Title: *Variation of tooth traits in ecologically specialized and sympatric morphs*

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Abstract

Differences in dentition between species relate to feeding specialisations, as examples of tetrapod dentition variation show clearly. The association of tooth traits and specialisations in non-mammalian vertebrates is less studied. We examined variation in dental traits in four sympatric morphs of Arctic charr (*Salvelinus alpinus*) which differ in feeding specialisations, head and jaw bone morphology. We studied tooth numbers in six bones (dentary, maxilla, premaxilla, palatine, vomer and glossohyal) and tooth angles in one bone (maxilla). We found fluctuating asymmetry in tooth numbers and angles and that the allometry of tooth numbers varied by bone but not morphs. The tooth numbers differed by morphs in four bones (dentary, palatine, vomer and glossohyal). In general, the morphs defined as pelagic had more teeth, and this relates partially to changes in bone shape. There was a difference in maxilla tooth angle, with benthic morphs having teeth which were angled more inwards. Dentary and maxilla tooth number correlated moderately with bone shape, maxilla tooth angle and premaxilla tooth number did not. While it is currently unknown what tooth characteristics are ancestral vs derived in these populations, the marked differences in specific bones presents an opportunity to explore rapid adaptive evolution in dentition.

Statement and Declaration

The authors declare that they have no conflict of interest

Introduction

Variation in dentition associates with various feeding specialisations in vertebrates (Gregory et al., 2016; Huysseune, 1995; Koussoulakou et al., 2009; Melstrom, 2017; Streelman et al., 2003). Many aspects of tooth traits vary, like numbers (Koussoulakou et al., 2009; Line, 2003), complexity (Melstrom, 2017), shape (Gregory et al., 2016; Streelman et al., 2003) and location in the jaw (Mihalitsis & Bellwood, 2019) and this can often be related to prey capture and processing. Among the best examples of ecologically specialized dentition are illustrated in extant and fossil mammals (Line, 2003), but similar adaptations and specialisations have also been found in non-mammalian vertebrates, like reptiles (Lafuma et al., 2021; Melstrom, 2017) and fishes (Carr & Motta, 2020; Huysseune, 1995; Streelman et al., 2003). Unlike mammals, who generally replace their teeth once throughout their life (diphyodonts), most non-mammalian lineages are thought to constantly replace their teeth (polyphyodont) (Fraser et al., 2006; Huysseune & Witten, 2008).

As basal vertebrates, fishes show great diversity in form and function of dentition, with many diverse shapes, like conical teeth which can pierce prey, molariforms used for grinding and crunching, and broad blade-like teeth useful for slicing (Gidmark et al., 2019). Individual variation in numbers of teeth per tooth bearing bones can also differ between species (Eastman, 1977; Märss et al., 2017) and among populations of the same species (Cleves et al., 2014; Ellis et al., 2015; Huysseune, 1995). Tooth numbers on paired bones can also differ within individuals (asymmetry) (Witten et al., 2005).

Individual variation, fluctuating asymmetry and allometry in tooth numbers

While bilateral symmetry is the general pattern in vertebrates (Klingenberg et al., 2002; Koeberle et al., 2020) individual organisms are rarely perfectly symmetric (Klingenberg et al., 2002). Fluctuating asymmetry, the non-directional deviations from perfect symmetry, is thought to stem from developmental noise generated by chance events, manifest in interactions of the genotype and environmental variation throughout ontogeny (Koeberle et al., 2020). For tooth numbers the degree of asymmetry varies by species and bone. Fossil records of *Tyrannosaurid* dinosaurs showed asymmetry for maxilla and dentary tooth numbers (P. Currie, 2003; P. J. Currie, 2003) but not the premaxilla (P. Currie, 2003; P. J. Currie, 2003; Miyashita & Currie, 2010). In some species of fish, the degree of asymmetry in tooth numbers varies throughout the life cycle. For example, there was minor asymmetry in dentary tooth number in pre-spawning salmon (*Salmo salar*, both first time spawners and repetitive spawners) but in post-spawning individuals the asymmetry was higher (Witten et al., 2005).

Although tooth numbers of non-mammalian lineages can be relatively static, they usually increase allometrically as the individual grows (Cleves et al., 2014; Ellis et al., 2015; Greer, 1991; Torres-Carvajal, 2007). Allometric variation in tooth numbers has been found in reptilia, for instance the order squamata (Greer, 1991; Torres-Carvajal, 2007), where allometric relationships differ depending on species and the bone carrying the teeth. Tooth numbers in fish are assumed to increase with body size, along with growth of the tooth bearing bones. In three-spine sticklebacks (*Gasterosteus aculeatus*), there is strong positive correlation between pharyngeal jaw plate area and tooth numbers (Cleves et al., 2014; Ellis et al., 2015).

Inter- and intraspecific variation in tooth traits

Between group differences in tooth numbers can be used for species identification (Eastman, 1977; Märss et al., 2017), but for some groups, the high level of intraspecific tooth number variation makes them unreliable for this (Eastman & Underhill, 1973). Intraspecific differences in tooth number have been shown in many fish lineages, for example cichlidae (Huysseune, 1995) and three-spine sticklebacks (Cleves et al., 2014; Ellis et al., 2015), often linked to diet (Huysseune, 1995). One study found that, tooth number (on the premaxilla of the oral jaw) and the allometric relationship varied between Pacific and Atlantic stickleback populations (Caldecutt et al., 2001). Differences were seen in the pharyngeal jaw between benthic freshwater sticklebacks and marine populations, with benthic fish having

more teeth (Cleves et al., 2014; Ellis et al., 2015), both in wild individuals (benthic fish with twice as many teeth, Cleves et al., 2014) and in the lab (benthic fish with 1.5x more teeth, Cleves et al., 2014; Ellis et al., 2015).

Rearing experiments indicate tooth numbers have a heritable component (Cleves et al., 2014), but may also be plastic (Karagic et al., 2020). A lab rearing study on oral jaw tooth numbers in the cichlid species *Archosargus Probatocephalus* did not reveal significant differences in tooth numbers based on diet treatment (but the trend was that specimens fed soft prey had more teeth, Worcester, 2012). Cichlids also have pharyngeal jaws with teeth. A study found differences in the tooth numbers and their allometric relationships (Huysseune, 1995) depending on diet treatments for the cichlid species *Astatoreochromis alluaudi*. Individuals fed a soft-food diet had more (but smaller) teeth - which increase rapidly with growth - compared to the individuals fed hard-food diets (Huysseune, 1995).

Ecological variation in salmonids

Salmonids are known for extensive phenotypic variability and in the case of Arctic charr (*Salvelinus alpinus*) polymorphism and plasticity (Klemetsen, 2010; Noakes, 2008). Arctic charr shows extensive phenotypic variation throughout its distribution (Klemetsen, 2010), and sympatric morphs that differ in feeding ecology are found in many lakes (Doenz et al., 2019; Østbye et al., 2020; Skoglund et al., 2015; Woods et al., 2013). Our study system is the four sympatric Arctic charr morphs in lake Þingvallavatn, Iceland. In the lake are two morphs that primarily feed on benthic invertebrates (large benthivorous (LB) and small benthivorous (SB) charr) and two morphs that capture prey in the water column (planktivorous (PL) and piscivorous (PI) charr), from here on called benthic and pelagic morphs for simplification. These morphs differ in many life-history and ecological traits (Jonsson et al., 1988; Malmquist, 1992; Malmquist et al., 1985; Sandlund et al., 1987; Skúlason, Noakes, et al., 1989; Skúlason, Snorrason, et al., 1989; Snorrason et al., 1989). They are known to differ in feeding ecology, with LB- and SB-charr feeding mainly on snails, PL-charr on crustacean zooplankton and PI-charr mostly on three-spined sticklebacks (Malmquist, 1992).

Comparatively few studies have explored tooth trait variation in salmonids, though tooth development and replacement has been described in rainbow trout (*Oncorhychus mykiss*, Fraser et al., 2006) and Atlantic salmon (*Salmo salar* L., Huysseune & Witten, 2008). Variation in tooth traits for Arctic charr has received some attention, with reports of differences between morphs in tooth traits during development (Pichugin, 2009) and between adults (Bryce et al., 2016). Ingimarsson (2002, unpublished 4th year honorary thesis at University of Iceland) looked at differences in counts and inclination angle of teeth in the four sympatric morphs from lake Þingvallavatn and found more teeth with increased size in four bones: maxilla, palatine, vomer and glossohyal. Ingimarsson (2002) also found significant differences in tooth numbers between morphs for two bones, the dentary and palatine, with the pelagic morphs having on average more teeth. Though no morph differences were found in maxilla tooth numbers, differences in the inward inclination (measured as an angle) of maxillary teeth were found between morphs, specifically more inward angled teeth in the benthic morphs (Ingimarsson, 2002). Our recent study on these same morphs found jaw bone shape differences between morphs, along a benthic-pelagic axis (Jónsdóttir et al., 2024). Importantly, the main differences were seen in the dentary bone of the lower jaw, where benthic morphs had significantly shorter dentaries.

The aim is to test if the ecologically divergent Arctic charr morphs of Þingvallavatn differ in tooth traits, that may relate to prey capture and handling. Specifically, we ask if variation in these traits relates to variation in size, ecological specialization of morphs and shape of specific bones. We studied tooth numbers on the dentary, maxilla, premaxilla, palatine, vomer and glossohyal and the angle of inclination of maxillary teeth. Based on prior findings (Ingimarsson, 2002; Jónsdóttir et al., 2024), we aim to address the following questions and hypotheses:

1. Do tooth traits (numbers and inclination angle) show fluctuating asymmetry (FA), and if so, does FA vary with fish size or morph?
2. We assume the numbers of teeth will increase with the size of the fish and that this relationship holds for all tooth bearing bones. We do not expect maxilla tooth angles to increase with size.
3. Do tooth numbers of tooth bearing bones and angles of maxillary teeth differ by morphs? We expect to find differences between morphs, in the two bones (dentary and palatine) linked to the biting movements.

Specifically, we expect to find fewer teeth and more angled maxillary teeth (inward leaning) in the benthic morphs (per Ingimarsson, 2002).

4. Do tooth traits of a specific bone correlate with the shape of that bone (and other tooth bearing bones)? We expect that tooth numbers of a specific bone covary with the shape of that bone.

Methodology

Tooth Imaging and Counts

To study divergence in tooth traits among Icelandic Arctic charr two datasets were utilized. The primary analyses were conducted on a large dataset of Arctic charr (N=240) caught in 2020 and 2021, consisting of adults of all four morphs from Lake Þingvallavatn (see details of sampling and processing in Jónsdóttir et al., 2024). To reassess patterns observed in the larger dataset, a replicate dataset (N=138) from the same morphs collected in 1997 (Ingimarsson, 2002) was examined, from here on referred to as the "replicate" dataset. All tooth counts and related data are provided as supplements (Online Resources 1 to 40) and as datafiles with analyses pipeline on Github (https://github.com/GudbjorgOskJ/CharrTeethTTV).

For the primary dataset, oral jaw bones (dentary, maxilla, premaxilla, palatine, vomer and glossohyal) were photographed (Canon EOS 77D, 50 mm Microlens) in stereotypical view with scale and teeth analysed. The dentary and maxilla were photographed in medial and ventral view, premaxilla in fronto-lateral view, palatine in lateral and medial view, vomer in lateral and ventral view and glossohyal in lateral and dorsal view (Fig. 1). Teeth were counted twice on each bone: March and May 2023, by same researcher (GOJ). For the symmetric bones (dentary, maxilla, premaxilla and palatine) we had four replicate measures (left-right, March and May) and two for the vomer and glossohyal. We used available landmark data for dentary, maxilla and premaxilla bones from Jónsdóttir et al. (2024) to examine the relationship between tooth traits and bone shape. All pictures of fish and bones are available on Figshare+ (doi:10.25452/figshare.plus.25118825).

For the "replicate" dataset, jaw bones (dentary, maxilla, premaxilla, palatine, vomer and glossohyal) were photographed (JVG camcorder, model E 5506DC, raw image files are not available), with scale in stereotypical view from both sides (left and right jaws). The dentary was photographed in medial view, maxilla in ventral view, premaxilla in fronto-lateral view, palatine in lateral view, vomer in lateral and glossohyal in dorsal view. Teeth were counted once for one side by the same researcher (FI).

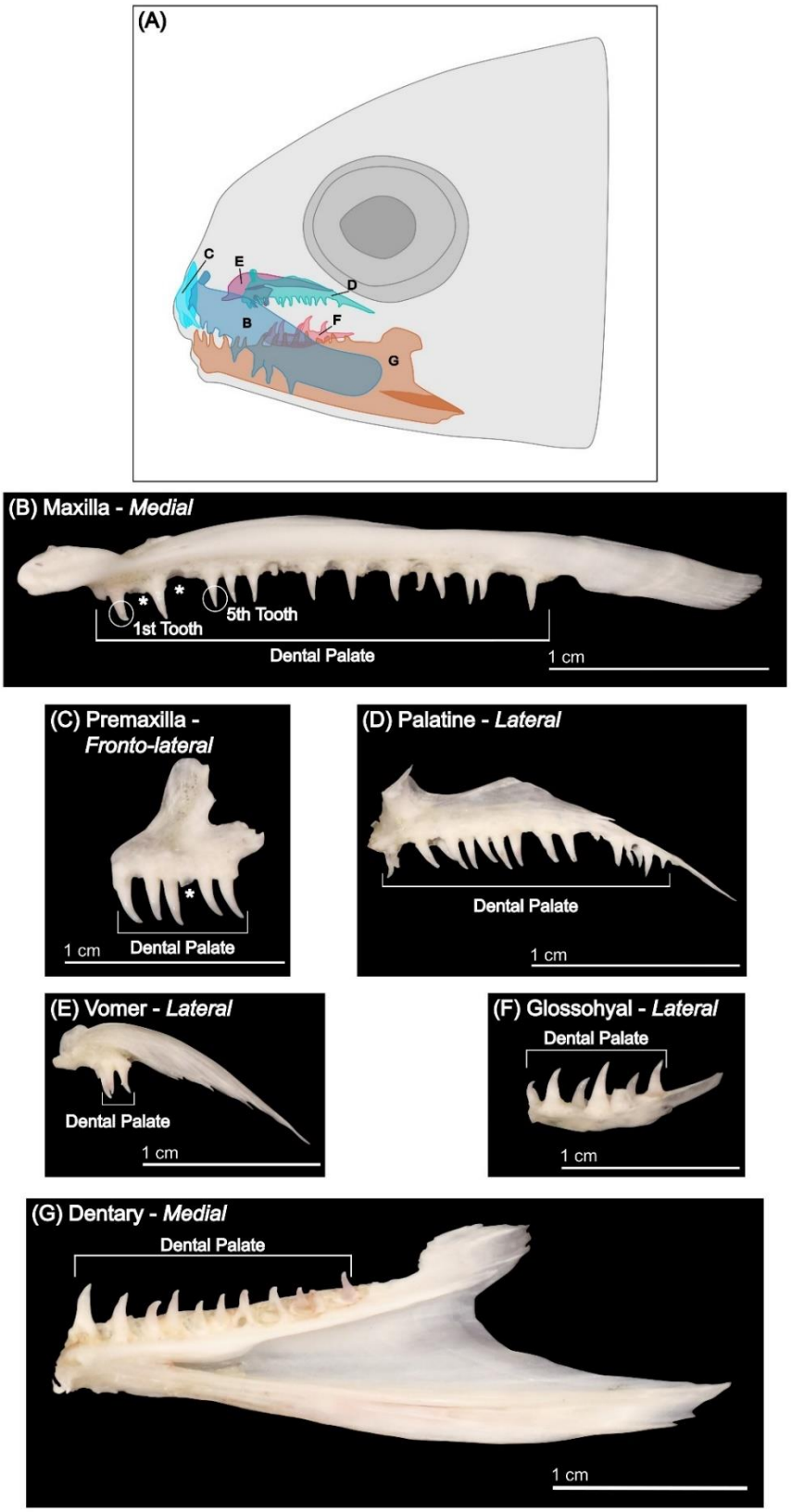

Fig. 1 *Morphology of the six craniofacial bones in Arctic charr, used in this study. (A) Locations of the six bones drawn on a schematic of a charr head, with front facing left. B- G) Images of the jaw bones used, with names and*

angle of photographing (italic). Dental palate (defined as distance from first tooth to last tooth) is indicated on each bone. Shown are bones of individual PC4386 (PI-charr, L = 36.6 cm and W = 435 g, male), inset scale (1 cm). B-C) White stars show examples of missing teeth, that were counted as true teeth here (may have fallen out during processing). Approach for measuring maxilla tooth angles is demonstrated in Online Resource 1

Maxilla tooth angles

Inspired by Ingimarsson (2002) we analysed maxilla tooth angles. To calculate the angles of maxillary teeth, we photographed the maxilla again using a stereomicroscope in three views: medial, ventral and fronto-lateral (focus on 1st tooth, Online Resource 1). To develop this trait, we tested two methods to measure angles. First, we used two photographs (medial and ventral view, here called "medial/ventral" method). The lengths of two teeth (1st and 5th) were measured from both medial and ventral view. These teeth were chosen for convenience and comparison to "fronto-lateral" method (see below). From these two measurements the tooth angle was calculated using the tangent:

$$Angle = \tan^{-1}\left(\frac{Perpendicular}{Base}\right) = \tan^{-1}\left(\frac{Ventral}{Medial}\right)$$

Second, we used a "fronto-lateral" method developed by Ingimarsson (2002). Using fronto-lateral view photographs of the maxilla we assessed the angle (1st tooth) directly with linear measures in Fiji (Schindelin et al., 2012). Repeatability of both methods was tested on a random subsample of ~60 individuals of three morphs (LB-, PI- and PL-charr, ~20 for each). All measurements were done by the same researcher. The "replicate" dataset came from photographs of the maxilla bones (again JVG camcorder, measured by FI). The angle was measured directly with Fiji on the tooth which was in best focus (the 2nd to 5th tooth) on each bone.

Statistical analyses of tooth counts

All statistical analyses were done in R (vs. 4.2.3, R Core Team, 2023) in R-studio (vs. 2023.06.1+524, RStudio Team, 2023). Figures were generated in R and formatted with Inkscape (vs. 1.3 (0e150ed6c4, 2023-07-21), Inkscape Project, 2020). Body size of individuals was estimated with fork length (FL, ln transformed). The repeatability of counts was assessed with a linear regression model (function *lm*), for all bones, left and right side independently (when applicable) and the mean (average number of teeth per fish). Pearson correlation was also estimated (*cor.test*).

To examine asymmetry in tooth numbers in the paired bones, we ran *glm* models on the differences between the left and right bone. The asymmetry in tooth numbers was calculated per individual (left – right, for the symmetric bones), which also allows estimation of the proportions of individuals with "perfect" symmetry (same number of teeth on both sides). Then, we asked whether these deviations correlated across bones (using *cor*), and if they deviated from 0 (directional asymmetry), varied by morphs, changed with body size (FL) and if asymmetry-allometry varied by morphs, with linear models in R:

$$T_D = M + LL + M \times LL + E$$

T_D: deviation in count, M: morph, LL: ln(FL). We used Bonferroni correction for four bones, $\alpha = 0.01$.

The variation in tooth numbers on the six bones was analysed using a generalized linear mixed model (GLMM) implemented with the Template Model Builder (TMB) (the *glmmTMB* package in R, version 1.1.7, Brooks et al., 2024). We tested the impact of morph, sex, and estimates of size (ln(FL)), assuming a gaussian distributed response variable (tooth number). The model was as follow:

$$T_C = M + Se + LL + M \times Se + M \times LL + Se \times LL + Si + Si \times Se + Si \times M + Rep(M \times Se \times Si) + E$$

In this model, T_C: tooth count, M: morph, Se: sex, LL: ln(FL), Si: side (left or right bone), with some interaction terms. Note, we accounted for repeated measures as a random term nested under fixed effects. Also, for bones with Centroid size estimates (C-size for dentary, maxilla and premaxilla, from geometric morphometric analyses, see Jónsdóttir et al. (2024)) we also ran the model with ln transformed C-size. As we studied six bones, a

Bonferroni corrected threshold $\alpha = 0.05/6 = 0.008$ was used. For the "replicate" dataset we ran the same model (dropping the side and replicate counts variables) to test effects of morph, sex and size.

Statistical analyses of maxilla tooth angles

Repeatability of the fronto-lateral method and medial/ventral method was tested on a subsample, 62 individuals, with three of the morphs (LB-, PI- and PL-charr, ~20 for each morph). We used a linear regression to gauge the repeatability of the methods. The results showed that the medial/ventral method was more repeatable (Online Resources 22 and 23). Therefore, tooth angle data for all individuals was collected with the medial/ventral method.

To examine the influence of morph, sex and size on maxilla tooth angles we again ran a generalized linear mixed model (GLMM) (*glmmTMB* package) with a gaussian distribution. The model was similar as above, except we added tooth as random variable (1st and 5th), and we did not have repeated measures per individual. Again, we used two proxies for size, ln FL, and ln C-size of the maxilla. As angle was only analysed on maxilla, we used $\alpha = 0.05$. For the "replicate" dataset we ran the same model (dropping the side and tooth variables).

Tests of shape and tooth trait covariation

We assessed the covariation of tooth traits (counts and angles) and shapes of tooth bearing bones (from published geometric morphometrics, (Jónsdóttir et al., 2024). Using two-block partial least squares (PLS) with the R function *two.b.pls* (*geomorph*), we compared tooth traits to shape of the same and other bones, both for all morphs together and each separately. Effect sizes were compared between bones (and between morphs within an individual bone), with *compare.pls.* For this analysis we used $\alpha = 0.007$ as a conservative threshold (Bonferroni correction for 7 traits, 6 tooth counts and 1 angle measurement).

Results

The four sympatric morphs of Arctic charr in Lake Þingvallavatn differ substantially in head morphology (Snorrason et al., 1989) and the shape of three jaw bones, with changes in the dentary and premaxilla being most dramatic (Jónsdóttir et al., 2024). All three are tooth-bearing jaw bones (Day, 1887), and we were keen to explore if tooth numbers also varied by morph, and also in three other bones in the salmonid skull that have teeth (Day, 1887).

Repeatability, fluctuating asymmetry and variation in tooth numbers

We counted tooth numbers on the dentary, maxilla, premaxilla, palatine, vomer and glossohyal, and estimated the angle of inward inclination of the maxilla teeth (see below). The counting of teeth was highly repeatable (Online Resources 2 - 4). There was substantial fluctuating asymmetry in tooth numbers in the four paired bones (Fig. 2 and Online Resource 5). Minority of individuals had the same number of teeth on both sides (from 15% for dentary to 21% for premaxilla). There was no indication of directional asymmetry, as left minus right tooth number did not differ from zero (Online Resource 8). Also, there was no indication of directional asymmetry by size nor as an interaction of morph and size (Online Resources 9 and 10). Finally, there was no indication of correlation of these deviations across bones (Online Resource 11).

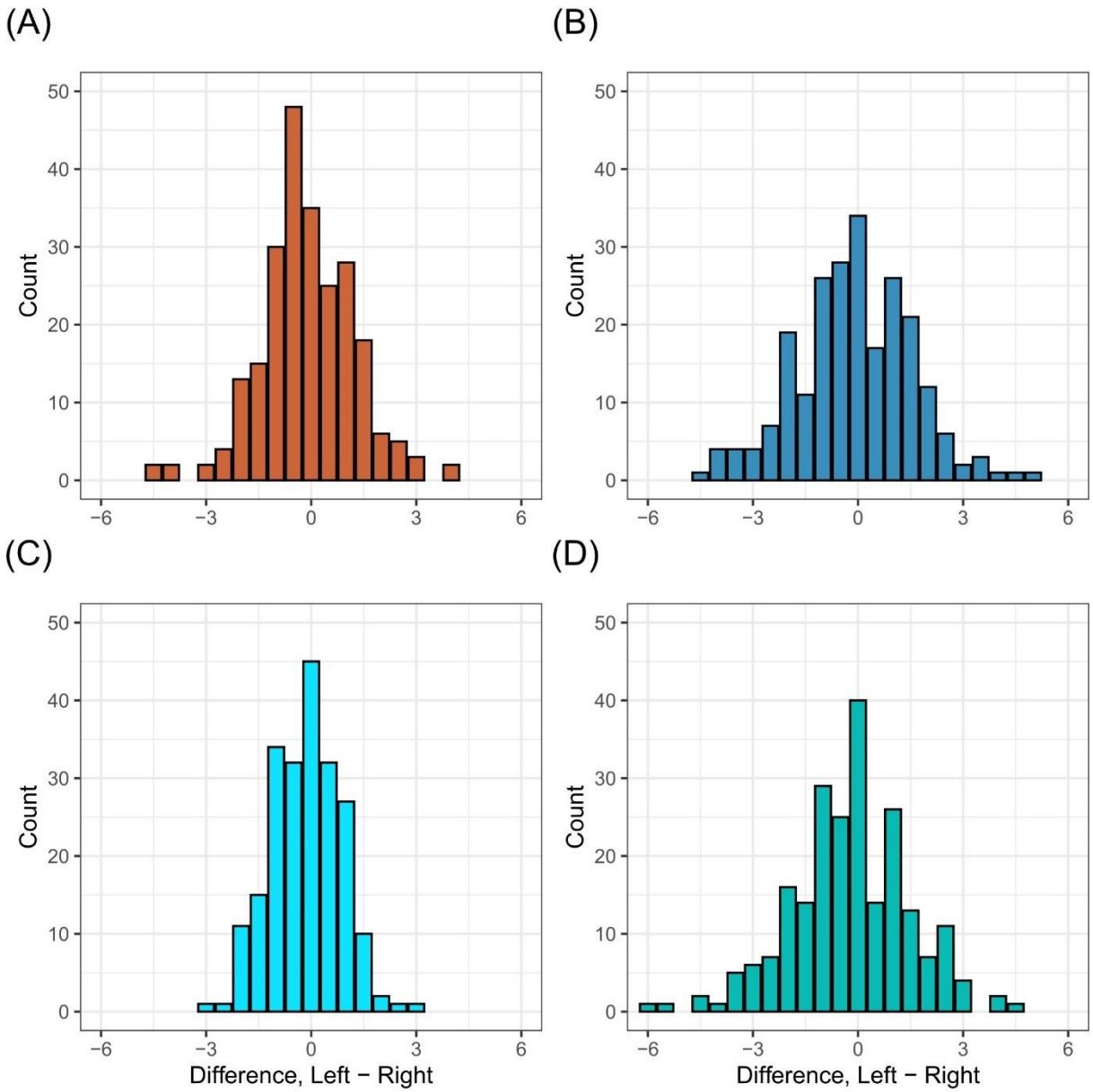

Fig. 2 *Fluctuating asymmetry in tooth numbers on four paired Arctic charr bones. Shown are histograms of the within individual variation, as the deviation in tooth numbers between the sides (subtracting right from left), for the dentary (A), maxilla (B), premaxilla (C) and palatine (D). X-axis shows the deviation between left and right and the Y-axis the number of individuals per bin. Note the scale is continuous, as averages from two measures per individual are represented*

As expected, the numbers of teeth varied greatly by bone (Fig. 3). The average tooth number, over all morphs and both sexes, was 14.7 for the dentary (range 9 to 26), 20.0 (12 to 28) for the maxilla, 7.5 (4 to 10) for the premaxilla, 16.5 (9 to 24) for the palatine, 4.5 (1 to 9) for the vomer and 12.0 (8 to 20) for the glossohyal (Online Resource 12). This reflects differences in types and sizes of bones, or possibly the dental palate (tooth-bearing part) - like in the dentary (Fig. 1g). When analysed by morphs and sexes, the dentary had the widest range, from average 11.7 teeth in SB-charr males to 17.1 in PI-charr males. The narrowest range (half a tooth) was in the premaxilla (7.05 in LB-charr males to 7.65 in LB-charr females). Predictably, bones with many teeth also had the highest variance (standard

deviation for maxilla was 2.26, compared to the premaxilla at 0.7 teeth). To study these apparent morph differences, we fitted mixed models (that utilized the repeated measures) with size as a covariate (cause the morphs differ greatly in size, Sandlund et al., 1992).

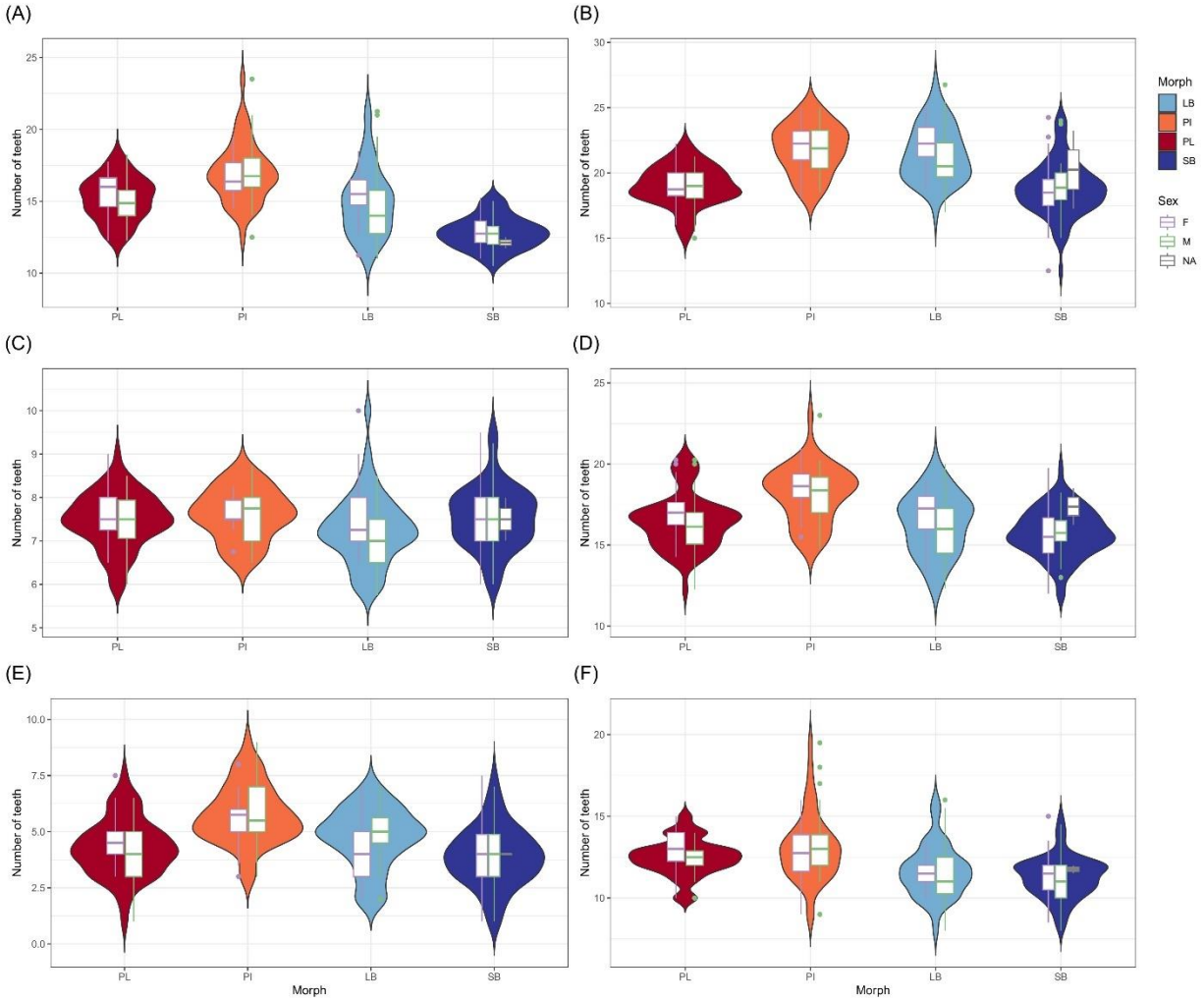

Fig. 3 *Distribution of tooth number in six bones by morph. Also shown are sex specific distributions as boxplots. The Y-axis indicates the number of teeth (average per individual for the paired bones A-D, average over repeated measures for the non-paired E-F). The bones are: dentary (A), maxilla (B), premaxilla (C), palatine (D), vomer (E) and glossohyal bone (F). Morphs are coloured: SB: dark blue, LB: light blue, PI: orange, PL: red (see also inset). Superimposed are boxplots for the sexes (female: purple, males: green, not sexed: grey, only in SB-charr)*

<u>Size and morph affect tooth numbers on most bones but not all</u>

We explored the effects of morph, sex and size on tooth numbers using both a mixed model framework and an ANOVA on size corrected tooth numbers (see methods). The mixed models showed size and morph had strong and consistent effects on tooth numbers in most bones, sex only having a minor effect, while no two-way or higher order interaction terms were significant (Table 1).

Body size strongly influenced tooth numbers in all bones, except premaxilla and glossohyal (Table 1, Fig. 4). The slope was steepest for the dentary and palatine (Online Resource 14), as an increase of an individual's length by 5 cm led to ~0.5 and ~0.4 extra teeth respectively. Using centroid size gave the same results (for the three bones with such estimates, Jónsdóttir et al., 2024, Online Resource 15). The analyses did not detect interaction of size with morph. Similarly, there did not appear to be consistent sexual dimorphism for tooth numbers (Table 1, sex effects minimal to non-existent) and no sex interaction term was significant (Table 1). The only trait with sex dependence was tooth angle (see below). More interestingly, the models indicated clear morph differences in four bones (dentary, palatine, vomer and glossohyal), largest for the dentary. In general, tooth numbers were highest in the large PI-charr and fewest in the small SB-charr (on average over all bones, Fig. 3). The complementary analyses, ANOVA on size corrected data, also showed morph differences. The LB-charr had the fewest teeth in most bones, and SB-charr had the highest numbers (Online Resource 13). The post hoc tests following the ANOVAs indicated stronger differences across all bones between the larger morphs (LB- < PI-charr), than the smaller ones (SB- and PL-charr). Finally, the "replicate" dataset (fish collected in 1997) gave generally consistent results (though the power was less), with the pelagic morphs having more teeth on average (Online Resources 33 - 36). These patterns will be described for each bone.

Table 1 *The influence of size, morph, sex and side (left or right) on tooth number in six jaw bones. Analysed with generalized linear mixed models (glmmTMB) using ln FL for size. P-values below the Bonferroni corrected threshold (α = 0.008) are shown in bold*

Terms		Dentary	Maxilla	Palatine	Premaxilla	Vomer	Glossohyal
Morph	χ^2	91.072	10.700	44.715	10.153	19.508	46.465
	df	3	3	3	3	3	3
	p-value	**< 0.001**	0.013	**< 0.001**	0.017	**< 0.001**	**< 0.001**
Sex	χ^2	0.406	0.121	0.030	4.907	2.090	0.036
	df	1	1	1	1	1	1
	p-value	0.524	0.728	0.862	0.027	0.148	0.851
FL (ln FL)	χ^2	31.124	8.747	18.434	1.677	8.814	3.769
	df	1	1	1	1	1	1
	p-value	**< 0.001**	**0.003**	**< 0.001**	0.195	**0.003**	0.052
Side	χ^2	1.745	0.910	4.909	5.273	NA	NA
	df	1	1	1	1	NA	NA
	p-value	0.187	0.340	0.027	0.022	NA	NA
Morph x Sex	χ^2	2.899	6.019	1.783	4.144	1.485	2.517
	df	3	3	3	3	3	3
	p-value	0.407	0.111	0.619	0.246	0.686	0.472
Morph x ln FL	χ^2	5.519	2.261	4.638	0.289	3.920	2.816
	df	3	3	3	3	3	3
	p-value	0.138	0.520	0.200	0.962	0.270	0.421
Sex x ln FL	χ^2	0.075	1.072	0.008	0.642	0.000	1.483
	df	1	1	1	1	1	1
	p-value	0.785	0.301	0.928	0.42305	0.992	0.223

NA: Each fish only has one copy of these bones, hence no "Side" term.

The dentary represents the anterior-most part of the lower jaw and its tooth numbers were affected both by size and morph ($p < 0.001$). The interaction term was not significant. The benthic fish had fewer teeth than pelagic fish matching in size. The pairwise tests (over both sexes) indicated significant differences between PL- and SB-charr ($p < 0.001$) and between LB- and PL-charr ($p < 0.001$), with PL-charr having ~3 more teeth than both SB- and LB-charr. The difference between LB- and PI-charr was ~2.3 teeth and near significance ($p = 0.02$, Online Resource 16). Palatine and vomer showed similar patterns, with significant size and morph effects on tooth numbers (more teeth on average in pelagic fish, Table 1). However, for neither bone did the pairwise tests of morphs reveal differences (Online Resources 16 and 17), suggesting subtler differences than in the dentary. The glossohyal tooth numbers were strongly

affected by morph, but not significantly by size. Again, the pelagic morphs had higher tooth numbers, shown by pairwise tests (significant differences between LB- and PL-charr, p = 0.003, with PL-charr having ~2.5 more teeth). The LB-PI-charr comparison pointed in the same direction (PI-charr > LB-charr) but was on the cusp of significance considering Bonferroni correction (unadjusted, p = 0.012, Online Resource 16). For both the maxilla and premaxilla (in upper jaw like the palatine), morph did not significantly affect tooth numbers. For the maxilla tooth numbers associated positively with size, however curiously, premaxilla tooth numbers did not.

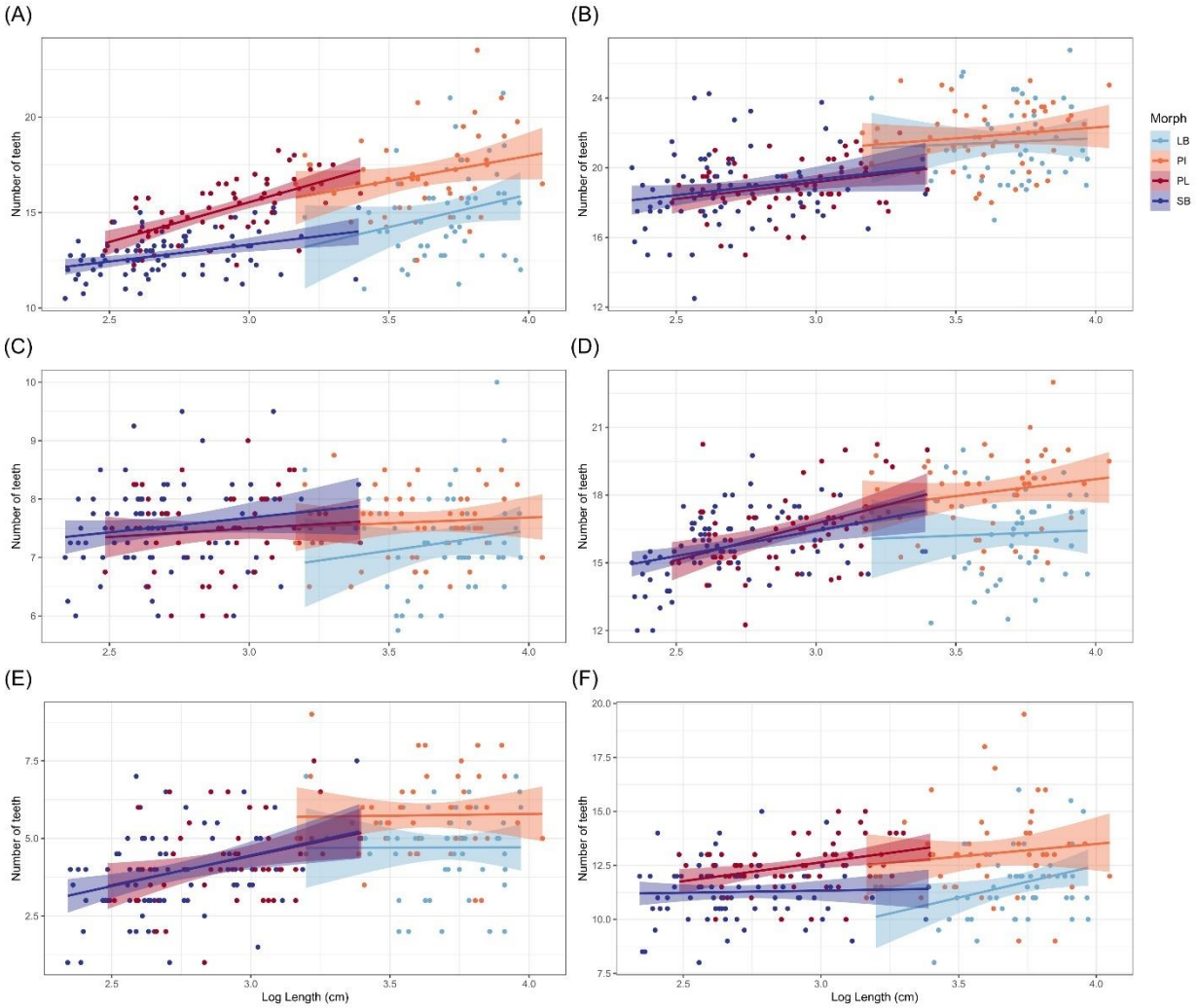

Fig. 4 *Influence of size and morph on number of teeth**.** The scatterplots show individual tooth numbers (Y-axis) against size (ln(FL in cm) X-axis) for the four morphs and six bones; A) dentary, B) maxilla, C) premaxilla, D) palatine, E) vomer and F) glossohyal. Note the numbers for symmetric bones (A-D) are averages for the left and right bones*

<u>Morph differences in maxilla tooth angles</u>

We explored the influence of morph, sex and size on maxillary tooth angles with *glmmTMB*. The strongest effects appeared to be morph, sex and an interaction of morph by tooth (difference between 1st and 5th tooth). First, the inward inclination of maxillary teeth differed by morph, with the mean angle (over both teeth) being largest in SB-charr

(50.9°) but smallest in PL-charr (32.4°). Differences in this trait among morphs was along a benthic-pelagic axis, with the benthic morphs having more inward angled maxillary teeth. Pairwise tests showed significant differences between the LB- and PI-charr, with LB-charr having more inward angled teeth ($p = 0.01$, Online Resource 25) as well as between the LB- and PL-charr ($p < 0.001$) and SB- and PL-charr ($p < 0.001$) always with the benthic morphs having more inward angled teeth (Online Resource 25). Second, while size influenced maxilla tooth numbers, it did not relate to their angles (Table 2, Online Resource 28). Third, several other terms were significant for maxilla tooth angle, notably the asymmetry, the two teeth measured and sex. Side (left vs right, estimate of asymmetry) was significant, with the left side being slightly more angled inward on average (estimated 2° differences, $p < 0.001$), but no interaction with side was significant (Table 2). Tooth angle did not differ between the 1st and 5th tooth, as the average angle, over the entire dataset, was 43.8° for the 1st maxilla tooth and 43.8°for the 5th tooth. However, there was significant interaction between the tooth and morph effects ($p = 0.001$). For the larger morphs (LB- and PI-charr) the 5th tooth was angled more inward, but for the smaller morphs (PL- and SB-charr) the 1st tooth was angled more inward. Within morphs this difference was only significant in the PI-charr (estimated 2.7° differences, $p = 0.017$) and PL-charr (estimated 2.7° differences, $p = 0.01$). Males tended to have less angled teeth, and this sex difference was most dramatic in the PL-charr (Fig. 5 and Online Resources 24, 26 and 27). This was the only tooth trait to show sex differences. While we found both significant morph and sex effects ($p < 0.001$) on tooth angle, there was no sex and morph interaction ($p > 0.07$).

Table 2 *The influence of size, morph, sex, side (left or right) and tooth (1st or 5th tooth) on the inward inclination of maxillary teeth. Analysed with generalized linear mixed models (glmmTMB) using ln (FL) for size*

Effect	χ^2	df	p-value
Morph	252.101	3	**< 0.001**
Sex	25.919	1	**< 0.001**
Size (ln FL)	0.222	1	0.638
Side	16.375	1	**< 0.001**
Tooth (1st v. 5th)	0.001	1	0.978
Morph x Sex	7.071	3	0.070
Morph x ln FL	3.439	3	0.329
Sex x ln FL	1.360	1	0.244
Morph x Tooth	16.416	3	**0.001**

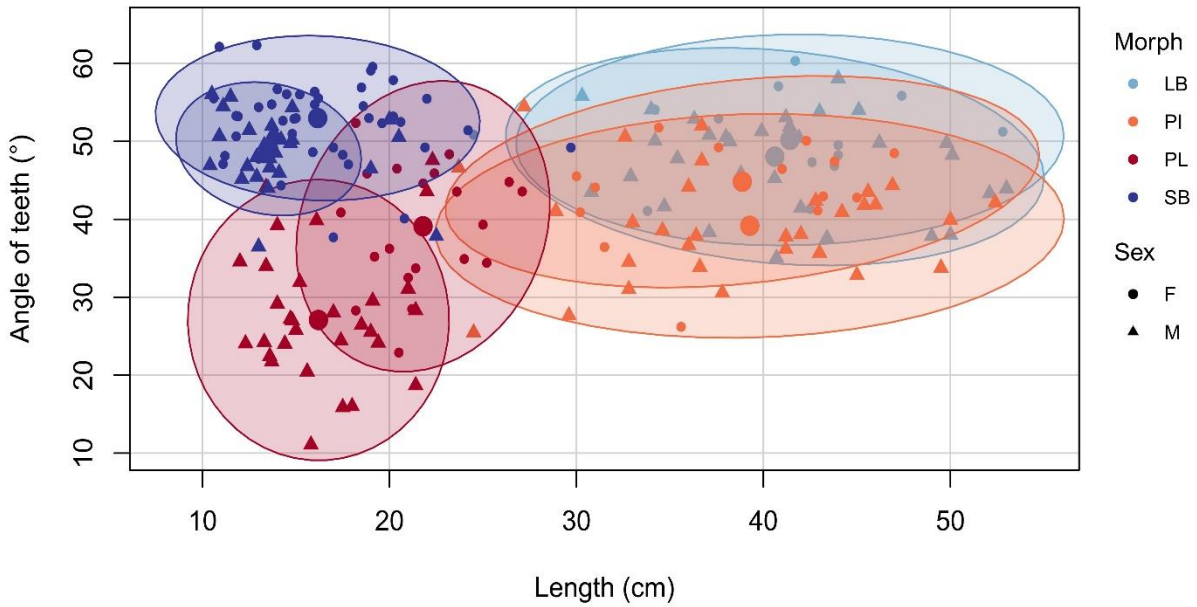

Fig. 5 *Effects of morph and sex on the inward inclination of maxillary teeth in the four morphs. The scatterplot shows average tooth angle for 1st and 5th tooth on both left and right bone (Y-axis) against size (fork length of fish, X-axis) for all individuals. The ellipses and shaded areas are 95% CI for the distributions and the large dots represent the means for the sexes within morphs*

Analyses of the "replicate" dataset showed the same patterns, the benthic morphs having more inward angled maxillary teeth than PL-charr (Online Resources 37 - 39). The mean angles in the groups differed substantially, between the datasets. Most likely this reflects methodological differences as the maxillary angles were measured with two different methods in the two datasets.

<u>Patterns of covariation between tooth traits and bone shape</u>

Lastly, we explored the patterns of covariation of tooth traits (counts and angles) on particular bones and the shape of the same bone, and others as a reference (by calculating the correlation coefficient from two block partial least squares, r-PLS). This was done for the entire dataset and morphs separately. For the entire dataset, nearly all pairs of bones and tooth traits were significant, with the degree of covariation generally being low to moderate (r-PLS ~ 0.38 – 0.72, $p < 0.001$, Online Resource 40). The strongest association was between dentary shape and dentary tooth number (r-PLS = 0.717, $p < 0.001$). However, the premaxilla tooth numbers did not covary with premaxilla, dentary or maxilla shape (Online Resource 40), confirming earlier results about low variation in this trait. We concluded that the pattern of covariation in the overall dataset likely reflects differences in bone size, shape and/or tooth numbers between morphs, and thus analysed the data by morph. Predictably, we found a lot fewer pairs of bone shape and tooth numbers significantly covarying (angle never covaried with shape). These covariations were primarily between tooth numbers and shape of the corresponding bone. For instance, dentary shape and dentary tooth number showed moderate but significant level of covariation in the two smaller morphs (SB- and PL-charr, r-PLS = 0.52 and 0.69 respectively, $p = 0.001$, table 3) and the maxilla shape covaried significantly and moderately with maxilla tooth number in two morphs (LB- and PL-charr, r-PLS = 0.53 and 0.55 respectively, $p \leq 0.004$, table 3). Notably, premaxilla shape did not covary with premaxilla tooth number. Other relationships were generally weak and non-significant, with some exceptions. Maxilla shape and dentary tooth numbers correlated in PI- and PL-charr (r-PLS = 0.54 and 0.62 respectively, $p \leq 0.005$, table 3), dentary shape and palatine tooth numbers in PL-charr, and premaxilla shape and maxilla tooth numbers

for SB-charr (r-PLS = 0.529, p = 0.003) and glossohyal tooth numbers for PI-charr (r-PLS = 0.599, p = 0.001). Finally, maxilla tooth angles did not covary with shape of the maxilla, nor the other two bones (Table 3).

Table 3 *Test of covariation between bone shape and tooth traits (numbers and angle) by morph. Traits were compared using partial least squares (PLS), within and among (All) morphs. Corresponding pairs of tooth counts and shape are underlined*

Covariation	Bone shape	Tooth counts	r-PLS	p-value				
				PL	PI	LB	SB	All morphs
Dentary shape to tooth traits	Dentary	Dentary	0.418 - 0.689	**0.001**	0.503	0.082	**0.001**	**0.001**
	Dentary	Maxilla	0.327 – 0.476	0.462	0.246	0.572	0.049	**0.001**
	Dentary	Premaxilla	0.253 – 0.422	0.33	0.528	0.559	0.729	0.784
	Dentary	Palatine	0.299 – 0.591	**0.001**	0.656	0.462	0.463	**0.001**
	Dentary	Vomer	0.319 – 0.378	0.433	0.954	0.554	0.180	**0.001**
	Dentary	Glossohyal	0.306 – 0.489	0.272	0.186	0.325	0.383	**0.001**
	Dentary	Angle	0.218 – 0.460	0.176	0.434	0.310	0.948	**0.001**
Maxilla shape to tooth traits	Maxilla	Dentary	0.383 – 0.622	**0.001**	**0.005**	0.137	0.015	**0.001**
	Maxilla	Maxilla	0.407 – 0.545	**0.002**	0.107	**0.004**	0.013	**0.001**
	Maxilla	Premaxilla	0.285 – 0.428	0.335	0.080	0.249	0.394	0.253
	Maxilla	Palatine	0.335 – 0.421	0.201	0.098	0.222	0.159	**0.001**
	Maxilla	Vomer	0.286 – 0.453	0.082	0.377	0.521	0.422	**0.001**
	Maxilla	Glossohyal	0.315 – 0.499	0.574	0.422	0.015	0.221	**0.001**
	Maxilla	Angle	0.267 – 0.497	0.024	0.686	0.325	0.608	**0.001**
Premaxilla shape to tooth traits	Premaxilla	Dentary	0.365 – 0.578	0.855	0.008	0.729	0.136	**0.001**
	Premaxilla	Maxilla	0.374 – 0.531	0.506	0.051	0.853	**0.003**	**0.001**
	Premaxilla	Premaxilla	0.358 – 0.490	0.049	0.710	0.335	0.096	0.273
	Premaxilla	Palatine	0.365 – 0.476	0.270	0.157	0.249	0.460	**0.001**
	Premaxilla	Vomer	0.399 – 0.420	0.392	0.520	0.563	0.238	**0.001**
	Premaxilla	Glossohyal	0.309 – 0.599	0.830	**0.001**	0.400	0.133	**0.001**
	Premaxilla	Angle	0.365 – 0.552	0.026	0.499	0.646	0.595	**0.001**

P-value, Bonferroni corrected threshold, $\alpha = 0.007$

Discussion

In fishes, variation in tooth number and form abounds, but tooth traits in salmonids have received limited attention. In general fishes tend to have uniform-shaped teeth and high intraspecific variation in tooth numbers (Gidmark et al., 2019). We explored variation in tooth numbers (and angle) among sympatric Arctic charr morphs in lake Þingvallavatn, known for extensive variation in external head and jaw bone morphology that apparently relate to their ecological specializations and life history characteristics (Jónsdóttir et al., 2024; Jonsson et al., 1988; Malmquist, 1992; Sandlund et al., 1987; Skúlason, Noakes, et al., 1989; Snorrason et al., 1989). Our data confirmed high variance for tooth numbers in six jaw bones (dentary, maxilla, premaxilla, palatine, vomer and glossohyal), and fluctuating asymmetry in the four paired bones. We assumed that tooth numbers on all bones would increase with the size of the fish. However, this did not apply to the premaxilla and glossohyal. We found significant differences in tooth number between morphs in four bones (dentary, palatine, vomer and glossohyal), with pelagic morphs having more teeth than benthic morphs in all cases. There was a significant difference in maxilla tooth angle, with benthic morphs having more inward angled teeth. We found significant covariations between these tooth traits and bone shape.

The Arctic charr morphs of Þingvallavatn, generally, had fewer teeth (3 to 7 teeth depending on bone) than Arctic charr morphs in Loch Rannoch, Scotland (Bryce et al., 2016), with the exception of the glossohyal (average 12 teeth in Iceland vs 8 in Scotland). Such differences are to be expected as resident populations of Icelandic and Scottish charr have been isolated from each other for a long time. While these differences in mean tooth number could reflect real differences between populations or countries (Icelandic vs. Scottish charr) or due to the size distribution of the sampled fish, as previously seen in sticklebacks (Caldecutt et al., 2001; Cleves et al., 2014; Ellis et al., 2015) and reptiles (Greer, 1991; Torres-Carvajal, 2007). Still, methodological factors (e.g. inclusion of missing teeth) must be considered.

Allometric relationships of tooth number vary by bone

Allometry of tooth numbers vary by bone within reptiles (Brown et al., 2015; Greer, 1991; Torres-Carvajal, 2007). Tooth numbers increase with size on the maxillary and mandibular bones but not the premaxilla in the Black Iguana (*Ctenosaura similis*, Torres-Carvajal, 2007) and *Ctenotus essingtoni* (Greer, 1991). As most (but not all) fish show asymptotic growth related to maturity (Beverton & Holt, 1959), one expects its features would change with size, and not all at the same rate. We assumed that tooth numbers would relate positively with body size for all bones, i.e. more teeth as the fish grows. The data showed relationships between size and tooth number for all bones, except the premaxilla and glossohyal. These relationships did not differ by morph, not even for the dentary, where the shape-size allometry differs markedly by morph (Jónsdóttir et al., 2024). No significant relationship was found between size and maxilla tooth angles.

Although allometry in glossohyal tooth number was not significant the slopes within the larger two morphs approached significance. The relative stability of the premaxilla tooth numbers over size is clear and confirms earlier studies (Bryce et al., 2016; Ingimarsson, 2002). Curiously, both the size and shape of this bone shows strong allometry with body size, and the shape differs between benthic and pelagic morphs (Jónsdóttir et al., 2024). This lack of allometry could relate to the relatively few teeth (4 to 10 in this study) on the premaxilla. With few teeth, an offset of one or two teeth could inflate the variance and reduce statistical power to estimate an allometric slope. However, the vomer has similar number of teeth (1 to 9) yet showed a significant relationship with size (and differences by morph). Therefore, we entertain the idea that the size independence of premaxilla tooth numbers in Arctic charr adults is biological. This could relate to a finite number of dental primordia being set early in premaxilla development, while in bones that gain teeth with increasing size, a stem cell niche may provide new precursor cells throughout the growth phase. Could this variance in the premaxilla be a conserved trait among Salmonids or related fishes? Perhaps not, as premaxilla tooth number increase with size in the three-spine stickleback, a relationship that differs between populations (Caldecutt et al., 2001). However, such invariance of premaxilla tooth numbers was found in the reptiles *Ctenotus essigtoni* (Greer, 1991), American alligator (*Alligator mississippiensis*, Brown et al., 2015) and Komodo dragon (*Varanus komodoensis*, Brown et al., 2015). Analyses of fossilized *Tyrannosaurid* dinosaurs indicate that

premaxilla tooth numbers were comparably stable within that group; not varying between taxa or individuals within a genus, though tooth numbers on other bones (maxilla and dentary) did (P. Currie, 2003; P. J. Currie, 2003). In the saltwater crocodile (*Crocodylus porosus*) studies found premaxilla tooth number to decrease with size (Brown et al., 2015). We add the caveat that even though premaxilla tooth numbers do not seem to vary between Arctic charr morphs (Bryce et al., 2016; Ingimarsson, 2002) other aspects of the premaxilla teeth (e.g. length, thickness, etc) could vary (Bryce et al., 2016). We call for further study of the allometries of teeth in more populations and species of salmonids.

Differences in tooth traits between morphs within species

Based on prior studies (Bryce et al., 2016; Ingimarsson, 2002; Jónsdóttir et al., 2024) we predicted morph differences in tooth number in the dentary and palatine. The data confirmed this prediction while tooth numbers in the maxilla and premaxilla did not differ among morphs. The data also revealed morph differences in the two bones forming the "tongue-bite" apparatus (vomer and glossohyal). Differences between Arctic charr morphs have previously been documented for the dentary, palatine (Bryce et al., 2016; Ingimarsson, 2002) and the glossohyal (Pichugin, 2009), but not the vomer. Confirming Ingimarsson (2002) findings, we report morph differences in the inward inclination of maxillary teeth, the angle being larger in the benthic morphs.

The pelagic morphs tended to have more teeth than the benthic morphs (in the dentary, palatine, vomer and glossohyal). This agrees with studies of adult Scottish Arctic charr and juvenile Siberian Arctic charr (Bryce et al., 2016; Pichugin, 2009). However, as the estimated differences are small (1 - 3 teeth) the question arises, how biologically important are they? With discrete characters like teeth and most bones having 10 or more teeth, addition or loss of one tooth may not affect fitness. Fishes use teeth for capturing and holding prey and some species use them to process prey into consumable parts (Gidmark et al., 2019). Teeth are believed to serve similar roles in salmonids (Camp et al., 2009; Konow et al., 2008). The functional significance of these differences in tooth number remains unclear (Bryce et al., 2016).

In the dentary (anterior-most lower jaw bone) the PL-charr had significantly more teeth than the two benthic morphs (SB- and LB-charr). This agrees with Bryce et al. (2016), which found the planktivore morph in Loch Rannoch had more dentary teeth than the benthivore morph (Bryce et al., 2016). However, they also saw significant differences between the planktivore and piscivore morphs in Loch Rannoch, with the planktivore morph having more teeth. This was not observed in Þingvallavatn. The functional importance of more teeth on the dentary for planktivorous charr morphs is unclear (Bryce et al., 2016). We previously found strong shape differences in the dentary bone between the Þingvallavatn morphs (Jónsdóttir et al., 2024) and hypothesized that shortening of the dentary (most likely the derived state) may aid benthic morphs in catching/holding their prey, from the stony littoral lake bottom. We found that dentary tooth numbers generally correlated with the dentary shape. Based on this we postulate that changes in dentary tooth number may themselves not be an adaptation but reflect a correlated response to the adaptive bone shape change (i.e. reduced size of the dental palate resulting in less teeth).

The palatine (upper jaw, medial to the maxilla) showed a similar pattern as the dentary, though weaker (the morph effect was significant, but none of the pairwise tests). The pattern was similar to that seen in Loch Rannoch (Bryce et al., 2016), with the piscivorous (PI-charr) morph having more teeth than the benthivorous (SB- and LB-charr) morphs. Based on this we hypothesize that more teeth on the palatine benefit the PI-charr, possibly making it easier to either grab or hold elusive pray (three-spine stickleback or juvenile charr). Assuming that the ancestor of the morphs was mostly likely also a piscivore (Bengtsson et al., 2023), the fewer teeth of the large benthivorous charr morph may be a derived trait. Under this hypothesis, the loss of teeth on the palatine in large benthivorous charr may be adaptive.

The vomer (positioned fronto-medially in the roof of mouth) also showed a consistent, but weak, pattern of morph differences (no pairwise tests were significant). The largest difference was between PI- and LB-charr, with the former having more teeth. Morph differences in vomer teeth have not been described before (as far as we know). For the glossohyal (positioned at the front of the tongue) we saw similar benthic vs. pelagic differences (significant only

between LB- and PL-charr, the latter having more teeth). Though insignificant for other pairs, the trend was for the pelagic morphs having more teeth. Prior studies have not found differences in glossohyal tooth number for adult Arctic charr (Bryce et al., 2016; Ingimarsson, 2002), however differences were found between size-matched juveniles of planktivorous and dwarf (feeding on planktonic and benthic prey seasonally) Arctic charr in Lake Davatchan, Transbaikalia (Pichugin, 2009). It is unknown if these difference manifest in adult morphology. More teeth on both the vomer and glossohyal in pelagic morphs could reflect adaptions for prey-processing, as both bones are part of the tongue-bite apparatus in salmonids (Camp et al., 2009; Konow et al., 2008). Stomach samples from LB- and SB-charr contain primarily whole snails (Malmquist, 1992), which suggests they rarely process their prey before swallowing. However, having more teeth on the same bones used for processing prey by other piscivorous salmonids (Konow et al., 2008; Konow & Sanford, 2008) may allow PI-charr to consume relatively large bodied prey by either increasing handling efficiency or by allowing prey to be broken down into smaller consumable parts (i.e. Konow et al., 2008). Though this has not been directly studied in this system. The increased tooth numbers on the same bones in the PL-charr, which primarily eats copepods (Malmquist, 1992), however, does not have a clear ecological benefit. Little is known of the raking behaviour in the Þingvallavatn charr morphs, and within the *Salvelinus* genus, modulation of muscle activity or raking kinematics of tongue-bite apparatus based on prey type is little studied (Konow et al., 2008). Therefore, we cannot confirm that the tooth differences in the vomer and glossohyal reflect adaptive differences in prey-processing.

Curiously, maxilla teeth were angled more inwards in the benthic morphs, despite no differences in tooth numbers. The strongest difference in tooth angles was between the smaller morphs (SB- and PL-charr), not the larger two (LB- and PI-charr) which differed mainly in tooth numbers in other bones. SB-charr had an average angle of 50.9°, while the PL-charr 32.4° (in between were LB-charr: 48.3°and PI-charr: 40.8°). Variation in tooth angle is understudied in salmonids and teleosts. Anatomical species descriptions indicate backwards curvature of glossohyal teeth in salmonids (Day, 1887) and inward curvature of premaxilla teeth in predaceous catfishes (Gosline, 1973), and the hypothesis is that this prevents prey from escaping the mouth (Gosline, 1973). It is unlikely that the inward pointing maxilla teeth in SB-charr serve a similar role, as snails (their main prey) are not particularly evasive prey. It is possible that angled teeth may assist in holding the prey or orienting it for swallowing. Ingimarsson (2002) proposed the inward angled teeth may allow benthic morphs to hook their teeth underneath snails from the lava bottom and dislodge them. This "soda-bottle opener" hypothesis could be tested with observations of feeding fish. We also note, the results suggested differences between angles of the 1st and 5th tooth, with the 5th tooth being more angled inwards in the larger morphs, but the 1st tooth leaning more inwards in the smaller morphs. However, these differences in angle by tooth were minor. Examining variation in tooth angles in multiple teeth may be another interesting research avenue. Studies of more snail eating charr morphs can evaluate the generalizability of these findings.

Other aspects of teeth not investigated here, like tooth length, width, pointedness and overall shape, could also be biologically meaningful (Karagic et al., 2020). These traits are known to differ among fish species (Gidmark et al., 2019; Streelman et al., 2003) and within Arctic charr (Bryce et al., 2016). For example, a long-standing proposition has been that piscivorous salmonids have larger teeth than non-piscivores (Day, 1887). Indeed, Bryce et al. (2016) reported piscivorous Arctic charr morph had longer teeth on the premaxilla, maxilla, dentary and glossohyal (Bryce et al., 2016). These results are quite interesting as neither we nor Bryce et al. (2016) found morph differences in tooth numbers for the premaxilla and the maxilla. Therefore, we speculate that other aspects of teeth may vary between Arctic charr morphs in Þingvallavatn and other lakes. As sympatric polymorphism is widespread among Arctic charr (Doenz et al., 2019; Østbye et al., 2020; Skoglund et al., 2015; Woods et al., 2013), it is possible that many other Arctic charr populations show similar differences in tooth traits.

Methodological aspects and future directions

Lastly, we discuss dataset differences, methodological limitations and suggest future directions. We analysed two datasets and despite qualitatively similar results, there were quantitative discrepancies (mainly fewer statistically significant terms in the "replicate" dataset). This could reflect differences in sample size, photography, layout of bones

(3D structures – photographed as 2D), measures of traits and biological reality. For both datasets we applied the same reasoning for counting teeth, though the image quality was worse for the "replicate" dataset and two different researchers counted each set. Generally, there were minor quantitative differences in tooth counts between sets, most likely reflecting differences in sample size (power of statistical analysis), size distribution of samples (can affect tooth number, see also Cleves et al., 2014; Ellis et al., 2015; Greer, 1991; Torres-Carvajal, 2007) and sampler effects (minor differences in tooth count due to non-identical humans). The notable exception was the glossohyal, ~7 more teeth were found in the new set. This most likely reflects the poor quality of the photos from 1997, causing tooth numbers to be underestimated. We also saw notable differences between datasets in the maxilla tooth angles, with more inwards leaning teeth in the new set. This likely reflects differences in approaches to estimate tooth angles. For the new dataset we measured angles with the medial/ventral method, as we found the fronto-lateral method (used for the "replicate" dataset) was not as repeatable (see above). Related to this, we measured the 1st and 5th teeth, but in the "replicate" dataset 2nd-5th tooth was measured, depending on focus. The indication of angle differences by tooth could influence this as well. However, it is possible that tooth numbers and maxilla angles have changed in these morphs in the 20+ years between the two studies.

We explored asymmetry and found it quite substantial for both tooth number and maxillary tooth angle. We examined adult spawning individuals, but previous research has shown that asymmetry can vary between pre-spawning and post spawning salmon (Witten et al., 2005). Whether such patterns also hold in Arctic charr is unknown and was not tested here. Neither did we analyse asymmetry for the bone shapes. In the future, a joint analysis of asymmetry in bone shape, size and tooth traits, in more groups (with larger sample sizes) would be an exciting research avenue. Related to this are the analyses of covariation of traits (not their asymmetry), bone shape and tooth numbers. We anticipated a relationship of tooth number and bone shape (Cleves et al., 2014; Ellis et al., 2015) and found support for this in the dentary and maxilla (not premaxilla) when analysed per morph. The analyses of the overall dataset indicated significant covariation of bone shape of the dentary, maxilla and premaxilla and tooth numbers on all the bones (except for premaxilla teeth). We think this stems from the landmark data retaining aspects of size (both body and bone size differ by morph, Jónsdóttir et al., 2024; Sandlund et al., 1992), bone shape and/or tooth number differences by morph. Statistical power may also have influenced these results, the total samples size was 240 specimens. With larger sample sizes it would be interesting to estimate if overall bone shape correlates with tooth traits in other bones (palatine, vomer and glossohyal), and whether tooth numbers relate strongly with specific axes (e.g. elongation of dental palate) of bone shape in charr and other salmonids. Previous research on three-spine sticklebacks found a positive correlation between tooth plate areas and tooth number on pharyngeal jaw (Cleves et al., 2014; Ellis et al., 2015). Something similar may be occurring in the dentary, where pelagic morphs with more elongated dental palates (tooth-bearing section, Jónsdóttir et al., 2024) and a higher dentary tooth number. Differences in strength of correlation of tooth numbers and dental palate length in specific bones (or populations) could reflect developmental or evolved differences in the pathways responsible for tooth formation and growth.

Conclusions

We discovered that tooth traits (both their numbers and angles on maxilla) vary between recently diverged sympatric Arctic charr morphs. We found significant morph differences in four bones, three of which were previously shown to differ in other Arctic charr morphs (Bryce et al., 2016; Pichugin, 2009) and were able to confirm a prior finding on maxilla tooth angle differences (Ingimarsson, 2002). The functional or evolutionary relevance of these variations remain unknown and calls for further comparative work and behaviour surveys. Although the allometry of tooth traits (both number and angle) did not differ between morphs it curiously varied markedly by bone. While some bones (e.g. dentary and palatine) showed strong allometry, others (e.g. premaxilla number and maxilla angle) had none. In particular, based on the relatively stable premaxilla tooth numbers one may ask whether this is a conserved trait among Salmonids or other teleosts? And what does this say about the development of tooth formation on these diverse bones, and how and when during vertebrate evolution did development of particular structures become more stereotypical

(and not affected by variation in size)? We lacked an outgroup, representing the ancestral state, to compare to the derived Þingvallavatn morphs. Questions on ancestral vs derived states and the developmental origins of these patterns are interesting to us. The great diversity in feeding and niche utilization in Arctic charr, provides a way to investigate the developmental origins of adaptive and non-adaptive divergence of bone shape and dental variation.

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Ethical Standards

This study builds on a previous study, and all the biological samples were ready in house. The previous study involved killing of wild fishes, and for such work a scientific fish fieldwork from the Directorate of Fisheries in Iceland (www.fiskistofa.is) was needed. Sigurður S. Snorrason and Arnar Pálsson, authors on both of these studies and in charge and present for all sampling efforts and, had for the period of the study, such scientific fish fieldwork permits (#0042/2014-2.13 and #0460/2021-2.0). The samples from 1999 were gathered before this law came into effect and are thus exempt from its stipulations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Supporting Information

Online Resource 1 *Morphology of two maxilla bones from three different views. Example of fronto-lateral view (A and B) ventral view (C and D) and medial view (E and F) images used to measure/calculate tooth angle. Shown are bones of individuals (A) PC3840 (male LB-charr, length = 37.1 cm, weight = 672 g), (B, D and F) PC3907 (female SB-charr, L = 15 cm, W = 24.5 g) and (C and E) PC3859 (male PL-charr, L = 14.8 cm, W = 36 g). Scale inset per picture is 1 cm*

Online Resource 2 *Linear regression was used to estimate repeatability of tooth counts for the A) dentary, B) maxilla, C) premaxilla, D) palatine, E) vomer and F) glossohyal. Scatterplot shows the relationship between the first (March) and second (May) counts. Equations for each line are provided in Online Resource 3*

Online Resource 3 *The results of liner regression models (using function lm) between the two teeth counts (March and May). Visualization of the datapoints and regression line are provided in Online Resource 2*

Online Resource 4 *Repeatability of tooth counts analysed with Pearson correlation (function: cor.test), for each morph separately. Table shows the estimate (r) and results from the test of correlation between the two teeth counts (March vs May). For paired bones the correlation was tested for both the left side and right side separately, and the average number of teeth over both sides. Only one estimate was done for the non-paired bones (vomer and glossohyal)*

Online Resource 5 *Fluctuating asymmetry in tooth numbers on four paired bones in four sympatric Arctic charr morphs. Graphed are histograms of the within individual variation in tooth number by morph. The distributions of deviation in tooth numbers between the sides (calculated by subtracted right from left), for the dentary (A), maxilla (B), premaxilla (C) and palatine (D). X-axis shows the deviation between left and right and the Y-axis the number of individuals per bin. Note the scale is continuous, as represented are the average from two measures per individual*

Online Resource 6 *Distribution of the repeated measures for each of the paired bones, for each morph. The scatterplots show the linear relationship between tooth numbers from the right and left bones (A: dentary, B: maxilla, C: premaxilla and D: palatine). Equations for each line can be seen in Online Resource 7*

Online Resource 7 *Results of linear regression model between tooth numbers of the right and left bones for all paired bones (dentary, maxilla, premaxilla and palatine). For visualization of relevant data, see Online Resource 6*

Online Resource 8 *Results from student t-test, testing whether the mean asymmetry in tooth numbers differs from zero. Bonferroni corrected threshold ($\alpha = 0.01$) to correct for tests on four bones*

Online Resource 9 *Test of the influence of size (ln fork length), morph and the interaction on fluctuating asymmetry in tooth numbers in the four symmetric bones. Results from GLM with ln FL for size, the morphs (four types) and the interaction term*

Online Resource 10 *The scatterplots show deviation in tooth numbers between left and right (Y-axis) against size (ln FL in cm, X-axis) for each of the four paired bones, dentary (A and B), maxilla (C and D), premaxilla (E and F) and palatine (G and H). The lines are a fitted linear model, with confidence intervals, nonsignificant by ANOVA on*

GLM models. Graphs on right (A, C, E and G) show all morph together and graphs on left (B, D, F and H) are coloured by morph

Online Resource 11 *Correlations (Pearson) between mean asymmetry in tooth numbers for each bone to each other and fork length*

Online Resource 12 *Average number of teeth for six bones and respective standard deviation (SD), calculated for all Arctic charr morphs. The numbers represent raw counts*

Online Resource 13 *The size corrected distribution of tooth number in six bones by morph. Also shown are sex specific distributions as boxplots. The Y-axis indicates the number of teeth corrected for with individual length (FL) (teeth/length). The bones are: dentary (A), maxilla (B), premaxilla (C), palatine (D), vomer (E) and glossohyal bone (F). Morph are coloured: SB: dark blue, LB: light blue, PI: orange, PL: red (see also inset). Superimposed are boxplots for the sexes (females: purple, males: green, not sexed: grey, only in SB-charr)*

Online Resource 14 *Result from emtrends, showing the different trends between tooth number and different length measurements (raw fork length, ln fork length, ln C-size)*

Online Resource 15 *The influence of size, morph, sex and side (left and right) on tooth number in six jaw bones. Analysed with generalized liner mixed model (glmmTMB) using ln C-size for size*

Online Resource 16 *Pairwise comparisons of teeth numbers between morphs, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 17 *Pairwise comparisons of teeth numbers between morphs within sex, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 18 *Pairwise comparisons of teeth numbers between sexes within morph, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 19 *Pairwise comparisons of teeth numbers between morphs, results from the generalized liner mixed models (glmmTMB), when using ln C-size for size*

Online Resource 20 *Pairwise comparisons of teeth numbers between morphs within sex, results from the generalized liner mixed models (glmmTMB), when using ln C-size for size*

Online Resource 21 *Pairwise comparisons of teeth numbers between sexes within morph, results from the generalized liner mixed models (glmmTMB), when using ln C-size for size*

Online Resource 22 *Linear regression (function lm) examining the repeatability of the two methods (fronto/lateral method and medial/ventral method) used when measuring the angle of maxillary teeth. This was analysed on 62 specimens from 3 morphs. The Medial/ventral method appeared to be more repeatable*

Online Resource 23 *Test of repeatability for estimation of maxillary teeth angle with linear regression. Scatterplots show the liner relationship between the two trials for both methods (fronto/lateral method and medial/ventral method) used to measure the angle. This was analysed on 62 specimens from 3 morphs. The Medial/ventral method appeared to be more repeatable*

Online Resource 24 *Average angle of maxillary teeth (± standard deviation) for each morph, also by sex*

Online Resource 25 *Pairwise comparisons of the inward inclination of maxillary teeth angles between morphs, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 26 *Pairwise comparisons of the inward inclination of maxillary teeth angles between morphs within sex, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 27 *Pairwise comparisons of inward inclination maxillary teeth angles between sexes within morph, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 28 *The influence of size, morph, sex, side (left or right) and tooth (1st or 5th tooth) on the inward inclination of maxillary teeth. Analysed with generalized liner mixed model (glmmTMB) using ln C-size for size*

Online Resource 29 *Pairwise comparisons of the inward inclination of maxillary teeth angles between morphs, results from the generalized liner mixed models (glmmTMB), when using ln C-size for size*

Online Resource 30 *Pairwise comparisons of the inward inclination of maxillary teeth angles between morphs within sex, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 31 *Pairwise comparisons of the inward inclination of maxillary teeth angles between sexes within morph, results from the generalized liner mixed models (glmmTMB), when using ln FL for size*

Online Resource 32 Effect of morph on the inward inclination of maxillary teeth in the four morphs. The scatterplot shows average tooth angle (averaged over 1st and 5th tooth on both left and right bone for each individual) (Y-axis) against size (fork length of fish, X-axis). The ellipses and shaded areas are 95% CI for the distributions and the large dots represent the means for each morph)

Online Resource 33 *Average number of teeth for six bones and respective standard deviation (SD), calculated for all Arctic charr from the 1997 "replicate" dataset. The numbers represent raw counts*

Online Resource 34 *Distribution of tooth number in six bones by morph, based on the 1997 "replicate" dataset. Also shown are sex specific distributions as boxplots. The Y-axis indicates the number of teeth. The bones are: dentary (A), maxilla (B), premaxilla (C), palatine (D), vomer (E) and glossohyal bone (F). Morphs are coloured: SB: dark blue, LB: light blue, PI: orange, PL: red (see also inset). Superimposed are boxplots for the sexes (female: purple, males: green)*

Online Resource 35 *The influence of size, morph and sex on tooth number in six jaw bones, from the 1997 "replicate" dataset. Analysed with generalized liner mixed models (glmmTMB) using ln FL for size. P-values below the Bonferroni corrected threshold ($\alpha = 0.008$) are shown in bold*

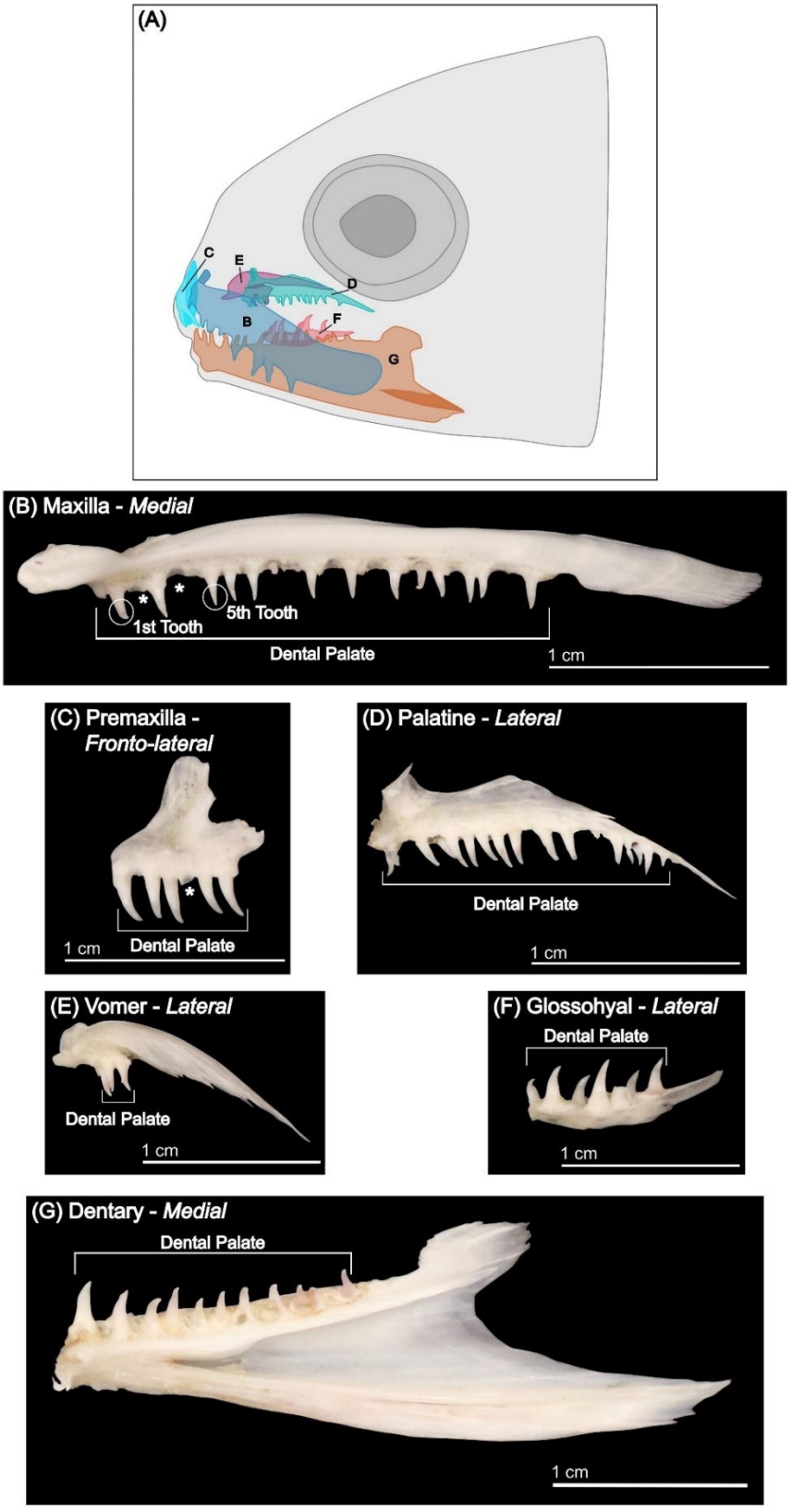
Online Resource 36 *Influence of size and morph on number of teeth from the 1997 "replicate" dataset. The scatterplots show individual tooth numbers (Y-axis) against size (ln FL in cm, X-axis) for the four morphs and six bones; A) dentary, B) maxilla, C) premaxilla, D) palatine, E) vomer and F) glossohyal*

Online Resource 37 *Average angle of maxilla teeth (± standard deviation) for each morph, also by sex from the 1997 "replicate" dataset*

Online Resource 38 *The influence of size, morph and sex on the inward inclination of maxillary teeth, from the 1997 "replicate" dataset. Analysed with generalized liner mixed models (glmmTMB) using ln FL for size*

Online Resource 39 *Maxilla tooth angle from the 1997 "replicate" dataset varies by morphs (A) and sex by morph (B). The scatterplots show average teeth angle for 1st and 5th tooth on both left and right bone (Y-axis) against size (Fork length of fish, X-axis). Maxilla teeth angle varies by morphs (A) and sex by morph (B). The ellipses and shaded areas are 95% CI for the distributions and the large dots represents the mean for each group. Two datasets were analysed, 240 fish from Jonsdottir 2024, combined data for tooth 1 and 5, with medial/ventral method (see figure 5 and Online Resource 32) and 138 fish from Ingimarsson 2001, measured on tooth 2-5 with fronto-lateral method*

Online Resource 40 *Test of integration between bone shape and tooth number. Traits were compared using partial least squares (PLS), within and among bones*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(B) Maxilla - Medial
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(C) Premaxilla - Fronto-lateral
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1 cm
Dental Palate
(D) Palatine - Lateral
Dental Palate
1 cm
(E) Vomer - Lateral
Dental Palate
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(F) Glossohyal - Lateral
Dental Palate
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(G) Dentary - Medial
Dental Palate
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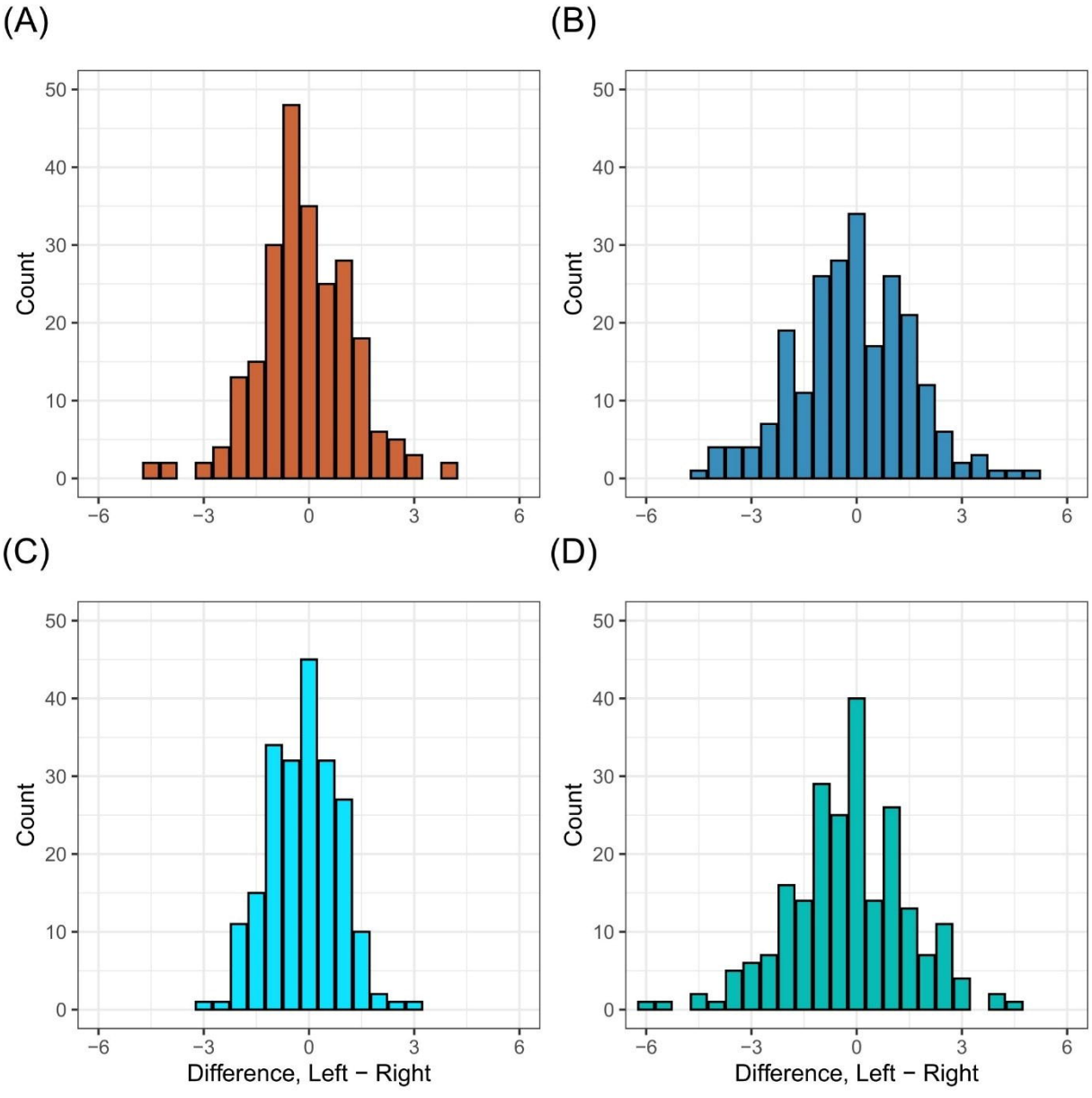
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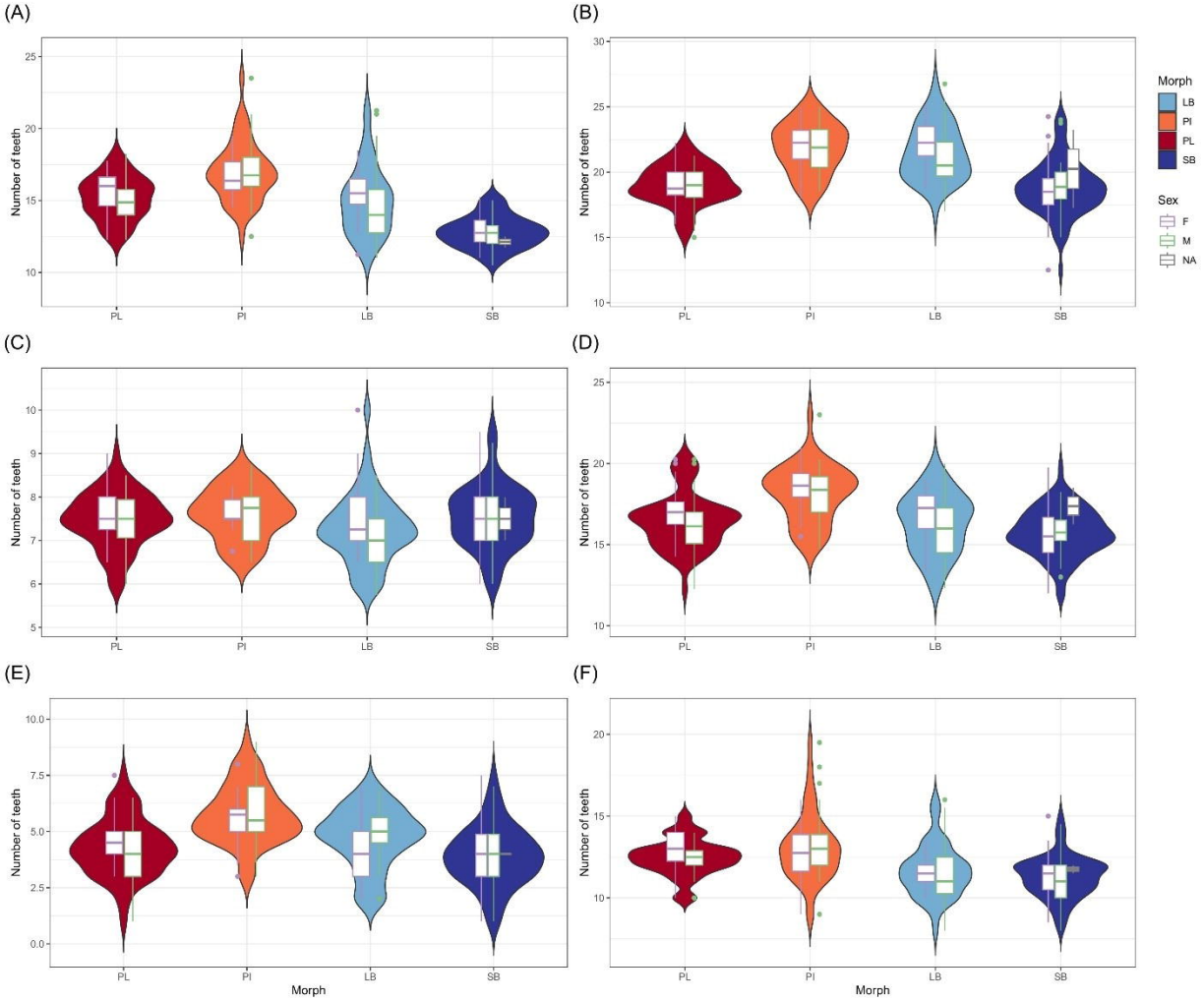
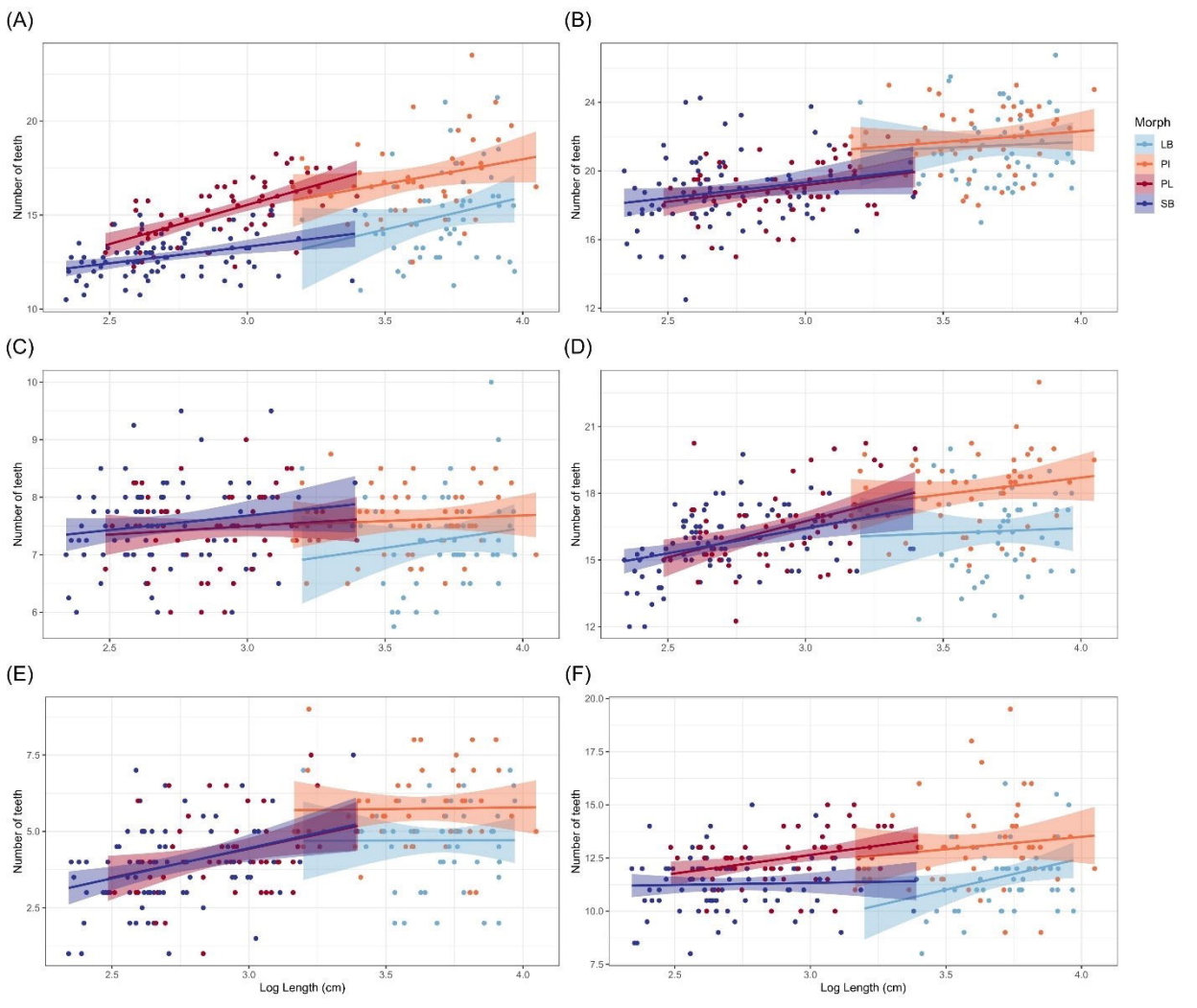
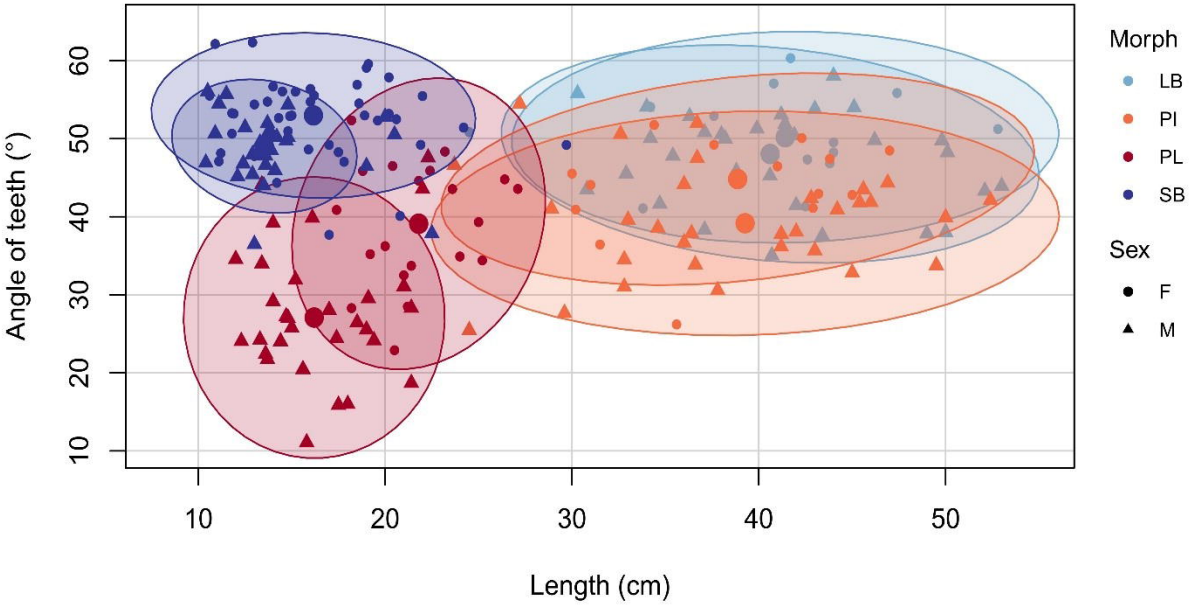
3
6
Difference, Left − Right
Difference, Left − Right


(A)
(B)
(C)
(D)
(E)
(F)
Number of teeth
PL
PI
LB
SB
Morph
Morph
LB
PI
PL
SB
Sex
F
M
NA


(A)
(B)
(C)
(D)
(E)
(F)
Number of teeth
Log Length (cm)
Morph
LB
PI
PL
SB


Angle of teeth (°)
10
20
30
40
50
60
10
20
30
40
50
Length (cm)
Morph
LB
PI
PL
SB
Sex
F
M