contents (Jónsdóttir, 2022; Skúlason, Noakes, et al., 1989; Snorrason et al., 1989). Otoliths were extracted from each fish and age determined under 4X magnification in a stereomicroscope. Six jaw bones (dentary, articular-angular, quadrate, premaxilla, maxilla, supramaxilla) were extracted using maceration protocols (Jónsdóttir, 2022) and photographed (Canon EOS 77D, 50 mm Microlens). The bones from the left side of the head were arranged stereotypically: the dentary and maxilla were photographed and landmarked in medial view, the articular-angular, quadrate and supramaxilla in lateral view, and the premaxilla in fronto-lateral view. To capture external morphology for morph classification, 37 landmarks (see Online Resource 2) were digitized for each individual using *tpsdig2* (http://www.sbmorphometrics.org/soft-dataacq.html). To investigate shape variation in internal feeding structures, we registered 2D landmarks on the six jaw bones with *tpsdig2* (Figure 1D-I: Online resource 3). All images of fish and bones are available on Figshare (XXX and YYY).

*Statistical analyses*

Landmark data were analysed using the R package *geomorph* version 4.0.0 (Adams et al., 2022; Baken et al., 2021). Landmark coordinates were aligned using Generalized Procrustes Analysis (GPA) via the function *gpagen*. Aligned shape variables were then used for all subsequent analyses. Outliers were identified using *plotOutliers* and removed if bone was damaged. Centroid size (CS) was calculated separately for each of the six bones ($CS_{bone}$; indicates use of CS of a bone, and applied to each bone separately), head ($CS_{head}$) and whole-body ($CS_{body}$). As size is an important predictor for shape in fishes (Frédrich & Sheets, 2009; Zelditch et al., 2004) and the morphs are known to differ in adult size and proportions (Sandlund et al., 1992), absolute size and the relationship between $CS_{body}$ and $CS_{head}$ as well as their relationship with each bone ($CS_{bone}$) were examined. We tested for differences in size and allometry of size measures between morphs using Procrustes ANCOVA using the function *procD.lm*. As salmonid morphology is influenced by sex and age (de la Cámara et al., 2023; Pakkasmaa et al., 1998), we investigated the influence of these variables on morph mean and allometry for size and shape. We then used pairwise comparisons using the *pairwise* function to test for significant differences between groups. External morphology (whole-body and head shape) was then examined using Procrustes ANOVA to characterize morph differences.

We tested for bone shape differences among morphs and differences in allometric relationships of bone shape and bone size across morphs using Procrustes ANCOVA, using *procD.lm*, with $CS_{bone}$, morph and their interaction as independent variables. A null (shape = $CS_{bone}$), reduced model (shape = $CS_{bone}$ + morph) and full model (shape = $CS_{bone}$ x morph) were compared to determine best fit. When the full model had the best fit, differences in allometric trajectories were investigated using *pairwise* with $CS_{bone}$ as a covariate. We tested for significant differences in both vector angles (vector correlation) and vector lengths (rate of shape change per unit covariate), considering significance among both tests as robust support for biological differences. For all tests $\alpha = 0.008$ was chosen, using the Bonferroni method as a conservative correction for multiple comparisons (six bone datasets). Additionally, we considered comparisons with Z scores <3 as poorly supported, and interpreted results below that as not biologically meaningful and chose the reduced model for further interpretation. Differences in allometry among morphs were investigated using *plotAllometry* and visualized using predicted values from regression analyses. Principal

Component Analysis (PCA, using *plotTangentSpace*) was used to visually represent shape variation among individuals and morphs. For bones that did not show significant differences in allometry among morphs, residuals from a regression of shape on size (size-corrected shape variables) via *procD.lm* were used for visualization. Shape variation along PC axes was summarized with deformation grids and vector plots from *shape.predictor*.

To analyze integration among bones, pairs of bones were compared using two-block partial least squares (PLS) analysis with the function *two.b.pls*. We compared elements within and among bones of the upper and lower jaws. The effect sizes were compared to determine if there was significant variation in levels of integration across different bone pairs or morphs using *compare.pls*. For all analyses we used randomized residuals in 1000 permutation procedures to assess statistical significance and estimate the effect sizes (*RRPP* implemented in *geomorph*, (Adams & Collyer, 2017). For integration tests $\alpha = 0.003$ was chosen as a conservative threshold (Bonferroni correction for 15 pairwise comparisons among bones). Figures were generated using R and formatted using Inkscape.

## Results

### *Differences in body size, sex and age of sympatric morphs*

To capture the morphological diversity in the four sympatric morphs from Lake Þingvallavatn we studied 240 sexually mature individuals. There was a significant difference between morphs in fork length (FL, F: 350.94, $p << 0.001$), log weight (F: 381.28, $p << 0.001$) and age (F: 56.75, $p << 0.001$, Online Resources 4, 5 and 6). SB- and PL-charr were smaller at sexual maturity than the larger LB- and PI-charr. The weight was influenced by sex ($p < 0.001$), and there was a significant sex by morph interaction ($p = 0.009$). The external whole-body ($p = 0.001$) and head ($p = 0.001$) shape differed by morph, with pelagic morphs having a more fusiform body shape and a more pointed snout (Online Resource 7). There was significant sexual dimorphism in whole-body shape ($R^2 = 0.04$, $p = 0.001$, Online Resource 8), with males having a more humped back compared to females (not a craniofacial trait). These results corroborate morphological descriptions of the morphs (Snorrason 1989) and known sexual dimorphism (de la Cámara et al., 2023). There was weak sexual dimorphism ($R^2 = 0.01$, $p = 0.007$) for head shape and all six bones ($R^2 \leq 0.02$, Online Resource 8) that will not be discussed further. Age was strongly correlated with FL ($R^2 = 0.7$), but always explained a lower proportion of variation as compared to FL (for external shape, bone size and shape variation). Therefore, FL was used as a covariate in allometry analyses of bone size relative to body size.

### *Allometry of bone size and shape*

Predictably, the size of all bones ($CS_{bone}$) increased (strong association) with body length ($\log_e$ FL, $R^2 \geq 0.94$, $p = 0.001$, Figure 2 and Online resource 6 and 9). Morph and morph by size interaction, while significant, accounted for a small fraction of the variation ($R^2 \leq 0.04$, Online Resource 6). Additional testing found small differences in size allometry between pairs of morphs (Online Resources 10 and 11). The differences in bone size ($CS_{bone}$) mostly reflected differences between larger (PI-charr) and smaller (SB-, PL-charr) morphs. PI-charr have significantly larger dentary bones than all other morphs ($p = 0.001$), as well as significantly larger articular-angular, maxilla, and supramaxilla bones than both LB- and PL-charr ($p < 0.002$). PL-charr also have a significantly smaller quadrate,

premaxilla and supramaxilla compared to SB-charr (p = 0.001). As $CS_{bone}$ was highly correlated with body size ($CS_{body}$ and $log_e$ FL) and a common allometric relationship was found among morphs, we used $CS_{bone}$ as size measures in subsequent analyses.

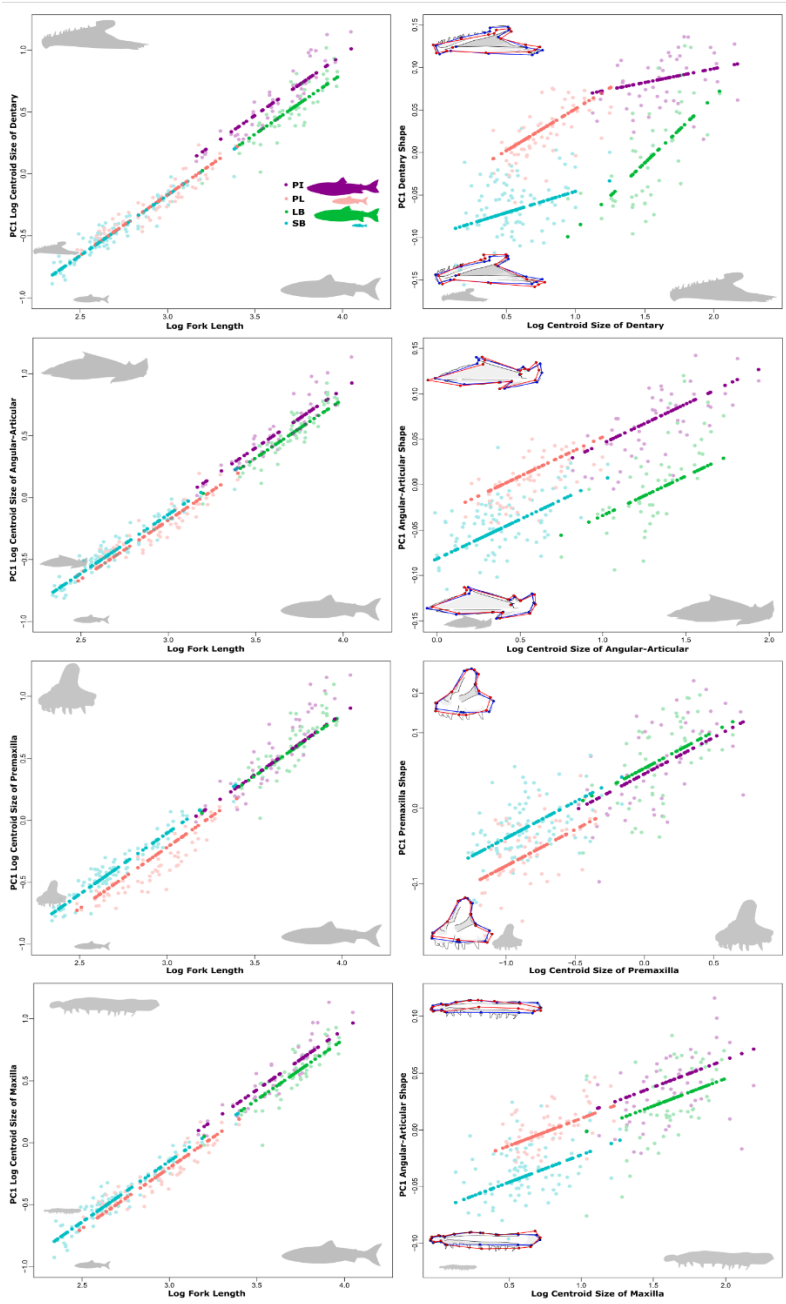


*Figure 2: Size and shape allometry of dentary, articular-angular premaxilla and maxilla (from top to bottom, inset pictures of each bone). Left panels: relationships between bone size and fork length by morph; Right panels: relationships between bone shape and bone size. Shown are values for individuals (open circles) and the predicted values (filled) of regressions for both bone size ($CS_{bone}$) vs body size ($log_e$ FL) or bone-shape versus bone-size. $Log_e$, natural log transformation.*

For all bones, shape was also significantly affected by size ($CS_{bone}$), the allometric relationship explained 20-30% of shape variation for dentary, articular-angular, quadrate, and maxilla ($R^2$ = 0.2-0.3, Table 1, for premaxilla and supramaxilla, bone size only explained 10-15%). Except for the quadrate, the allometry of all bones varied by morph (p = 0.001, Table 1), though the effects were small for maxilla and supramaxilla. Furthermore, comparisons of vector correlation angles and lengths indicated that only in the dentary did the allometric relationships vary markedly by morph (Online Resources 12, 13 and 14). For the dentary, while the common allometry and morph effects on shape were much larger than the interaction, there was moderate support for allometric differences among several morph pairs (Online Resource 12). Specifically, the PI-charr differed significantly in allometric trajectory orientation from all other morphs ($p \geq 0.003$), converging on a similar shape as LB-charr at larger size. We also found that while the allometric trajectories of LB- and SB-charr were parallel, the rate of shape change was higher in LB- compared to SB-charr (p = 0.001). Below we present the mean shape differences among morphs using the full model ($CS_{bone}$ x morph) for the dentary but for all other bones the reduced model ($CS_{bone}$ + morph) results are reported, as both common allometry and morph affected shape variation significantly (p = 0.001). All visualizations, unless otherwise noted, have been corrected for size to remove the effects of allometry on shape. We focus first on the lower jaw bones and then the upper jaw.

*Table 1: The influence of size (bone centroid size) and morph on shape variation in 6 jaw bones, analysed with Procrustes ANCOVA, testing for main and interaction terms. For all bones, except quadrate, was the full model with interaction the best fit.*

| Bone Shape | $Log_e$ CS Effect | | | | Morph Effect | | | | Morph x $Log_e$ CS Interaction Effect | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | F | Z | P | $R^2$ | F | Z | P | $R^2$ | F | Z | P |
| Dentary | 0.237 | 107.58 | 5.29 | **0.001** | 0.228 | 34.49 | 6.35 | **0.001** | 0.026 | 3.89 | 5.09 | **0.001*** |
| Articular-angular | 0.190 | 72.95 | 5.26 | **0.001** | 0.184 | 23.43 | 7.43 | **0.001** | 0.020 | 2.61 | 3.56 | **0.002** |
| Quadrat | 0.298 | 102.95 | 6.32 | **0.001** | 0.020 | 2.35 | 2.98 | **0.002** | - | - | - | - |
| Premaxilla | 0.165 | 55.15 | 9.14 | **0.001** | 0.110 | 12.27 | 9.53 | **0.001** | 0.030 | 3.33 | 5.19 | **0.001** |
| Maxilla | 0.199 | 67.56 | 7.75 | **0.001** | 0.096 | 10.87 | 7.54 | **0.001** | 0.022 | 2.53 | 2.83 | **0.001** |
| Supramaxilla | 0.099 | 27.66 | 6.27 | **0.001** | 0.074 | 6.90 | 5.29 | **0.001** | 0.027 | 2.55 | 2.75 | **0.003** |

*Pairwise tests (Online Resource 13 and 14) indicated only the dentary had true differences in allometry by morph.

$R^2$ = coefficient of determination; $F$ = F-statistic; Z = Z-statistic; P = P-values, ≤ 0.008 in bold (Bonferroni correction).

*Divergence in shape of lower jaw bones*

For the dentary and articular-angular, the morph effect on shape variation was similar in magnitude to the common allometric effect (20-30% of shape variation, Table 1). In contrast, the morph effect on quadrate shape was very low relative to the other bones ($R^2 = 0.02$) and weakly supported ($Z < 3$, Table 1). Pairwise tests for mean quadrate shape differences among morphs and visualizations (Table 2 and Online Resource 15) revealed no strong shape divergence among morph pairs and will not be discussed further.

Pairwise tests on morphs revealed differences in dentary shape between all morph pairs across the benthic-pelagic axis, except among the two smaller morphs (SB- and PL-charr, $p = 0.119$, Table 2). Also, the comparison PI-SB-charr was weakly supported ($Z < 3$). No significant shape differences were found within morphotype (LB-SB-charr, PL-PI-charr). The dentary shape differences by morph were visualized using PCA (Figure 3), and due to variation in allometry among morphs, size effects were not removed. PC1 (50% of the total variation) represents elongation of the dentary bone, with positive values representing a more elongate shape, with extension of the dental palate containing the teeth, and negative PC1 values representing a compact shape with reduction in the length of the dental palate. Positive values were also associated with a more acute angle between the dorsal and central ridges, reducing the size of the Meckelian notch, as well as a more vertical inclination of the lingual palate. The morphs separated along a benthic-pelagic axis on PC1, with pelagic morphs, on average, having an elongated dentary, increased length of the dental palate, more acute angle between ridges, and vertically inclined lingual palate relative to benthic morphs. PC2 explained 13% of the total variation, and mainly split the morphs by size (the larger morphs (LB-, PI-charr) with positive values and taller bones than small morphs (SB-, PL-charr)). The associated shape changes mainly affected the height of the whole bone, with negative PC values associated with thinner bones overall, reduced height of the mandibular symphysis, coronoid process, and ventral shelf. Together, PC1 and PC2 separated all morphs clearly in shape space, but there was some overlap among groups (possibly due to overlapping sizes of particular individuals and/or more size variation within LB-charr).

*Table 2: Morph differences in bone shape estimated by pair-wise distances in mean shape for morphs, from the best fit ANCOVA models for each bone (Table 1).*

| Bones | Morph pairs | *d* | UCL (95%) | Z | P-value |
|---|---|---|---|---|---|
| Dentary | LB-PI | 0.176 | 0.129 | 3.91 | **0.001** |
| | SB-PL | 0.106 | 0.110 | 1.24 | 0.119 |
| | LB-PL | 0.154 | 0.131 | 3.15 | **0.001** |
| | PI-SB | 0.131 | 0.117 | 2.65 | **0.005** |
| | LB-SB | 0.070 | 0.072 | 1.47 | 0.070 |
| | PI-PL | 0.042 | 0.051 | 0.60 | 0.270 |
| Articular-angular | LB-PI | 0.088 | 0.023 | 6.77 | **0.001** |
| | SB-PL | 0.063 | 0.022 | 4.43 | **0.001** |
| | LB-PL | 0.104 | 0.035 | 5.12 | **0.001** |
| | PI-SB | 0.059 | 0.038 | 3.04 | **0.001** |
| | LB-SB | 0.055 | 0.039 | 2.72 | **0.005** |
| | PI-PL | 0.038 | 0.035 | 1.98 | 0.021 |
| Quadrat | LB-PI | 0.024 | 0.025 | 1.54 | 0.065 |
| | SB-PL | 0.022 | 0.021 | 1.73 | 0.045 |
| | LB-PL | 0.037 | 0.037 | 1.58 | 0.050 |
| | PI-SB | 0.048 | 0.038 | 2.65 | **0.006** |
| | LB-SB | 0.038 | 0.04 | 1.43 | 0.080 |
| | PI-PL | 0.046 | 0.035 | 2.91 | **0.004** |
| Premaxilla | LB-PI | 0.094 | 0.036 | 5.62 | **0.001** |
| | SB-PL | 0.087 | 0.030 | 6.30 | **0.001** |
| | LB-PL | 0.086 | 0.054 | 3.70 | **0.001** |
| | PI-SB | 0.098 | 0.053 | 4.56 | **0.001** |
| | LB-SB | 0.034 | 0.054 | -0.38 | 0.653 |
| | PI-PL | 0.049 | 0.053 | 1.31 | 0.090 |
| Maxilla | LB-PI | 0.024 | 0.019 | 2.35 | 0.010 |
| | SB-PL | 0.051 | 0.017 | 5.93 | **0.001** |
| | LB-PL | 0.053 | 0.028 | 4.06 | **0.001** |
| | PI-SB | 0.048 | 0.031 | 3.28 | **0.001** |
| | LB-SB | 0.033 | 0.032 | 1.83 | 0.037 |
| | PI-PL | 0.050 | 0.028 | 4.04 | **0.001** |
| Supramaxilla | LB-PI | 0.022 | 0.038 | 0.002 | 0.490 |
| | SB-PL | 0.083 | 0.033 | 4.60 | **0.001** |
| | LB-PL | 0.061 | 0.056 | 1.89 | 0.028 |
| | PI-SB | 0.102 | 0.061 | 3.41 | **0.001** |
| | LB-SB | 0.096 | 0.059 | 3.37 | **0.001** |
| | PI-PL | 0.051 | 0.056 | 1.33 | 0.092 |

UCL = upper confidence level; Z = Z-statistic; P-values ≤ 0.008 in bold (Bonferroni correction)

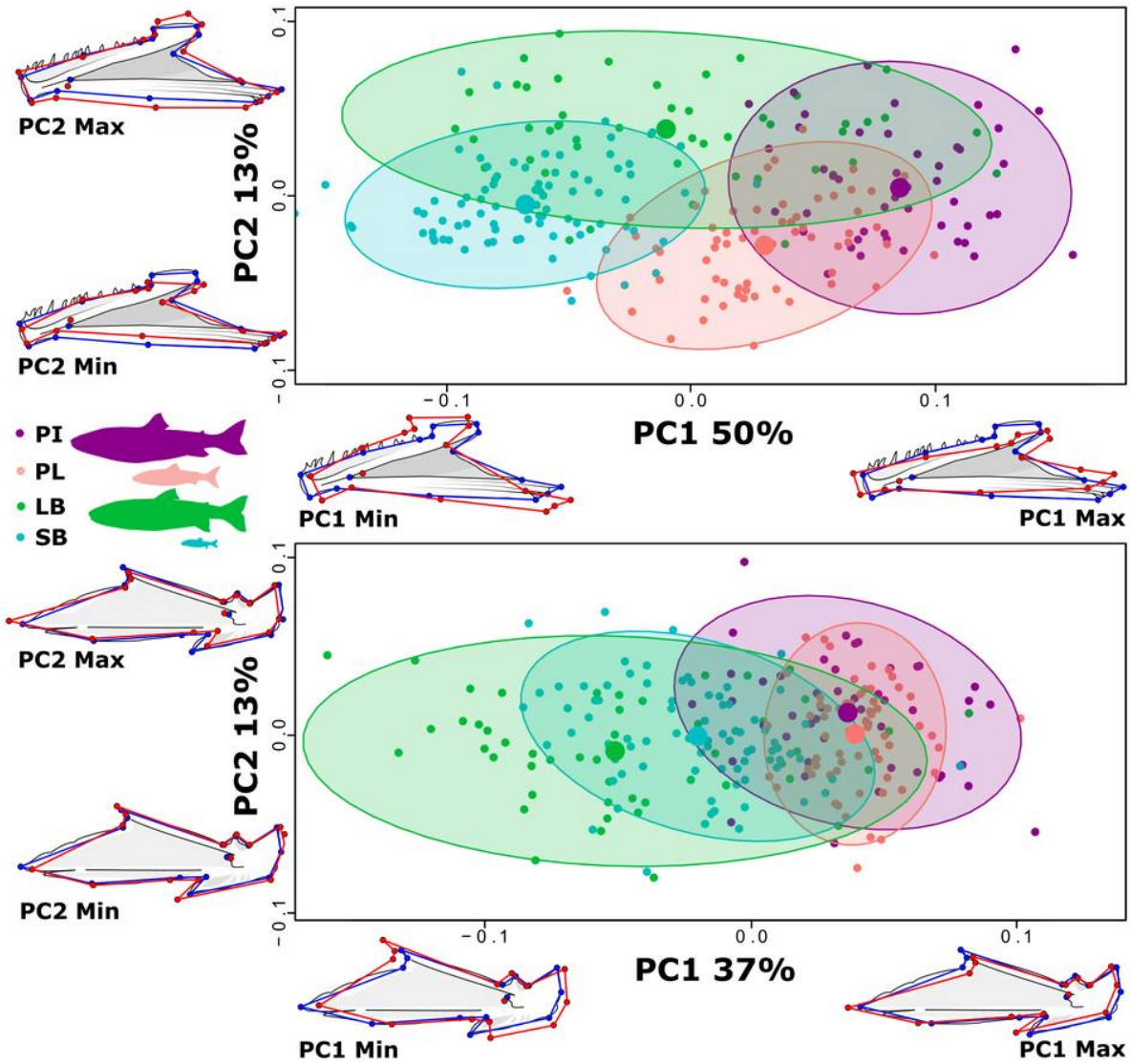


*Figure 3: Strong morph effects on shape variation in dentary (top) and notable on articular angular (bottom). For the dentary (top), size effects were not removed, but effects of size were removed for articular angular (and other bones, see below). PC-plots of specimens and deformation grids show how shape differences by morphs. None of the shape warps (X- and Y-axis) are magnified. Each dot represents an individual and the ellipses 95% CI for the distribution by morph (large dot represents the mean for each morph in these dimensions). PC1 and 2 explain 50% and 13% of the variation respectively. For the articular angular (bottom) PC1 and 2 explain 37% and 13% respectively.*

Pairwise tests for articular-angular shape also revealed differences between all morph pairs across the benthic-pelagic axis. Biologically meaningful differences were not found within morphotypes (Z<3, see Table 2). PC1 explained 37% of the variation and represents changes in relative sizes of the anterior process of the angular 'wing', retroarticular, and quadrate facet (Figure 3). Individuals with positive PC1 values show anterior extension of the

anterior process of the angular “wing”, increase in the size of the anterior process of the retroarticular but dorso-ventral compression of the retroarticular, and narrow quadrate facet. Negative values associate with a more compact shape overall, particularly with restrictions to the length of the anterior process of the angular "wing”, however the coronoid process is extended anteriorly, a more square retroarticular, and wider quadrate facet. The morphs aligned along a benthic-pelagic axis on PC1. The two pelagic morphs were largely overlapping (positive values), with elongated anterior process of the angular “wing”, shorter coronoid process, and narrower quadrate facet. The benthic morphs had more shape variation (especially the LB-charr, as in dentary) but had a more compact bone shape and wider quadrate facet overall, with LB-charr individuals being most compact. PC2 explains 13% of the variation and mainly represents differences in the ventral process of the retroarticular, with individuals with negative PC values with a shorter process. The morphs did not separate along PC2.

*Divergence in shape of upper jaw bones*

For the upper jaw bones the magnitude of the morph and size effects on shape were similar (~10-15%, Table 1) but notably lower than in the dentary and articular-angular. The morph effect was strongest in the premaxilla and weakest in the supramaxilla.

Pairwise tests for mean premaxilla shape differences among morphs indicated significant differences in all morph pairs across the benthic-pelagic axis ($p \leq 0.001$, Table 2). No differences were found between pairs within morphotype. PC1 explained 25% of the variation and captures morphological shape differences of the posterior and anterior limbs (Figure 4). Positive values associated with elongated and narrow posterior and anterior limbs, while negative values with more compressed and wider posterior and anterior limbs. The morphs separated along a benthic-pelagic axis on PC1. The PL- and PI-charr had positive PC1 values with elongated and narrow limbs, while SB- and LB-charr overlapped with more negative PC1 values, having compact and wide limbs. PC2 explained 14% of the variation, representing variation due to differences in the curvature of ascending limb but did not separate morphs.

For maxilla shape, pairwise tests of mean shape between morphs revealed significant separation between all morphs ($p = 0.001$), except the SB-LB-charr and LB-PI-charr (Table 2). Therefore, maxilla diverged neither along the benthic-pelagic axis nor the morph size gradient. PC1 explained 33% of the total variation, but it represents technical variation (either due to rotation or tilting of the bone during photographing, that was randomly distributed with respect to morph). PC2 explained 23% of the total variation, representing biological variation due to differences in the curvature of the maxillary head and angle of the maxillary neck (Figure 4), but did not separate morphs. Individuals with positive values exhibited posterior expansion of the caudal lobe and reduced length of the dental palate. The morphs separated on the benthic-pelagic axis on PC3 (10% of variation). The component represents the height of the maxilla (positive: tall and negative: short), but the distributions overlapped substantially. In combination, PC2 and PC3 indicate that PL-charr has reduced morphological variation and appears to have a thinner maxillary body and caudal lobe than the other morphs.

Pairwise comparisons of mean supramaxilla shape differences among morphs indicated SB deviated from all other morphs (p = 0.001, Table 2). PC1 and PC2 explained 62% of variation, but no combination of PCs revealed how SB-charr shape deviated from the other three morphs (Online resource 15). We note the morphological variation in SB appeared reduced relative to other morphs.

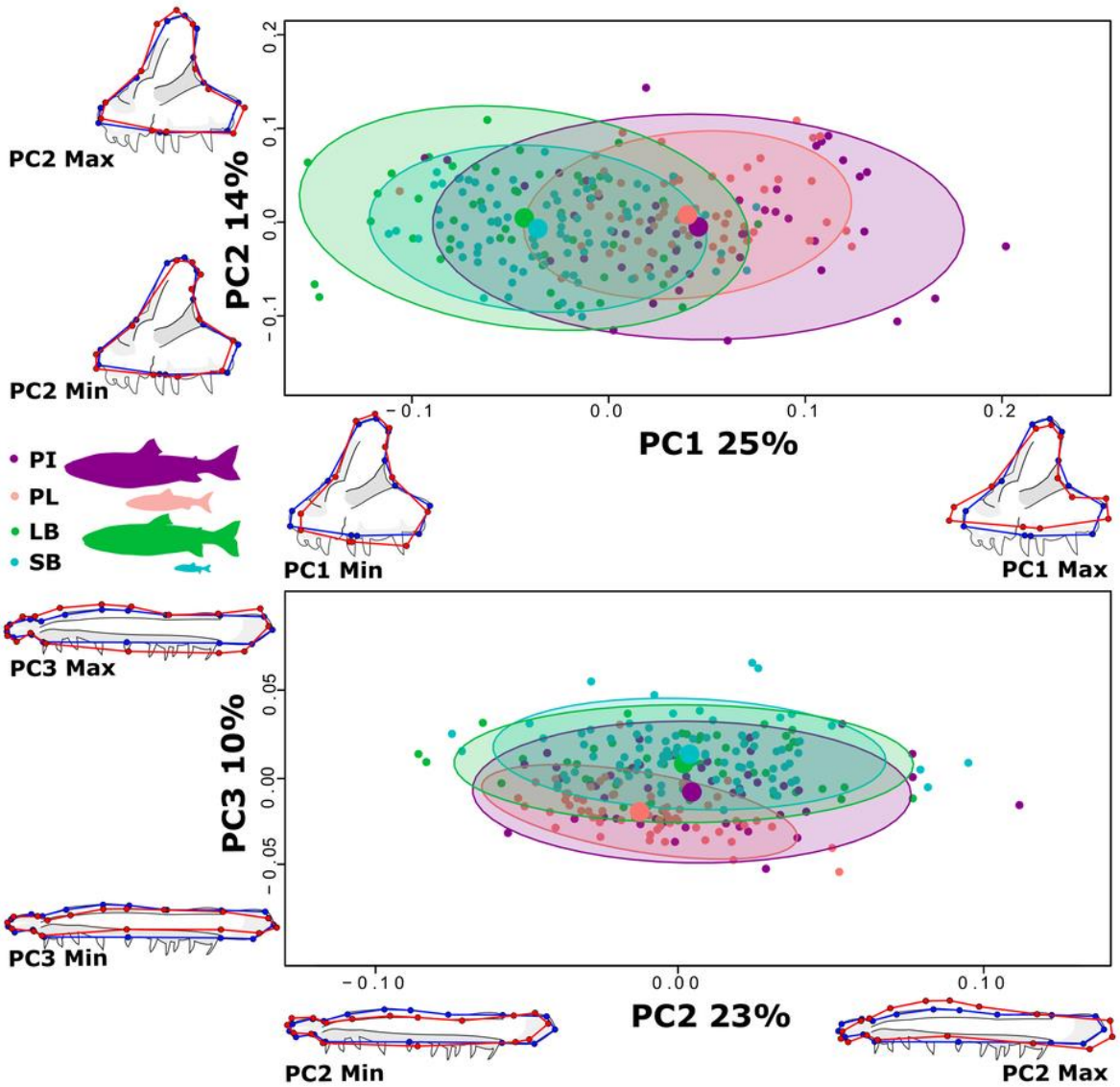


*Figure 4: Size corrected PC-plots of specimens and deformation grids showing how the morphs differ in mean (top) premaxilla and (bottom) maxilla shape. None of the shape warps (X- and Y-axis) are magnified. Each dot represents an individual and the ellipses represent 95% CI for the distribution by morph (large dot represents the mean of each morph in these two dimensions of shape). For premaxilla (top) PC1 and PC2 explain 25% and 14% of the variation respectively. For maxilla (bottom) PC1 and PC2 explain 23% and 10% respectively.*

*Covariation in shape change among bones*

Finally, we tested for covariation in shape among bones. Within the upper jaw and within the lower jaw, shape covaried significantly among all bones (p = 0.001). The degree of covariation was greatest for the dentary and articular-angular (r-PLS = 0.92; Z = 7.08, Online Resource 16) and much higher compared to the other pairs (r-PLS = 0.54-0.69). Among the jaws, all covariation among bones was significant (p = 0.001) and the degree of covariation was moderate (r-PLS ~ 0.54-0.78, Online Resource 16). We did not find significant differences in effect size among pairs in the lower jaw, however in the upper jaw effect size between the premaxilla and supramaxilla was found to be lower (p = 0.006) than all other comparisons, and effect size between the maxilla and supramaxilla was found to be higher (p = 0.002) (Online Resource 16). In addition, the data did not reveal significant differences in effect size between morphs for any of the bone pairs (comparisons limited to bones that showed significant covariation or biological importance as described above, quadrate and supramaxilla excluded, data not shown).

## Discussion

Evolutionary divergence along the benthic-pelagic axis occurs repeatedly among fishes (Burress et al., 2017; Friedman et al., 2021; Hulsey et al., 2013; Rincon-Sandoval et al., 2020). Body elongation is a consistent trend along the benthic-pelagic axis in marine and freshwater fishes (Friedman et al., 2021) as well as fin adaptations for sustained swimming in pelagic environments and manoeuvrability in benthic habitat (Astudillo-Clavijo et al., 2015; Rincon-Sandoval et al., 2020). Craniofacial responses to benthic and pelagic prey resources are much more complex (Buser et al., 2019; Cooper et al., 2010; Ferry-Graham et al., 2002; Gidmark et al., 2019). Prey specialization along this axis leads to morphological adaptation of multiple traits that can be either integrated or modular, with known examples of functional convergence via a range of morphological solutions (Alfaro et al., 2005). Salmonids are known for extensive variation in feeding specializations and morphology but to date few studies have investigated variation in internal structures among or within a salmonid species (except see, Eiríksson et al., 1999; Esin et al., 2018; Ingimarsson, 2002; Kapralova et al., 2015; Kimmel et al., 2015; Küttner et al., 2013; Muir et al., 2014). Notably, in addition to pioneering work of Ingimarsson (2002), one study reported differences in dentary and articular-angular shape in parr, between several salmonid species and also within rainbow trout (*Oncorhynchus mykiss*, Kimmel 2015). We report morph differences in body, head and bone shape, with bones showing more pronounced differences. The whole-body and head shape differences by morph corroborates previous studies (de la Cámara et al., 2023; Snorrason et al., 1989). We examined six cranial bones tied directly to feeding and prey capture in recently evolved sympatric morphs of arctic charr and found the strongest benthic-pelagic contrast in the most anterior bones in both jaws (lower: dentary and upper: premaxilla). These are bones that interact directly with prey, but shape differences were also seen in the articular-angular and maxilla. For the dentary, allometry also varies by morph, and dramatically the morph effects (benthic-pelagic axis, on PC1) were stronger than the effects of morph size (PC2). In contrast, the shape of the posterior bones, quadrate and supramaxilla, did not vary strongly between morphs. We discuss these results in the context of functional feeding morphology and its mechanics, the putative

genetic and environmental sources of this sympatric diversity and suggest future studies of craniofacial diversity in salmonids.

*Bone shape variation and functional divergence*

We found divergence along the benthic-pelagic axis in several cranial bones. We found clear allometric components for shape variation in all six bones, but allometry only varied between morphs for the dentary. This may indicate dentary shape variation is more strongly influenced by morphotype, driven primarily by ecological function, than size differences between morphs. Here we focus on cranial bone shape and potential relationships to prey and mechanics of prey capture, but acknowledge that postcranial divergence allows access to preferred prey habitat but also aids in locating, approaching, and capturing individual prey (Gidmark et al., 2019; Rincon-Sandoval et al., 2020).

While adult morphology should reflect long-term prey consumption (e.g., higher pelagic prey consumption associates with stronger pelagic features), variation in the mechanisms of capturing and processing of different prey items within and between sympatric charr morphs, and potential impacts on morphological variation, is not well understood. To our knowledge biomechanical studies of salmonids have not yet been done on fishes that specialize on such divergent prey as snails and zooplankton. Pelagic feeding has been compared between PL- and SB-charr, revealing reduced efficiency of zooplankton feeding in SB-charr juveniles due to lower attack rates (Malmquist, 1992), increased handling time (Skúlason, 1991), increased ejection of prey (Skúlason, 1991) and better retention in the branchial apparatus likely due to longer gill rakers (Malmquist, 1992). Both morphs likely capture zooplankton using a similar strategy, i.e., suction (Malmquist, 1992), however may exhibit differences in capture efficiency as a result of morphological differentiation associated with ecological divergence. Prior studies are limited to biomechanics of piscivorous feeding in brook trout (*Salvelinus fontinalis*) (Camp et al., 2009) or theoretical models of suspensorium and lower jaw movement (Olsen & Westneat, 2016). Salmonid jaw elements are likely to be largely immobile due to rigid attachments of the components (Gromova & Makhotin, 2016). Therefore, the use and efficiency of suction is likely limited compared to pelagic fishes with highly mobile jaws (Westneat, 2005), and perhaps not highly differentiated among arctic charr morphs.

Benthic-pelagic divergence can be associated with divergence in lower jaw length relative to head length (Cooper et al., 2010), as seen in subterminal mouths of benthic arctic charr (Kristjánsson et al., 2012; Sandlund et al., 1992; Snorrason et al., 1989). Shelled freshwater snail (*Lymnaea peregra*) is the main prey of the derived SB- and LB-charr in Þingvallavatn (Malmquist, 1992; Sandlund et al., 1992). In general, hypertrophied pharyngeal jaws (Huckins, 1997; Lauder, 1983; Osenberg et al., 2004; Wainwright, 1991, 1996), molariform pharyngeal teeth (Burress, 2015; Keast, 1978), and decreased gill raker length and density (Amundsen et al., 2004; Malmquist, 1992; Snorrason et al., 1989; Werner & Hall, 1976) are all morphological adaptations widely employed across fishes that consume large quantities of hard-shelled benthic prey. Additionally, mechanisms for capturing attached prey vary widely among species (Copus & Gibb, 2013; Ferry-Graham et al., 2008; Ferry-Graham et al., 2002). Use of strong suction, gripping and biting, lateral movements, and highly protrusible jaws are commonly utilized traits (Copus & Gibb, 2013; Ferry-Graham et al., 2008; Ferry-Graham et al., 2002). However, details of the intersection of these

biomechanical and behavioural components for feeding on divergent prey resources in Þingvallavatn morphs is currently unexplored.

While we expected adaptions to divergent prey resources to be expressed in shape divergence of cranial elements among morphs, other morphological modifications can occur that do not impact bone shape as studied here. Internal bone size and architecture has been shown to diverge along the benthic-pelagic axis, with benthic feeders exhibiting more robust hollow cranial bones with complex internal structure, similar to mammalian long-bones, and planktivores having thin solid bones (Albertson et al., 2012). Bones of benthic scrapers also have higher rates of mineralization (Albertson & Kocher, 2001) and are more resistant to bending (Albertson et al., 2012). We found benthic morphs (LB- and SB-charr) to have thicker and more robust bones overall (relative to PL- and PI-charr), but variation in their internal structure and mineralization is unknown. The differences in adult bone size and shape found here may associate with differentiation in muscle mass and surface area of attachment sites, and as skeletal and musculature may contribute independently to biomechanical diversity (Roberts et al., 2018), adaptive changes to soft tissues may both be more critical in foraging divergence (i.e. proportionally larger impact on biomechanics of feeding related to speed, force, and mode of prey capture) and be more apparent than associated changes in bone shape in these morphs. Given our focus on cranial bone shape and difficulty in comparing salmonids to other groups (e.g., cichlids or wrasses), we speculate on functional outcomes of cranial bone divergence among morphs.

We found strong divergence in the lower jaw along the benthic-pelagic axis. The characteristic subterminal mouth of the benthic morphs associates with a shortening of the dentary and articular-angular (relative to the quadrate facet). Specifically, the anterior region of the dentary around the mandibular symphysis is also angled ventrally relative to pelagic fishes (Figure 3). This may aid in creating a more optimal angle to grip attached prey or perform scraping behaviours on a surface to dislodge prey, such as snails. The benthic morphs may use a combination of biting and ramming behaviours, as suction forces are likely weak. Taller dentary and articular-angular bones could be adaptations for feeding on benthic prey, increasing size of the mouth cavity and efficiency of consuming benthic organisms (Saltykova et al., 2015). The coronoid processes of the dentary and articular-angular are enlarged in benthic charr, which could associate with a larger mandibular portion of the adductor mandibulae and a larger aponeurosis maxillaris (Gromova & Makhotin, 2016), thus allowing for greater bite forces in benthic morphs which could aid in dislodging attached prey items (Copus & Gibb, 2013; Ferry-Graham et al., 2008; Ferry-Graham et al., 2002). Relative elongation of the lower jaw is expected in pelagic morphs. A previous study on lower jaw bone shape in juveniles of *O. mykiss* found the anadromous population (considered to have a pelagic lifestyle) possessed a more elongated and streamlined lower jaw than the resident population (Kimmel et al., 2015). The authors speculated that elongated, streamlined lower jaws could be adaptations to the pelagic ocean environment.

In the upper jaw, we found premaxilla shape to diverge along the benthic-pelagic axis more strongly than the maxilla. Relative to SB-, PL-charr had smaller premaxilla overall, with shorter ascending limbs and narrow anterior and posterior limbs. Reductions in the height of the ascending process and elongation of the anterior-posterior axis of the premaxilla has been found in other pelagic fishes (Buser et al., 2019; St. John et al., 2022; Yabe,

1985) that use ram feeding in the water column (Norton, 1995) while suction feeders with highly protrusible jaws have long ascending limbs (Cooper et al., 2017). Moreover, shortening and steeper angles of the ascending limb of the premaxilla can also be associated with increased biting forces (Witte, 1983) and possibly linked to biting behaviours. The ascending limb of the premaxilla has been found to extend more dorsally in pelagic fish (Munyandamutsa et al., 2021) and in highly protrusible jaws (Hulsey et al., 2010), however, in our study the ascending limb was proportionally longer in the benthic morphs. As the premaxilla, and the skull as a whole, is relatively rigid in salmonids (sensu Gromova & Makhotin, 2016), the biomechanics of similar morphological changes in fishes with protrusible jaws could be incomparable. We also note feeding mode (e.g., ram, suction) is not characterized in these morphs. For the maxilla, it was the third shape axis that associated with the benthic-pelagic axis (as in the premaxilla and dentary). The maxilla is generally involved in the protrusion of the upper jaw maxillary apparatus, but such function may not be present in salmonids (Gromova & Makhotin, 2016) and the proposed movement of these components to aid in feeding (Sanford, 2000) has been criticized (Gromova & Makhotin, 2016). However, the maxilla is a movable element in the upper jaw in salmonids, however, impacts on feeding (e.g., suction efficiency) have not been investigated.

The quadrate nor supramaxilla did not diverge along a benthic-pelagic axis (but showed some weak morph differences). While shape variation in them could reflect biomechanical differences due to size (Herrel & Gibb, 2006; Kolmann & Huber, 2009), they did not vary along a morph size gradient. In salmonids, the function of the supramaxilla (if any) is unknown. The morph differences in supramaxilla may reflect correlations with linked bones (like the maxilla), as observed high levels of integration between these bones indicates. In contrast, the quadrate is a critical linkage in the suspensorium, its main function to allow rotation of the lower jaw. Since changes to the maximum angle of opening potentially needed to capture different prey would likely only require subtle morphological changes in this bone, it is unlikely we could detect these differences given our methods, or divergent selection may not act strongly on quadrate shape.

In other groups of fishes, strong benthic-pelagic ecological divergence has led to modification in morphology and behaviour to allow for optimal feeding performance (Berchtold et al., 2015; Cooper et al., 2017; Cooper et al., 2010; Ferry-Graham et al., 2002; Friedman et al., 2021; Friedman et al., 2020; Kusche et al., 2014; Munyandamutsa et al., 2021; Østbye et al., 2006; Rincon-Sandoval et al., 2020; Ronco et al., 2021; St. John et al., 2022). The degree to which internal morphology and behaviour has evolved concordantly (or discordantly) in arctic charr in Iceland is largely unknown. Clearly, investigation of the behavioural and biomechanical strategies individuals and morphs use to both capture and process prey, and bone shape in the same individuals, is needed to reveal how arctic charr specialize on such divergent prey types.

*Influence of extrinsic and intrinsic factors on bone variation within and among morphs*

The observed shape variation in the jaw bones within and between morphs could be influenced by differences in their ecology and food preferences (see above, Malmquist, 1992; Sandlund et al., 1987), genetic composition of the morphs (Guðbrandsson et al., 2018; Kapralova et al., 2011) and interactions of specific genes and environmental factors.

Adaptive plasticity may contribute to ecological and morphological divergence among sympatric and allopatric charr (Kristjánsson et al., 2018; Skúlason et al., 2019; Skulason & Smith, 1995). We studied wild caught individuals of the four morphs that mostly operate in different habitats and feed on different prey items that vary seasonally in abundance and nutrition, all factors that may influence their development and adult morphology (Malmquist, 1992; Sandlund et al., 1987; Snorrason et al., 1994). Furthermore, the morphs differ in the location, timing and synchronicity of spawning (Skúlason, Snorrason, et al., 1989). Thus, as juveniles they likely both receive different maternal provisions and develop in different conditions. Abiotic and biotic environmental factors could thus induce differences in bone shape by morphs. Arctic charr shows developmental plasticity in body shape and size in response to benthic vs. pelagic diet treatment (Kristjánsson et al., 2018; Parsons et al., 2011). Morphological differences between (and/or within) morphs could to some extent reflect this developmental plasticity. These adaptation can also be influenced by the behaviour associated with habitat choice, prey capture and processing (Berchtold et al., 2015). For example, in sheepshead wrasse (*Archosargus probatocephalus*), individuals consuming a higher proportion of hard-shelled prey had larger adductor mandibulae and jaw bones but also employed biting mode of feeding to dislodge prey as well as buccal manipulations to crush shells and separate hard and soft parts before ingestion (Cutwa & Turingan, 2000). Similarly, preference for snail prey was associated with increased pharyngeal jaw size and muscles, specifically the levator posterior responsible for bite force of pharyngeal jaws (Lauder, 1983; Wainwright, 1996) across populations and individuals compared to more planktivorous counter parts (Berchtold et al., 2015). However, it must be noted that some of the environmentally induced variation in bone shape (plastic response) may not be adaptive (Ghalambor et al., 2007).

Genetic differences by groups can be assessed with experiments and population genetics. Common garden rearing of offspring of Þingvallavatn morphs indicate genetic influence on many aspects of juvenile and adult morphology (de la Cámara et al., 2023; Eiríksson, 1999; Eiríksson et al., 1999; Kapralova et al., 2015; Skúlason, Noakes, et al., 1989), though maternal and/or transgenerational parental effects also play a role (Leblanc et al., 2016). Three of the morphs (PL-, SB-, and LB-charr) are genetically separable (Kapralova et al., 2011) and whole genome methods revealed substantial allele frequency differences between them on nearly all linkage groups (Brachmann et al., 2021; Guðbrandsson et al., 2019). We saw the strongest morph differences in the dentary and premaxilla bones of the lower and upper jaws, respectively, that may reflect alleles influencing these traits specifically. Models indicate these three morphs have been separate for thousands of generations, with limited gene flow between them (Brachmann et al., 2021; Kapralova et al., 2011). Curiously, the PI-charr is genetically more heterogeneous, most individuals are genetically similar to PL-charr, but others have either LB-charr or mixed PL-/LB-charr ancestry (Guðbrandsson et al., 2019). One hypothesis is that PI-charr result from ontogenetic niche shifts of juvenile PL-charr that learn to catch sticklebacks, abandoning zooplankton feeding, a radical change which may allow individuals to attain larger size and develop piscivorous characteristics (Snorrason et al., 1989). The genetics (Guðbrandsson et al., 2019) suggest juvenile LB-charr and/or LB/PI hybrids may also shift from benthic feeding to piscivory. For most bones studied here, the PI-charr tended to align with PL-charr (consistent with ancestry), except in the maxilla where PI clustered closer to LB-charr. This heterogeneity of phenotypes in PI-charr could either reflect variation in genes, or the impact of piscivory on development of specific bones. Piscivory, a radical change in

lifestyle, and associated size increase may place high functional demands on bone shape (Collar et al., 2009) and enhance divergence in shape and even suppress morphological variation among piscivorous charr of different genetic origins. Admixture mapping of traits influencing bone shape variation in PI-, LB- and PL-charr might shed light on this, and more generally identify alleles and pathways of relevance for bone shape variation in salmonids.

*Interaction of extrinsic and intrinsic factors may influence shape bone variation among morphs*

Interactions between particular alleles and specific environmental factors, acting at specific times or over broader developmental windows (Hallgrímsson et al., 2019), may produce the observed morph differences in bone shape. Considering a hypothetical case, SB-charr may have alleles that influence the development of the dentary and articular-angular (lower jaw bones), that will induce the subterminal morphology if they eat snails as juveniles. Tests of such hypotheses require joint study of genetic and environmental influences on specific aspects of how bones develop and acquire their shape and functions. To distinguish between genetic and transgenerational environmental effects, crosses between morphs and multigenerational studies are needed. Genetic mapping can identify loci, genes or specific polymorphisms impacting variation in shape (Palsson et al., 2005; Pitchers et al., 2019; Zimmerman et al., 2000). Such approaches could enable examination of the sensitivity of shape-alleles to environmental factors, like specific juvenile food types (e.g., benthic or pelagic). Analyses of gene expression can also highlight variation in developmental pathways that differ by environment or morph. Indeed, RNA-sequencing of embryos (PL-, LB- and SB-charr) reared in a common garden revealed ~2000 genes to be differentially expressed by morphs during development (from formation of the gill-arches to establishment of the viscerocranium primordia) (Guðbrandsson et al., 2018). Similarly, the developmental methylome differs (for the same developmental window), mainly between benthic and pelagic morphs in the lake (Matlosz et al., 2022). Finally, the developmental miRNA transcriptome differs by genotype in one study comparing SB-charr to aquaculture charr (Kapralova et al., 2014). This indicates the morphs differ in genes influencing multiple developmental systems and could influence bone shape differences between them. Also, as common garden experiments have revealed ossification of skull elements in juveniles starts earlier in SB- compared to PL-charr (Eiríksson, 1999), it would be interesting to explore effects of genes and environmental factors on ossification and bone shape. In sum, most likely interactions of specific environmental attributes and genetic composition cause the observed morph differences in bone shape, for instance via differences in patterns of growth or differentiation in the bone primordia or linked tissues.

*Future avenues in research of bone shape variation in salmonids*

We studied variation in six dentary bones in sexually mature fish of derived morphs from one lake. This study could be expanded in several ways. First, size, age and sex can influence traits (Sandlund et al., 1992; Tamate & Maekawa, 2006). We chose to study sexually mature individuals, but average age and size varies quite substantially by morph. It would be interesting to study the emergence of these bone shape variations during ontogeny in these morphs. This could be done by sampling age classes from the lake and genotyping individuals (the major unknown being the PI-charr). For bone shape, size (fork length) had stronger effect on shape than age. Also, despite significant difference among morphs in allometries (age-size relationship as well as between size measures of head,

body, bones), these effects were rather small, and we concluded bone size was a robust proxy for overall size (and was used to account for size variation). But with larger samples it might be possible to disentangle and better estimate these effects. The same would apply for potential sexual dimorphism or morph specific sex-dimorphism in bone size or shape allometry. Secondly, due to sampling of four derived morphs, the observed phenotypes could not be polarized with respect to an outgroup. Studies of anadromous charr, from Iceland and or elsewhere, could identify ancestral vs. derived characters, and even reveal variation among putative ancestral populations. Arctic charr in Iceland have repeatedly evolved along a benthic-pelagic axis and subsequently experienced modification to allow for capture and processing of diverse prey items (e.g., benthic macroinvertebrates, fish, snails) (Brachmann et al., 2021; Gíslason et al., 1999; Kristjánsson et al., 2012; Woods et al., 2013). It would be interesting to study the degree of parallelism in bone shape variation and integration in more sympatric benthic-pelagic morphs or the miniaturized SB-charr. Thirdly, the salmonid head contains several dozens of bones and the cranial case. Ingimarsson (2002) found a signal (but weak) of morph differences in linear measures of several other bones, but geometric morphometrics on these (and other) bones may reveal this more systemically and highlight potentially adaptive changes. We studied shape with 2D landmarks, but in the future CT-scans and 3D morphometrics may offer better resolution and characterization of shape variation (Cardini, 2014; Vidal-García & Keogh, 2017; Zelditch et al., 2004) and biomechanical traits allowing divergence of prey preference in salmonids. These methods and other staining approaches may enable analyses of associated variation in soft tissues (muscles and ligaments), cartilage and ossification. We found rather high integration in shape and size variation among bones within and between the upper and lower jaws. However, covariation of shape and univariate traits (like teeth numbers) or variation in cranial kinesis, how the bones lie together in the skull and how they move together (Camp et al., 2009), was not analysed here. The feeding structures of fishes are highly functionally integrated with one another (Gidmark et al., 2019; Westneat, 2005). Research on the seven dolly varden trout (*Salvelinus malma*) morphs in Lake Kronotskoe indicated morph specific combinations of morphological traits but their benthic-pelagic ecologies manifest in mouth position and jaw length divergence early in ontogeny (Esin et al., 2018). They also found three cranial bone sets with coordinated shifts in ossification, which were as follows: jaws (premaxilla, maxilla and dentary), intraoral tongue-bite apparatus (vomer and lingual bone, gill arch elements) and skull dermal bones (supraethmoid, frontal and preopercle). They hypothesized that differences in early ossification led to these morphological differences between the morphs (Esin et al., 2018). Studies on additional internal skull elements and their integration are needed to understand the functional capacity of different jaw elements and how they relate to feeding divergence in arctic charr.

*Conclusions*

The diversity of feeding morphology in fishes provides opportunities to examine variation in functional elements, to determine rates of evolution, integration among morphological units, and how specialization occurs along a benthic-pelagic axis. We gathered unique data on variation in bones related to feeding that represents within species polymorphism. The results indicate a clear benthic-pelagic separation that was most prominent in the dentary bone. Further work is needed to understand the causes of variation in feeding elements within species, how it evolves and

develops. Are the axes of jaw bone shape variation in salmonids shared with other fishes or even other vertebrates? Work along these lines will aid in our understanding of the processes that still generate the great biodiversity we observe and must protect.

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