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Natural selection for body shape in resource polymorphic Icelandic Arctic charr

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Abstract

Resource polymorphisms exhibit remarkable intraspecific diversity and in many cases are expected to be maintained by diversifying selection. Phenotypic trade-offs can constrain morphologically intermediate individuals from effectively exploiting both alternate resources, resulting in ecological barriers to gene flow. Determining if and how phenotypic trade-offs cause fitness variation in the wild is challenging because of phenotypic and environmental correlations associated with alternative resource strategies. We investigated multiple pathways through which morphology could affect organismal performance, as measured by growth rate, and whether these effects generate diversifying selection in polymorphic Icelandic Arctic charr (*Salvelinus alpinus*) populations. We considered direct effects of morphology on growth and indirect effects via trophic resource use, estimated by stable isotopic signatures, and via parasitism associated with trophic resources. We sampled over three years in (lakes) Thingvallavatn and Vatnshlíðarvatn using the extended selection gradient path analytical approach and estimating size-dependent mortality. We found evidence for diversifying selection only in Thingvallavatn: more streamlined and terminally-mouthed planktivore charr experienced greater growth, with the opposite pattern in small benthic charr. However, this effect was mediated by parasitism and non-trophic pathways, rather than trophic performance as often expected. Detection of between-morph differences in the presence (Vatnshlíðarvatn) and direction (Thingvallavatn) of size-dependent mortality, together with non-trophic effects of shape, suggest that a morphological trophic performance explanation for polymorphism is insufficient. This rare insight into selection during early diversification suggests that a complex of interacting local factors must be considered to understand how phenotype influences fitness, despite morphological variation reflecting intuitive trade-off explanations.

Keywords: microevolution, phenotypic selection, path analysis, selection differential, performance gradients, adaptation, natural selection, intraspecific competition, sympatric diversification

Introduction

Numerous taxa show dramatic phenotypic polymorphisms, many of which involve the specialisation of discrete intraspecific morphs toward use of alternative resources (e.g. Price, 1987; Smith & Skúlason, 1996; Martin & Pfennig, 2010). Natural selection is thought to contribute to resource polymorphisms such as where morphs consume alternate resources but intermediate morphologies suffer reduced relative fitness due to incompatibilities between shape and either resource (Smith & Skúlason, 1996; Arnegard *et al.*, 2014). Such diversifying selection, where natural selection increases phenotypic diversity, could maintain discrete morphs through post-zygotic ecological isolation (Nosil, 2012), and play a role in initiating polymorphisms (Ackermann & Doebeli, 2004; Herron & Doebeli, 2013). Studies under semi-natural conditions support a role for diversifying selection in maintaining such morphological diversity (Martin & Pfennig, 2010; Arnegard *et al.*, 2014) but evidence in the wild is limited due to difficulties estimating fitness, multiple correlated environmental factors, and the numerous pathways through which traits can affect fitness (Mitchell-Olds & Shaw, 1987; Ghalambor *et al.*, 2003; Morrissey *et al.*, 2010; Franklin & Morrissey, 2017).

In many cases, morphological specialisation is thought to result adaptively from the effects of traits on fitness where phenotypic trade-offs exist. Phenotypic trade-offs are where a trait that is beneficial for a particular ecological activity is detrimental to performance of an alternative activity (Bolnick *et al.*, 2003). Hypothesised phenotypic trade-offs are typically well-grounded in the scientific literature: for example, lever systems involve force/velocity trade-offs whereby phenotypes maximising jaw closing speed result in weaker closing forces (Wainwright & Richard, 1995; Herrel *et al.*, 2009). Such biomechanical trade-offs could contribute to diversification where alternate resources demand alternative foraging strategies. However, consideration must also be given to other potential fitness costs associated with alternate ecological activities. For example, exploiting alternate resources may increase susceptibility to predation or parasitism (Langerhans & Reznick, 2009; Karvonen & Seehausen, 2012). Recent theory reconciling the general framework of path analysis with the concept of selection gradients now facilitates quantification of the effects of focal

traits on fitness both directly and indirectly via other traits or performance measures (Morrissey, 2014). In addition to generating estimates of natural selection for focal traits, this approach can reveal through which functional pathway(s) traits influence fitness.

Phenotypic trade-offs are thought to have contributed to the evolution of numerous postglacial fish species (Robinson & Wilson, 1994; Schluter & Rambaut, 1996). Trade-offs between terminal and subterminal mouth position affect gape and prey capture ability (Keast & Webb, 1966; Wainwright & Richard, 1995), and trade-offs between deep- and streamlined-bodies affect maneuverability and efficiency of continuous swimming (Webb & Weihs, 1983; Ellerby & Gerry, 2011). Such morphological trade-offs are expected to constrain individuals to forage on either patchily-distributed pelagic prey or larger benthic prey within a complex substratum, resulting in selection for shape through prey capture efficiency (herein referred to as trophic performance). Arctic charr (*Salvelinus alpinus*) exhibit variation in morphology and resource-use that is consistent with hypothesised trade-offs associated with benthic and pelagic foraging. Coexisting morphs in Iceland inhabit ecosystems with few interspecific competitors and predators and often overlap in spawning times and locations (Skúlason *et al.*, 1989a; Jónsson & Skúlason, 2000; Woods *et al.*, 2012). The ability in these systems to gather individual-level data on diet, parasitism, and growth, permits insight into the various pathways through which trade-offs can influence fitness and facilitates estimates of diversifying selection in the wild.

We investigated multiple pathways by which morphology could affect overall organismal performance, as measured by growth rate, and investigated whether these effects generate net diversifying selection in populations of Arctic charr inhabiting two lakes in Iceland in which early stages of putatively adaptive phenotypic differentiation are well-documented. We considered direct effects of morphology on growth, simultaneously with possible indirect effects via habitat preferences (as reflected by stable isotope analysis), and rates of parasitism that we expected would be linked to habitat use. We conducted our analyses of morphology in a geometric morphometrics

framework, and utilised the extended selection gradient path analytical approach (Morrissey, 2014) to quantify direct and indirect effects of morphology on fitness. We found evidence for diversifying selection in the lake in which greater differentiation already exists, and found that this diversifying selection is largely mediated by parasitism, rather than being an effect of morphology on trophic performance.

Methods

Study system

We separately investigate two lakes, Thingvallavatn and Vatnshlíðarvatn, that contain charr morphs exhibiting differentiation associated with morphological trade-offs. Arctic charr, long recognised for intraspecific diversity (Klemetsen, 2010), exhibit degrees of phenotypic and genetic diversification across Icelandic lakes (Gíslason *et al.*, 1999; Kapralova *et al.*, 2011; Kristjánsson *et al.*, 2011; Woods *et al.*, 2012). Since colonisation 10,000 years ago, many lakes became inaccessible to migrants due to lava flows, landslides and isostatic rebound, and likely experienced no subsequent colonisation (Norddahl & Einarsson, 2001; Wilson *et al.* 2004; Geirsdóttir *et al.*, 2009). Thingvallavatn is a spatially complex, spring-fed environment containing four charr morphs, distinguished as benthic or pelagic and further by size (small or large benthic) or diet (planktivore or piscivore), as well as threespine stickleback and brown trout (Jónasson, 1992). The small benthic and planktivore charr on which we focus overlap in spawning times and locations and exhibit morphological differences consistent with expectations for benthic and pelagic niches, respectively (Webb, 1984; Skúlason *et al.*, 1989a; Wainwright & Richard, 1995). Vatnshlíðarvatn is a simple, shallow, runoff-fed lake with two charr morphs and no other fish (Jónsson & Skúlason, 2000). The silver and brown morphs exhibit life-history differences and contrast in size and shape associated with the breadth of prey taxa used. The smaller, deeper bodied brown charr is a benthic Cladocera-specialist, while the larger, more streamlined silver charr is a trophic generalist (Jónsson & Skúlason, 2000; Franklin, 2017). In

Vatnshlíðarvatn, cannibalism occurs infrequently (Jónsson & Skúlason, 2000) and avian predators are usually present (personal observation). Morphological differentiation has been considered an adaptation to alternate trophic resources in both lakes (Malmquist *et al.*, 1992; Jónsson & Skúlason, 2000), and we investigate the lakes separately as two examples of shape selection in resource polymorphisms.

Sampling

In July 2014, 2015 and 2016, we sampled Arctic charr from specific locations within Thingvallavatn and Vatnshlíðarvatn. In Thingvallavatn we sampled small benthic (n=359) and planktivore (n=611) charr from the littoral zone around Mjóanes peninsula (Sandlund *et al.*, 1992; Kapralova *et al.*, 2011). In Vatnshlíðarvatn we sampled brown (n=199) and silver (n=943) charr along the north shore. We aimed to sample a range of immature charr to capture morphological variation unrelated to sexual traits, using Nordic mixed nets with mesh size 5 to 55 mm. On-site morph classifications, distinguished by size, shape, texture, and colour, were supported by objective classification using Gaussian mixture models (Franklin, 2017).

Fish were sacrificed and, at the laboratory, individuals were weighed, their left sides photographed, and caudal muscle tissue was removed and frozen. Individual fish were frozen until sampling concluded, whereby we thawed them, extracted otoliths, determined their sex, and weighed their gonads. For a random subset of each morph (planktivore n = 349, small benthic n = 300, silver n = 489, brown n = 123) we quantified *Diphyllbothrium spp.* in the body cavity and *Diplostomum spp.* in the right eye, using light microscopy for identification. Caudal muscle tissue was oven-dried at 60°C and ground to send 500ug to GLIER, University of Windsor, to quantify $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios. Age information was obtained by counting annuli of whole otolith sagittae submerged in water overnight and viewed with reflected light on a dark surface. Where we could not confidently assign fish sex due to underdeveloped gonads (208 fish), we used the male-specific marker sdY and

procedure described in Yano *et al.* (2012) to confirm sex. DNA was extracted from caudal muscle tissue and then PCR and agarose electrophoresis were used to identify presence of sdY.

Shape, trophic resource, and performance data

Shape, trophic resource, and performance metrics were required to estimate effects and function of shape within our path analytical hypothesis (Fig. 1a). Our focal morphs differ in life history characteristics, including our performance metric of growth (Malmquist *et al.*, 1992; Jónsson & Skúlason, 2000). Therefore, to explore natural selection at the most relevant level, path analyses were assessed within each morph. However, to facilitate interpretation of shape selection between coexisting morphs, our shape variables were calculated by pooling coexisting morphs within lakes. Strong covariance of within-morph shape variation with across-morph shape variation suggests that our variables are appropriate summaries of shape to explore within-morph patterns (Franklin, 2017). We therefore have two sets of shape variables, one per lake, because a formal comparison of shape variation between lakes is beyond the scope of this study.

Shape data were summarised in each lake using linear measures of pectoral fin length and geometric morphometric relative warps one (RW1) to five (RW5), which account for around 75% of shape variation captured by 20 landmarks (Fig. S1; Franklin, 2017). Relative warps, principal components of shape space interpretable as shape transformations along major axes of observed variation, were computed using TPS software (F. James Rohlf; <http://life.bio.sunysb.edu/morph>), from size- and position- corrected Procrustes scores (as Franklin, 2017). Briefly, the greatest axis of shape variation (RW1T; where T references Thingvallavatn) in Thingvallavatn describes variation in relative head size and elongation of the body, particularly in the caudal region, and variation in mouth position, whereas RW2T reflects mid-body depth. The greatest axis in Vatnshlíðarvatn (RW1V) describes variation in relative head size, with RW2V reflecting depth of body (Fig. 2). In both lakes, coexisting

morphs can be differentiated by morphology along relative warp axes (Fig. S2, Franklin. 2017).

Although we explored effects of RW1 to RW5 in each lake, here we present results for RW1 and RW2 which differentiate coexisting morphs and represent shape differences consistent with the expected variation in body-shape and mouth-position associated with trophic performance (Malmquist *et al.*, 1992; Jónsson & Skúlason, 2000; Franklin, 2017). Results for both lakes' RW3 to RW5 are contained in the appendix.

As a performance measure for path analyses, growth was estimated as the increase in backcalculated fork length for the last complete year of growth. This was measured by the most recent full otolith annulus, which relates more directly to observed shape and trophic proxies (below) than lifetime growth. Estimates of back-calculated length-at-age were obtained through relative sizes of otolith annuli, under the assumption that the relationship between somatic and otolith growth is linear throughout the age range in question (Casselman, 1990; Campana & Jones, 1992). To account for potential bias associated with statistical adjustments (Campana, 1990), we used a biological intercept of length at hatching of 20mm and otolith diameter of 0.1mm (from *Salvelinus malma*; Radtke *et al.* 1996). We backcalculated length-at-age as

$$L_i = L_c + (O_i - O_c) (L_c - L_o) (O_c - O_o)^{-1},$$

where L_i is estimated fish fork length at age i , L_c is fork length at capture, O_i is measured otolith radius at age i , O_c is otolith radius of outermost complete band at capture, L_o is fork length at biological intercept, and O_o is otolith radius at biological intercept, with all lengths in mm. Mean absolute error of observed with backcalculated size at age, calculated as $(L_i - L_c) / L_c * 100$, was 1.73% (standard deviation 2.28) in Vatnshlíðarvatn, and 1.60% (standard deviation 2.23) in Thingvallavatn. As an independent performance measure, we calculated condition factor as residuals of a regression of total body mass on fork length within each morph (Jakob *et al.*, 1996).

We used estimates of recent growth and condition factor to estimate effects of shape on fitness. Due to difficulties assessing fitness directly in aquatic populations, we use performance measures related to our focal traits and trophic performance, and which we expect to affect fitness. Although there are exceptions, generally growth rate and condition factor have positive effects on fitness in fish through survival, mating success and fecundity (e.g. Dickerson *et al.* 2002; Foote 1990; Morita & Takashima 1998; Wootton 1973; Bolnick & Araújo 2011). Because we lack estimates of growth-fitness or condition factor-fitness relationships in our study populations, our estimates are performance gradients (Arnold, 1983) and cannot be interpreted quantitatively as selection gradients. However, under the assumption that these relationships are monotonic, performance gradients should reflect qualitative features of the true selective surface (Franklin & Morrissey 2017).

Our proxies of trophic resource use are stable isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and counts of *Diphyllbothrium* and *Diplostomum* parasite infections. Benthic primary producers integrate less ^{12}C (greater $\delta^{13}\text{C}$) than pelagic producers due to less turbulence in benthic boundary layers (Hecky & Hesslein, 1995), and ^{15}N is enriched (greater $\delta^{15}\text{N}$) with each additional trophic level (Minagawa & Wada, 1984). Together, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provide estimates of time-integrated use of trophic resources (e.g. Knudsen *et al.*, 2014; Berchtold *et al.*, 2015). In Thingvallavatn, lower $\delta^{13}\text{C}$ values associate with consumption of Copepoda and greater $\delta^{13}\text{C}$ values associate with *Lymnaea*, while Cladocera and Chironomidae exhibit intermediate values. Planktivore and small benthic charr exhibit variation in $\delta^{13}\text{C}$ corresponding to planktivorous and benthic diet expectations, with a degree of overlap potentially due to use of larval (small benthic) or emerging (planktivore) Chironomidae (Malmquist *et al.*, 1992; Franklin, 2017). In Vatnshlíðarvatn, greater $\delta^{13}\text{C}$ associates with increased resource breadth (more taxa identified in gut contents), and morphs differ in $\delta^{13}\text{C}$ values consistent with specialist and generalist diets (Franklin, 2017). In addition to quantifying resource use over several months using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Guelinckx *et al.*, 2007), counts of trophically-transmitted parasites are a proxy for cumulative consumption of Copepoda and *Lymnaea* (Bérubé & Curtis, 1986;

Wilson *et al.*, 2002; Voutilainen *et al.*, 2008; Henriksen *et al.*, 2015). *Diphyllbothrium* intensity increases with cumulative consumption of Copepoda, and is greater in the planktivore charr of Thingvallavatn and the silver charr of Vatnshlíðarvatn, whereas intensity of *Diplostomum*, transmitted via the water column from *Lymnaea* hosts, is greater in small benthic and brown charr (Franklin, 2017), controlling for size in both cases. Although *Diplostomum* can cause cataracts, and *Diphyllbothrium* become encysted in the body cavity (Bérubé & Curtis, 1986; Voutilainen *et al.*, 2008), the greater parasite intensities observed in larger fish, including within cohorts (Franklin, 2017), suggests that energetic benefits of resource consumption may generally outweigh detrimental effects of parasitism.

Performance-based inference of selection

We estimated directional and quadratic performance gradients (Arnold 1983) using lakewide-standardised (across morph) data to assess indications of diversifying selection independent of morph designations. Within each lake our performance measure of growth was relativised by dividing by mean growth across morphs and regressed on z-standardised shape traits (RW1 to RW5 and relative pectoral fin length), including covariates of age, sex, residual gonad mass, and fork length; residual gonad mass are estimates of gonadal investment calculated as residuals of regressions of gonad mass on total body mass. Positive quadratic coefficients along with identification of a growth minimum within the range of phenotypes are indicative of diversifying selection. We then used a path analytical approach, using within-morph data throughout, to explore functional relationships among shape, resource use, and relative growth. The total effects of shape on relative growth can be considered ‘extended performance gradients’ in the manner of extended selection gradients (η ; Morrissey, 2014). These total effects are the sum of all effects of shape on growth, via measured or unmeasured pathways, and can qualitatively reflect selection for shape (Franklin & Morrissey, 2017). Total effects can be divided into indirect and direct effects on growth:

indirect effects involve mediating variables determined by functional hypotheses (here, trophic proxies of isotopic signatures and parasite infections; Fig. 1a); direct effects are any effects for which intermediate variables are not included (Shipley, 2000). This partitioning allows us to identify the presence of diversifying selection (total effects), and determine if shape's effects are via trophic performance, parasitism, or unmeasured non-trophic functions.

Our path model (Fig. 1a) relating traits to growth presents a hypothesis whereby relative growth is affected by shape traits and resource use, represented by parasite infections over the long term and stable isotopic signatures over several months. Parasite infections are affected by shape traits and stable isotopic signatures. Stable isotopic signatures are affected by shape traits. Relative growth is individual growth divided by mean growth within morph, parasite infections are log counts, shape variables are relative warps 1 through 5 and residuals of pectoral fin length regressed on fork length. In addition, each path includes covariates of fork length (mm), sex (binary), residual gonad mass, and age (continuous covariate). The path-model equations are detailed in the appendix. Although effects on growth rate might vary inter-annually, we assume cumulative growth over several years is positively associated with fitness, allowing us to pool sampling years and improve statistical power. We log-transformed parasite data prior to inclusion in the path model, ensuring that there were minimal (and no qualitative) differences between the log-transformed models and log-link negative binomial models. We then z-standardised all variables except relative growth, and used the lavaan package in R (Rosseel, 2012) to carry out path analyses with bootstrapped standard errors on the data as provided. We repeated path analyses using condition factor as an independent response variable. We refer to our trophic pathways as capturing effects via differential prey resource use, yet there is invariably trophic variation that we cannot include such as physiological variation (Afik & Karasov, 1995; Pörtner *et al.*, 2013), or variation within isotopic signatures (e.g. different-sized prey may not differ in isotopic signature, but may differ in profitability). Despite these limitations, we

expect to observe effects of shape on growth via our trophic pathways, as implied by prior research suggesting adaptation to differential use of prey taxa (e.g. Malmquist *et al.* 1992; Jónsson & Skúlason 2000). Having estimated path coefficients, we compared coefficients between coexisting morphs using two-sample z-tests, $z = (y_a - y_b) / \sqrt{(se_a^2 + se_b^2)}$, where y is the path coefficient, se is the associated standard error, and subscripts denote compared morphs.

Size-dependent mortality

To address a component of the growth-fitness relationship we explored size-dependent mortality within morphs by comparing frequency distributions of backcalculated length at a given age for fish caught in 2014 to those caught in 2016. The 2014 sample of a particular cohort represents an initial population, while the 2016 sample of the same cohort represents the subset of the population that survived a two-year selective period. A systematic difference in mean length (measured at a common age) suggests size-dependent mortality: if selection were for larger individuals, as is generally expected in fishes (e.g. Sogard, 1997; Morita & Takashima, 1998), the 2016 sample of fish would have a greater mean back-calculated length at the given age. This approach allowed us to account for inter-annual variation in size-dependent mortality by comparing only fish from the same cohort. For example, we compared age 5+ caught fish in 2016 (after selection) with age 3+ caught fish in 2014 (before selection) with length back-calculated to age 3+ in both cases. We calculated directional selection differentials within each morph by deducting the 2014 size data from the 2016 size data, using z-standardised size data, and assessing deviation from zero with t-tests ($p < 0.05$). Nonlinear selection differentials were calculated by deducting the variance of 2014 size data from the variance of the 2016 size data, then adding the squared value of the directional selection differential to account for reductions in variance associated with directional selection; F-tests assessed significant deviation from zero at $p < 0.05$.

Results

Lakewide performance gradients revealed effects of shape on growth that are consistent with diversifying selection in both lakes, but only in Thingvallavatn did they reflect morphology associated with trophic expectations. In Thingvallavatn, quadratic RW1T was positive ($g_{936}=0.051$, $se=0.020$, $p=0.012$; Table 1) indicating intermediate shapes experiencing reduced growth relative to extremes on an axis summarising head size, mouth position, and body elongation. In Vatnshlíðarvatn, quadratic RW1V and RW5V were negative, indicating intermediates experiencing increased relative growth; although RW4V was positive ($g_{1199}=0.040$, $se=0.016$, $p=0.011$; Table 1), this reflects an aspect of shape for which no *a priori* expectations exist. These nonlinear effects, including whether they are mediated by trophic resource use, were further investigated by exploring within-morph patterns in path analyses.

(a) Total effects of shape on growth

Shape affects growth in opposing directions between coexisting morphs in Thingvallavatn but not in Vatnshlíðarvatn. In Thingvallavatn, consistent with expectations, RW1T has a positive total effect on growth in planktivore charr ($b_{305}=0.039$, $se=0.020$, $p=0.046$) but a negative total effect in small benthic charr ($b_{269}=-0.047$, $se=0.024$, $p=0.051$; Table 2 details effects of RW1T and RW2T, see Tables S2 and S3 for further shape variables). These morph estimates differ significantly from each other ($p=0.006$, z-test, Table 2). Because RW1T involves variation in head size, mouth position, and elongation of the caudal region, these results support *a priori* predictions of diversifying selection for shape. Additionally, a negative effect of RW2T on growth detected in small benthic charr was absent in planktivore charr, representing an effect of mid-body depth differing significantly between morphs ($p=0.007$, z-test, Table 2). In Vatnshlíðarvatn, RW1V had positive effects on growth in both morphs while RW2V has negative effects significant only in silver charr (Table 3; Tables S4 and S5 for further shape variables). Brown and silver charr do not differ in total effects on growth for any shape variables ($p>0.05$, z-tests, Table S6). Rather, relatively larger heads (RW1V) and shallower bodies

(RW2V) associate with increased growth in both morphs. These total effects allow inferences regarding the form of selection. To explore function, we examined relationships among trophic resources and growth.

(b) Effects of trophic resource use on growth

Trophic resource use appears to affect growth only in planktivore and silver charr, largely due to detrimental effects of parasitism. No resource proxies significantly associated with growth for small benthic charr, though $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ positively associate with *Diplostomum* infections (Table 2a). For planktivore charr, isotopic signatures associate with growth only through *Diphyllbothrium* and *Diplostomum* infections, which have negative effects (Table 2a, Fig. 1b). In Vatnshlíðarvatn, though trophic resource proxies had no significant associations with each other or with growth for brown charr, $\delta^{13}\text{C}$ did have a marginally non-significant ($p=0.08$) negative effect on growth (Table 3a). A significant negative effect of $\delta^{13}\text{C}$ on growth was identified in silver charr, in addition to increasing *Diplostomum* infections which further negatively affected growth (Table 3a).

(c) Effects of shape on growth via trophic resources

Although shape affected growth via trophic resources in planktivore and silver charr, the mechanism is not consistent with expectations of trade-offs influencing trophic performance. For planktivore charr, greater RW1T increased growth by increasing $\delta^{13}\text{C}$, which reduced detrimental *Diphyllbothrium* infections (Fig. 1b, Table 2). Although covariance of shape and growth is as predicted, the details differ: more streamlined individuals with terminal mouths (positive RW1T) have increased growth because they consume fewer Copepoda (increased $\delta^{13}\text{C}$) and experience lower copepod-transmitted parasite infections. Other aspects of shape that affect planktivore charr growth via trophic pathways, RW2T and RW3T, follow the same pattern (Table 2, Table S3). In silver

charr RW3V and RW4V affect growth by increasing $\delta^{13}\text{C}$ and *Diplostomum* infections, respectively ($p < 0.05$; Table S5 a-c). However, these aspects of shape did not have a significant total effect on growth, suggesting opposing effects via non-trophic pathways. In small benthic and brown charr, trophic resources did not associate with growth, and so there were no effects of shape on growth via trophic pathways. There were however associations suggesting shape-dependent resource-use: greater RW3T increased *Diplostomum* infection, and RW4T and pectoral fin length influenced isotopic signature in small benthic charr (Table S3a), while for brown charr greater RW1V increased *Diplostomum* infection (Table 3). The lack of effects on growth via trophic resources and the presence of total effects of shape suggest non-trophic effects of shape for these morphs also.

Direct effects of shape on growth

Effects of shape on growth independent of trophic resource pathways are large compared with effects via trophic pathways. The total effects of RW1T and RW2T on growth in small benthic charr that oppose those in planktivore charr are largely due to differing direct effects (RW1T $p = 0.022$, RW2T $p = 0.001$, z-test, Table 2), rather than effects via trophic pathways. The magnitude of direct effects in both Vatnshlíðarvatn morphs are considerably larger than effects via trophic pathways, and do not differ between morphs (Table 3, Tables S4 and S5). In addition, effects of shape via trophic pathways in planktivore and silver charr are often opposed by direct effects, nullifying their net influence on growth (e.g. planktivore RW2T, Table 2a and c; silver RW3V and RW4V, Table S5a and c). The associations between shape and growth that could not be explained by trophic resource pathways suggest that non-trophic functions of shape may play an important selective role.

Repeating path analyses with condition factor as a response revealed no qualitative differences via trophic pathways to those presented above. Although direct effects of shape on condition differed to the equivalent effects on growth, due to covariance of deeper bodies with greater condition factor, condition factor revealed no additional opposing effects among morphs (data not shown).

Size-dependent mortality

Differences in size-dependent mortality between coexisting morphs were evident in both lakes.

Small benthic charr experienced selection for smaller sizes between 2014 and 2016, significant in age comparisons of 2 to 4 (before selection: 101 mm, after: 89 mm, $S=-0.607$, $se=0.134$, $t_{83}=-3.78$; Table 4) and 3 to 5 (before: 121 mm, after: 109 mm, $S=-0.474$, $se=0.125$, $t_{81}=-3.13$), whereas planktivore charr experienced selection for larger sizes over the same period, significant in age comparisons of 4 to 6 (before: 135 mm, after: 152 mm, $S=0.323$, $se=0.072$, $t_{379}=3.47$) and 5 to 7 (before: 161 mm, after: 181 mm, $S=-0.540$, $se=0.098$, $t_{165}=4.59$). Although we obtained no statistically significant selection differentials in other age classes, effect sizes suggest it is advantageous for small benthic charr to be smaller at times when larger planktivore charr are advantaged. In Vatnshlíðarvatn, silver charr experienced selection of larger sizes in all age-classes, whereas there was no size selection in brown charr that significantly differed from zero, though estimates were positive (Table 4). There were no significant estimates of nonlinear size-dependent mortality in any morph (data not shown).

Discussion

Complex relationships among various ecological factors promote morphological diversity even in relatively simple and young polymorphic systems, as demonstrated through our novel use of the extended selection gradient path analytical approach. Contrary to general expectations (e.g. Malmquist *et al.*, 1992; Robinson & Wilson, 1994; Arnegard *et al.*, 2014), morphological trade-offs contributed little to diversifying selection via effects on trophic performance. In Thingvallavatn, the lake with greater phenotypic differentiation, morphologically intermediate individuals experienced reduced relative growth, indicating disruptive selection of shape if growth is monotonically related to fitness (Franklin & Morrissey, 2017). This diversifying selection is mediated by detrimental effects of *Diphyllbothrium* (Copepoda-transmitted tapeworms) and effects of shape on growth independent of resource use variation. In Vatnshlíðarvatn we found no support for diversifying

selection and shape predominantly affected growth independent of resource use. Our observations of parasite-mediated diversifying selection, non-trophic effects of shape, and contrasting patterns of size-dependent mortality between morphs, suggest a limited role for morphological-trade offs influencing fitness via trophic performance under natural conditions.

Effects via trophic resource use

Effects of shape on growth via trophic resource use are mediated by parasitism and suggest alternative functional explanations to that of prey capture efficiency. In Thingvallavatn, streamlined-bodied planktivore charr (increased RW1T) experience greater growth by consuming fewer Copepoda (greater $\delta^{13}\text{C}$), thus experiencing lower *Diphyllbothrium* infections. This may reduce subsequent internal haemorrhaging (Bérubé & Curtis, 1986). The shape-dependent use of Copepoda, and subsequent parasite exposure, appears not to result from biomechanical limitations involving prey capture: Thingvallavatn planktivore charr frequently consume *Daphnia* which are less parasitised and more easily captured than Copepoda (Malmquist, 1992; Malmquist *et al.*, 1992; Franklin, 2017). Alternative explanations for shape-dependent resource use may involve hydrodynamic demands associated with behaviours such as increased search behaviours, and/or boldness in foraging farther from the shoal (Ehlinger, 1990; Mikheev *et al.*, 1996; Adams, 2004; Edelsparre *et al.*, 2013). Shape-dependent parasitism was also evident in small benthic and brown charr, but without subsequent effects on growth. Variation in parasitism may result from differential use of the spatial environment, as suggested by the prevalent *Diplostomum* infections of brown charr despite rare consumption of *Lymnaea* (Jónsson & Skúlason, 2000; Franklin, 2017).

Where resource use variation did affect growth independent of parasitism, it did not contribute to diversifying selection. In Vatnshlíðarvatn consumption of fewer taxa associated with greater growth, contrary to expectations for the generalist silver charr (Jónsson & Skúlason, 2000). In addition, shape did not influence variation in resource breadth as would be necessary to support a morphological

trade-off hypothesis between the specialist and generalist morphs. Therefore, morphological trade-offs do not contribute to Vatnshlíðarvatn diversification via resource use under contemporary conditions, notwithstanding fluctuations over timescales greater than our three-year study (e.g. Wilson & Yoshimura, 1994).

Non-trophic effects

Shape affected growth independent of resource use variation, and we suggest this arises in part from biomechanical constraints of swimming behaviours associated with predator avoidance and escape (Webb, 1975; Bolnick & Araújo, 2011; Ellerby & Gerry, 2011; Samways *et al.*, 2015).

Interindividual variation in predator-avoidance behaviour often associates with morphology (Mikheev *et al.*, 1996; Hawley *et al.*, 2016), and in Thingvallavatn behavioural differences are evident. Planktivore charr exhibit shoaling, reluctance to forage without conspecifics, and spend considerable time hovering in the water column. Small benthic charr travel independently, are often stationary on the substrate and utilise refuges within the complex lava substrate (Malmquist, 1992; Sandlund *et al.*, 1992; Skúlason *et al.*, 1993; Kristjánsson *et al.*, 2011). Such behavioural variation could result in diversifying selection of shape via energetic costs of locomotion, independent of trophic resource use.

Predation may play a role in polymorphism in Thingvallavatn and Vatnshlíðarvatn through size-selective mortality and behavioural responses. Predation as a cause of divergence has received considerable support (Rundle *et al.*, 2003; Langerhans & Reznick, 2009; Scharnweber *et al.*, 2013) and our selection differentials indicate that size-dependent mortality differs in direction (Thingvallavatn) or magnitude (Vatnshlíðarvatn) between coexisting morphs. Predation on intermediate-sized fish could act as a diversifying mechanism and could promote diversification in charr behavioural responses (Doucette *et al.* 2004; Parsons, 2008). Subsequent contrasting locomotive costs, prey and parasite exposure, or spawning behaviour, could have morphological

implications (Reznick, 1983; Stearns, 1989; Hutchings, 1993). In both lakes, differences between coexisting morphs in behaviour associated with predation (Malmquist, 1992; Skúlason *et al.*, 1993; Parsons, 2008), in growth rate and life history (Sandlund *et al.*, 1992; Jónsson & Skúlason, 2000), and our data suggesting non-trophic costs of shape and size-dependent mortality, may together suggest a diversifying role for predation.

Implications

We found little support for morphological trade-offs contributing to polymorphism through trophic performance under contemporary conditions. However, colonising charr 10,000 years ago experienced different conditions to today's populations, and a complex of interacting ecological factors may have contributed to initiation and maintenance of polymorphism. Whether or not biomechanical constraints influencing trophic performance were an initial cause of morphological polymorphism, this study has demonstrated that resource polymorphisms can persist in its absence, despite potential gene flow.

Our sampling approach allowed us to make inferences regarding selection via trophic resources, but naturally there were limitations. In Thingvallavatn it is possible that morph subpopulations exist, which might vary in phenotype, resource availability, and gene flow (Sandlund *et al.*, 1992; Kapralova *et al.*, 2011). We sampled in the littoral zone, obtaining planktivore charr that were likely more similar ecologically to small benthic charr compared with those in pelagic zones. This allowed us to examine selective processes where overlap in phenotype and resource use was most likely, and therefore to speculate on the fitness of potential inter-morph hybrids. During sampling we were also unable to sample the youngest cohorts adequately and may have overlooked selection via trophic pathways in young of the year. Although we expect competition in early life stages to be strong (Perez & Munch, 2010), reduced variation in morphology and feeding performance early in ontogeny (Skúlason *et al.*, 1989b; Parsons, 2008) and limited juvenile diet breadth (Sandlund *et al.*, 1988)

suggest that selection likely results from interference competition and variation in size rather than shape. However, as suggested by differences in size among morphs, competition in the nursery grounds might contribute to morph formation if size thresholds trigger faster growing individuals to switch to pelagic environments with different ecological conditions (Byström *et al.*, 2014).

We estimated performance gradients, which are qualitatively indicative of selection gradients when growth and fitness are monotonically related in the wild (Arnold, 1983; Franklin & Morrissey, 2017).

Although the general expectation for fish is that increased growth results in greater fitness, this is not always the case (Dickerson *et al.*, 2002; Foote, 1990; Morita & Takashima, 1998; Barber *et al.*, 2001; Carlson *et al.*, 2008), and our data indicate that increased size can reduce survival of small benthic charr. Furthermore, differences in age at maturity and asymptotic size between coexisting morphs in both Thingvallavatn and Vatnshlíðarvatn (Jonsson *et al.* 1988; Skúlason *et al.*, 1996; Jónsson & Skúlason, 2000) are suggestive of fitness component trade-offs resulting from different growth-fitness relationships between morphs (Reznick, 1983; Stearns, 1989; Hutchings, 1993). The potential for non-monotonic growth-fitness relationships may have implications for the forms of selection experienced in these systems, though this would not invalidate our observations regarding the roles of morphology via trophic performance. Measurement of growth-fitness relationships in the wild remains necessary to quantify fitness effects of parasitism and morphology via non-trophic functions.

In conclusion, this study demonstrates that phenotype contributes to performance via multiple functions and that consideration of a complex of local ecological factors is necessary to understand diversification. We identified parasitism, non-trophic effects of shape, and size as factors likely influencing fitness in resource polymorphisms, with indications of disruptive selection of shape in Thingvallavatn and differing size-dependent mortality between morphs in both lakes. Because alternative functions can produce similar trait-performance covariance patterns, we stress the importance of measuring the nature of selection in the wild. Our observations using path analyses

and individual-level data revealed effects of shape inconsistent with assumptions based on intuitive morph categories. Phenotypic trade-offs influencing trophic performance have been identified or assumed in numerous systems (e.g. Price, 1987; Schluter, 1995), yet there remain few studies of diversifying selection in the wild, within few focal systems (Kingsolver *et al.*, 2001; Siepielski *et al.*, 2013). This study therefore represents a valuable contribution to understanding early diversification in nature. Finally, if morphological trade-offs do not influence trophic performance under contemporary conditions, this suggests either that in these relatively simple and young systems, processes contributing to initial morphological diversification differ from those that now maintain them, or that such trade-offs did not contribute to morphological diversification. In either case, this highlights the need for a more nuanced understanding of the selective processes that result in intraspecific diversity.

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Tables and Figures

Table 1: Standardised lakewide (across-morph) performance gradients of relativised growth regressed on variance-standardised shape traits, for Arctic charr from the Icelandic lakes

Thingvallavatn and Vatnshlíðarvatn. RW refers to relative warps, Pec fins to pectoral fin length, and square terms indicate quadratic estimates, p values below 0.05 are bold. Growth is relativised by dividing by mean growth across morphs within each lake, covariates included were age, sex, fork length and residual gonad mass. The presence of nonlinear effects in these lakewide comparisons suggests selection may operate in opposing directions between morphs. See Fig. S3 for wireframe depictions of RW1-5.

Variance-standardised traits	Thingvallavatn			Vatnshlíðarvatn		
	Estimate	Std. error	p. value	Estimate	Std. error	p. value
RW1	0.065	0.017	0.000	0.061	0.021	0.003
RW2	0.023	0.011	0.033	-0.102	0.015	0.000
RW3	-0.008	0.010	0.457	0.013	0.012	0.265
RW4	-0.002	0.010	0.855	0.045	0.013	0.000
RW5	-0.046	0.010	0.000	-0.006	0.013	0.653
Pec fins	0.008	0.014	0.560	-0.042	0.019	0.028
RW1 ²	0.051	0.020	0.012	-0.041	0.020	0.038
RW2 ²	0.009	0.014	0.509	-0.014	0.020	0.483
RW3 ²	-0.006	0.014	0.672	0.010	0.015	0.495
RW4 ²	0.003	0.010	0.800	0.040	0.016	0.011
RW5 ²	-0.008	0.014	0.567	-0.047	0.015	0.002
Pec fins ²	0.017	0.012	0.177	0.013	0.013	0.346

Table 2: Path coefficients detailing effects of shape (RW1T and RW2T) on growth within samples of small benthic and planktivore Arctic charr morphs from the Icelandic lake Thingvallavatn, and z-tests comparing effects between morphs. Standardised path coefficients (a), compound path coefficients (b), effects via trophic proxies (c), and total effects (d) from the path diagram relating shape (Fig.2) to relative growth (Fig. 1a), with covariates of age, sex, residual gonad mass, and fork length. Coefficients are displayed (standard errors in parentheses) except z-test columns which display p-values. Bolded values are significant at $p=0.05$ (significant path coefficients differ from zero; z-test significance indicates morph coefficients differ). Growth was relativised by dividing by mean growth within morph. Greater $\delta^{13}\text{C}$ indicates a benthic diet in Thingvallavatn, greater $\delta^{15}\text{N}$ indicates higher trophic level, *Diplostomum* are flukes with snail intermediate hosts, *Diphyllbothrium* are tapeworms with copepod intermediate hosts. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are included separately in (a) for univariate coefficients. Because $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ together produce an estimate of resource use, in compound path coefficients (b) they are not considered independently.

	small benthic						planktivore						z-test	
	RW1	RW2	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>Diplo.</i>	<i>Diph.</i>	RW1	RW2	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>Diplo.</i>	<i>Diph.</i>	RW1	RW2
(a) path coefficients, detailing effect of column variable on row. Bottom row is direct effects of traits or trophic proxies on relative growth													p-values	
$\delta^{13}\text{C}$	-0.106	-0.108					0.148	-0.150					0.015	0.650
	(0.078)	(0.064)					(0.069)	(0.067)						
$\delta^{15}\text{N}$	0.021	-0.029					-0.139	0.211					0.121	0.009
	(0.073)	(0.059)					(0.073)	(0.070)						
<i>Diplostomum</i>	0.115	-0.056	0.260	0.130			-0.019	0.027	0.355	0.045			0.097	0.245
	(0.061)	(0.050)	(0.047)	(0.051)			(0.053)	(0.051)	(0.043)	(0.041)				
<i>Diphyllbothrium</i>	0.007	0.008	-0.037	-0.058			-0.056	0.091	-0.322	0.250			0.494	0.287
	(0.082)	(0.067)	(0.063)	(0.068)			(0.042)	(0.040)	(0.034)	(0.032)				
Relative growth	-0.045	-0.062	0.007	0.010	-0.021	0.010	0.025	0.033	0.013	0.014	-0.049	-0.109	0.022	0.001
	(0.024)	(0.020)	(0.020)	(0.021)	(0.024)	(0.018)	(0.019)	(0.019)	(0.019)	(0.016)	(0.021)	(0.026)		

(b) compound path coefficients. Summaries of indirect effects of traits (columns) on relative growth via trophic pathways (rows)

Effect on relative growth via:

<i>isotopes</i>	-0.001 (0.002)	-0.001 (0.002)	0.000 (0.004)	0.001 (0.005)	0.823	0.710
<i>isotopes via Diphyllbothrium</i>	0.000 (0.000)	0.000 (0.000)	0.009 (0.004)	-0.011 (0.004)	0.024	0.006
<i>isotopes via Diplostomum</i>	0.001 (0.001)	0.001 (0.001)	-0.002 (0.002)	0.002 (0.002)	0.180	0.655
<i>Diplostomum</i>	-0.002 (0.003)	0.001 (0.002)	0.001 (0.003)	-0.001 (0.003)	0.480	0.579
<i>Diphyllbothrium</i>	0.000 (0.001)	0.000 (0.001)	0.006 (0.005)	-0.010 (0.005)	0.239	0.050

(c) effects of traits on relative growth via trophic pathways (sum of indirect effects)

Relative growth	-0.002 (0.004)	0.001 (0.003)	0.014 (0.007)	-0.019 (0.007)	0.047	0.006
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(d) total effects of traits on relative growth, reflecting extended performance gradients (sum of all indirect & direct effects of traits on growth)

Relative growth	-0.047 (0.024)	-0.061 (0.020)	0.039 (0.020)	0.014 (0.019)	0.006	0.007
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Table 3: Path coefficients detailing effects of shape (RW1V and RW2V) on growth within samples of brown and silver Arctic charr morphs from the Icelandic lake Vatnshlíðarvatn, and z-tests comparing effects between morphs. Standardised path coefficients (a), compound path coefficients (b), effects via trophic proxies (c), and total effects (d) from the path diagram relating shape (Fig. 2) to relative growth (Fig. 1a), with covariates of age, sex, residual gonad mass, and fork length. Coefficients are displayed (standard errors in parentheses) except z-test columns which display p-values. Bolded values are significant at $p=0.05$ (significant path coefficients differ from zero; z-test significance indicates morph coefficients differ). Growth was relativised by dividing by mean growth within morph. Greater $\delta^{13}\text{C}$ indicates greater resource breadth in Vatnshlíðarvatn, greater $\delta^{15}\text{N}$ indicates higher trophic level, *Diplostomum* are flukes with snail intermediate hosts, *Diphyllbothrium* are tapeworms with copepod intermediate hosts. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are included separately in (a) for univariate coefficients. Because $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ together produce an estimate of resource use, in compound path coefficients (b) they are not considered independently.

	brown						silver						z-test	
	RW1	RW2	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>Diplo.</i>	<i>Diph.</i>	RW1	RW2	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	<i>Diplo.</i>	<i>Diph.</i>	RW1	RW2
(a) path coefficients, detailing effect of column variable on row. Bottom row is direct effects of traits or trophic proxies on relative growth													p-values	
$\delta^{13}\text{C}$	-0.020	0.024					0.034	-0.033					0.691	0.665
	(0.124)	(0.122)					(0.056)	(0.049)						
$\delta^{15}\text{N}$	-0.015	-0.172					-0.010	-0.078					0.973	0.515
	(0.131)	(0.129)					(0.074)	(0.065)						
<i>Diplostomum</i>	0.270	-0.084	0.067	-0.048			0.121	-0.238	0.220	0.088			0.170	0.143
	(0.097)	(0.096)	(0.077)	(0.073)			(0.049)	(0.043)	(0.041)	(0.031)				
<i>Diphyllbothrium</i>	0.129	-0.224	-0.062	0.167			0.034	-0.188	0.004	0.001			0.522	0.801
	(0.129)	(0.128)	(0.102)	(0.097)			(0.073)	(0.064)	(0.062)	(0.047)				
Relative growth	0.088	-0.034	-0.064	0.051	0.025	0.006	0.092	-0.084	-0.064	0.014	-0.061	0.008	0.939	0.323
	(0.046)	(0.045)	(0.036)	(0.034)	(0.045)	(0.034)	(0.025)	(0.023)	(0.021)	(0.016)	(0.024)	(0.016)		

(b) compound path coefficients. Summaries of indirect effects of traits (columns) on relative growth via trophic pathways (rows)

Effect on relative growth via:

<i>isotopes</i>	0.001	-0.010	-0.002	0.001	0.781	0.385
	(0.010)	(0.012)	(0.004)	(0.004)		
<i>isotopes via Diphyllbothrium</i>	0.000	0.000	0.000	0.000	1.000	1.000
	(0.000)	(0.001)	(0.000)	(0.000)		
<i>isotopes via Diplostomum</i>	0.000	0.000	0.000	0.001	1.000	0.480
	(0.000)	(0.001)	(0.001)	(0.001)		
<i>Diplostomum</i>	0.007	-0.002	-0.007	0.015	0.268	0.018
	(0.012)	(0.004)	(0.004)	(0.006)		
<i>Diphyllbothrium</i>	0.001	-0.001	0.000	-0.002	0.808	0.907
	(0.004)	(0.008)	(0.001)	(0.003)		

(c) effects of traits on relative growth via trophic pathways (sum of indirect effects)

Relative growth	0.008	-0.014	-0.010	0.015	0.318	0.088
	(0.017)	(0.015)	(0.006)	(0.008)		

(d) total effects of traits on relative growth, reflecting extended performance gradients (sum of all indirect & direct effects of traits on growth)

Relative growth	0.096	-0.048	0.082	-0.069	0.789	0.675
	(0.046)	(0.045)	(0.025)	(0.022)		

Table 4: Size-dependent mortality within cohorts from 2014 to 2016 for Arctic charr morphs from two Icelandic lakes, Thingvallavatn and Vatnshlíðarvatn. Estimated mean cohort fork length before selection, after selection, directional selection differentials (*S*), with bolded values significant at $p < 0.05$ (t-test), standard errors, and p-values. Age comparisons are within cohorts of each morph, using back-calculated length at a given age to estimate the difference between mean length of the cohort before selection (caught at younger age) to that of individuals that survived selection (caught at older age).

Thingvallavatn										
age comparison	before, mm	after, mm	small benthic			before, mm	after, mm	planktivore		
			<i>S</i>	<i>se</i>	p-value			<i>S</i>	<i>se</i>	p-value
2 to 4	101	89	- 0.607	0.134	0.000	100	95	- 0.034	0.096	0.798
3 to 5	121	109	- 0.474	0.125	0.002	114	121	0.127	0.086	0.251
4 to 6	137	133	- 0.129	0.174	0.534	135	152	0.323	0.072	0.001
5 to 7	143	158	0.454	0.259	0.127	161	181	0.540	0.098	0.000
6 to 8						186	191	0.189	0.215	0.411

Vatnshlíðarvatn										
age comparison	before, mm	after, mm	brown			before, mm	after, mm	silver		
			<i>S</i>	<i>se</i>	p-value			<i>S</i>	<i>se</i>	p-value
2 to 4	116	124	0.097	0.121	0.555	112	140	0.510	0.049	0.000
3 to 5	149	153	0.205	0.289	0.511	169	191	0.473	0.059	0.000
4 to 6	170	170	0.021	0.345	0.949	209	224	0.583	0.107	0.000

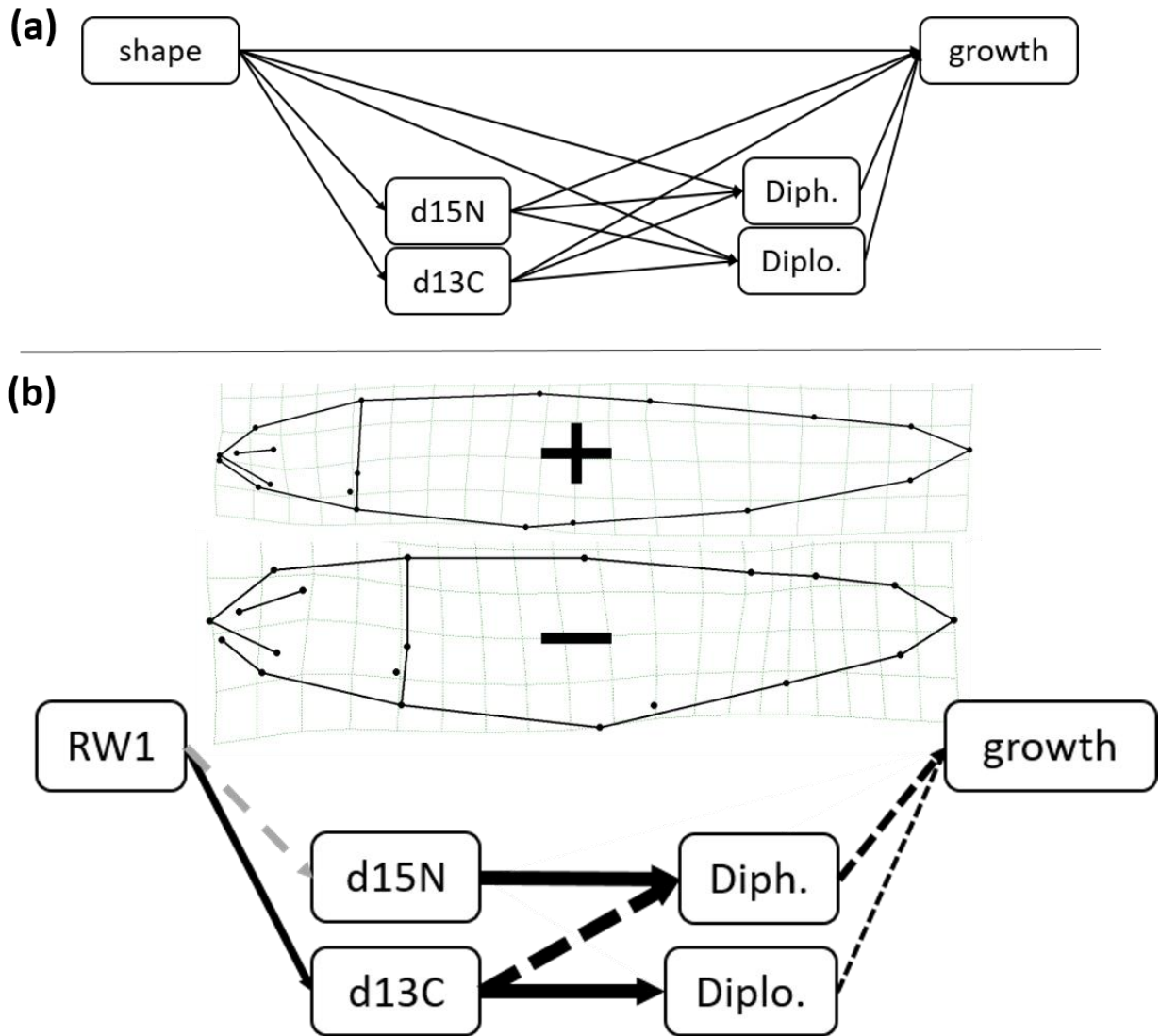


Figure 1: Path diagrams illustrating (a) all path coefficients incorporated into models, and (b) effects estimated for the first relative warp of the planktivore Arctic charr morph from Thingvallavatn, Iceland. In (a) arrows connecting shape, stable isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), and parasite infections (Diph., Diplo.), show how shape could indirectly affect growth via trophic pathways. The arrow directly connecting shape and growth accounts for non-trophic effects of shape on growth. The sum of all pathways reveals the total effects of shape on growth. (b) Wireframe depictions and path diagram of Thingvallavatn first relative warp (RW1) summarising effects of shape variation on growth in planktivore charr. Diph refers to *Diphyllbothrium*, Diplo to *Diplostomum*, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are stable isotopic signatures, and growth is relative back-calculated growth over one year. Solid lines depict positive relationships, dashed lines negative, with thickness reflecting the magnitude of path coefficients. Black lines are significant at $p < 0.05$, grey lines are $p < 0.10$, other path coefficients omitted; see Tables 2 - 3 (RW1 and RW2) or Tables S2 - S5 (all shape variables) for full details. Notably, effects of stable isotopic signatures on growth are mediated by parasitism, and effects of shape on growth are accounted for by trophic pathways.

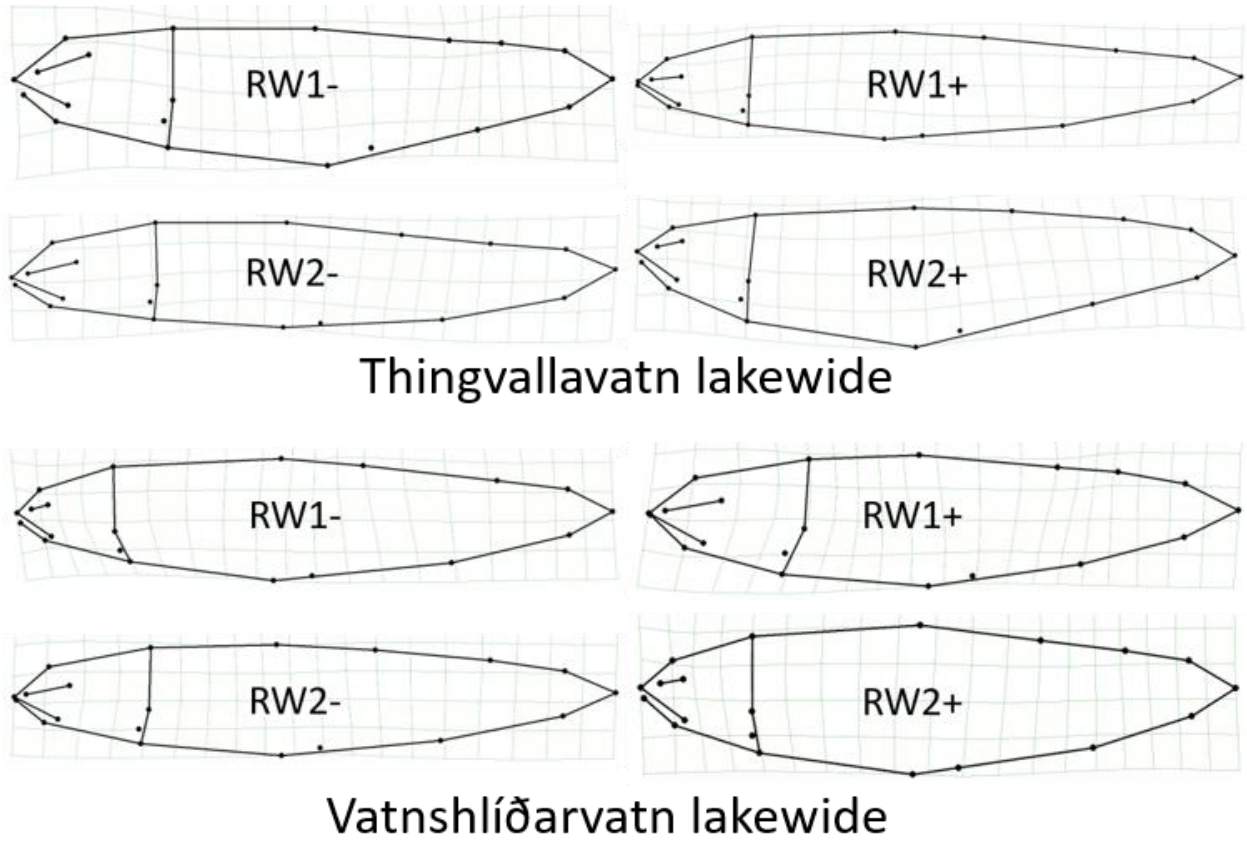


Figure 2: Wireframe representations summarising the first two principal axes of shape variation (RW1, RW2) of Arctic charr morphs from the Icelandic lakes Thingvallavatn and Vatnshlíðarvatn. Thingvallavatn RW1T summarises 43.98%, and RW2T 10.34%, of lakewide shape variation captured by landmarks. Vatnshlíðarvatn RW1V summarises 31.05%, and RW2V 19.18%, of lakewide shape variation captured by landmarks. Wireframe depictions of RW3 to RW5 are in supplementary materials (Fig. S3)