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Intraspecific diversity in Arctic charr, *Salvelinus alpinus*, in Iceland: II. Which environmental factors influence resource polymorphism in lakes?

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ABSTRACT

Background: The mechanisms causing resource polymorphism are not well understood, but likely include frequency-dependent selection. However, other selection mechanisms could also explain the development of resource polymorphism. Comparative analyses of polymorphic and monomorphic systems are uncommon, making it difficult to distinguish the effects of geography, frequency-dependent selection, niche expansion, and species interactions. Detailing ecological conditions associated with the development of resource polymorphism is necessary to discern demographic and environmental processes that may cause it.

Goal: Test for environmental correlations with (a) the presence of resource polymorphism and (b) the degree of differentiation in polymorphic systems, to evaluate the hypotheses that the development of resource polymorphism results from (1) frequency dependence, (2) expansion to include a zooplanktivorous niche, or (3) lower survivorship due to predation on intermediate trait values. Trends in prey consumption, as they related to the presence of polymorphism and limnetic lake characteristics, were also analysed.

Organism: Arctic charr (*Salvelinus alpinus*) populations from lakes sampled in 1994–2004 across Iceland.

Methods: Random forest and multiple regression models assessing the presence and degree of resource polymorphism using environmental variables reflecting physical, chemical, and biological conditions. Prey consumption was fitted to the presence of polymorphism and brown trout abundance in negative binomial generalized linear models. The proportion of individuals consuming zooplankton within monomorphic versus polymorphic populations was also measured to test the idea that more widespread zooplankton consumption reflects niche expansion.

Results: In Iceland, polymorphic populations tended to occur in cooler lakes with few brown trout (*Salmo trutta*), a trophic competitor, and in lakes where Arctic charr consumed more zooplankton. Lakes with greater limnetic habitat, fewer nutrients, and greater potential to consume zooplankton appeared to promote resource polymorphism, thereby supporting the presence of niche expansion. The overall results supported the frequency dependence hypothesis, as well as the niche expansion hypothesis in most cases. However, morphs that

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differed in consumption of fish or chironomid pupae rather than zooplankton were also evident, indicating that mechanisms other than niche expansion may also be important. Relative resource availability and its link with the environment need to be accounted for when trying to predict the occurrence of polymorphism.

Keywords: competition, divergent selection, ecological speciation, individual heterogeneity, limnology, prey availability, resource polymorphism, salmonid.

INTRODUCTION

Resource polymorphism is characterized by the occurrence of discrete forms within a population that differ by one or more phenotypic attributes associated with resource use, such as growth rate, age at first maturity, or morphological traits related to feeding (Skúlason and Smith, 1995). It is most simply observed as bimodality in a population trait. In general, frequency-dependent selection is thought to be the primary evolutionary factor leading to bimodality (van Valen, 1965; Dieckmann *et al.*, 2004). Under frequency-dependent selection, resource polymorphism may arise when a single population increases in density, after which intraspecific competition for a preferable resource increases due to decreasing per capita availability of the resource. At some critical point, it becomes beneficial to be efficient at obtaining less preferable but more abundant resources, and selection becomes disruptive on phenotypic attributes associated with foraging. The relative fitness expected of individuals possessing a morphological characteristic therefore depends on its frequency in relation to individuals with alternative morphological characteristics, resulting in stable frequency-mediated selection (Dieckmann *et al.*, 2004).

Although frequency dependence is difficult to measure, experimental studies have shown patterns that strongly indicate its presence in polymorphic populations. Genetic characteristics have been detected as dependent on their own frequency in a polymorphic system (Schluter, 2003), and intraspecific competition can lead to morphological divergence (Schluter, 1994; Bolnick, 2004; Svanbäck and Bolnick, 2007; Bolnick and Lau, 2008). Niche expansion can also occur alongside mechanisms of frequency dependence; populations at high densities may consume a wider range of resources (Svanbäck and Persson, 2004). Therefore, divergence is thought to occur under ecological conditions that promote niche expansion, i.e. the greater use of other available resources not used in ancestral populations (Knudsen *et al.*, 2006).

However, frequency dependence is not the only explanation for bimodality in a population. For example, predation risk can promote differential habitat use, leading to divergent selection (Abrams, 2000; Vamosi and Schluter, 2002; Rundle *et al.*, 2003; Doucette *et al.*, 2004; Langerhans *et al.*, 2004). Predation has been suggested as a mechanism promoting size divergence of Arctic charr (*Salvelinus alpinus*), as large forms can escape gape-limited predators and small benthic forms can hide among substrate (Snorrason and Skúlason, 2004; Kristjánsson *et al.*, 2011). On the other hand, cannibalism by Arctic charr can provide an advantage to reaching large sizes (Griffiths, 1994).

In our companion paper (Woods *et al.*, 2012, this issue), we present standard methods for detecting resource polymorphism and defining the degree of divergence between morphs within populations of Arctic charr. To understand how such intraspecific diversity arises, a natural question following the detection of polymorphism would be 'What characteristics of the environment promote the development of resource polymorphism, and why do

populations differ in their degree of polymorphism?' The goal of this study is to determine which characteristics of the lake environment best predict the presence and degree of polymorphism in Arctic charr among lakes in Iceland. The availability of biological and limnological data from a wide diversity of lakes found within the database of the Ecological Survey of Icelandic Lakes (ESIL) project (Malmquist *et al.*, 2000; Kristjánsson *et al.*, 2011; Woods *et al.*, in press) yielded a rare opportunity to test the link between resource polymorphism in Arctic charr and environmental conditions. We tested the hypotheses that resource polymorphism would be found under environmental conditions that promote (1) frequency dependence (hypothesis 1), (2) niche expansion (hypothesis 2), or (3) greater predation risk (hypothesis 3). To test these relationships, we analysed patterns in (a) the presence of polymorphism and (b) the relative degree of differentiation between morphs (restricted to lakes with two morphs) across lakes in Iceland.

As it is difficult to predict which characteristics of the lake environment should reflect these three phenomena, the present study is a preliminary analysis in which a wide variety of environmental characteristics were explored. We paid particular attention to environmental features used to support the three hypotheses in past studies. Thus, if polymorphism is attributable to frequency-dependent selection (hypothesis 1), then polymorphism and degree of divergence should be associated with the following patterns:

(1a) High conspecific densities (Griffiths, 1994), indicating greater intraspecific competition, which is thought to influence frequency dependence (Schluter, 1994; Bolnick, 2004; Dieckmann *et al.*, 2004; Svanbäck and Bolnick, 2007).

(1b) Low competitor densities (i.e. the brown trout, *Salmo trutta*, in Iceland), allowing for greater niche breadth. Only when these niches are available (i.e. prey populations are not suppressed by predators other than Arctic charr) is it possible for the frequency of Arctic charr phenotypes specializing on these prey to increase (Griffiths, 1994, 2006; Robinson and Wilson, 1994; Skúlason and Smith, 1995; Schluter, 1996; Skúlason *et al.*, 1999). This also reduces the chance of habitat displacement by brown trout, a dominant competitor (Langeland *et al.*, 1991; Forseth *et al.*, 2003).

(1c) High altitudes. High-altitude sites generally have lower species diversity, because they are more isolated and more recently deglaciated than sites at lower altitude (Griffiths, 2006). However, these regions not only have fewer competitors (see 1b), they also act as a filter: only those species that have the highest colonization potential (i.e. are the most adaptable to new conditions and able to bypass barriers to migration) persist there (Bernatchez and Wilson, 1998; Griffiths, 2006). Perhaps these regions have also promoted adaptation to high environmental variability on geological time scales (Bernatchez and Wilson, 1998; Robinson and Schluter, 2000). High adaptability to new environments in these species then more readily initiates the process of divergence (Bernatchez and Wilson, 1998; Robinson and Schluter, 2000).

If polymorphism occurs as a result of niche expansion (hypothesis 2), then we would expect that a greater frequency or degree of polymorphism would be associated with expanded use of available resources relative to an ancestral form (Knudsen *et al.*, 2006). Although this has been studied in Arctic charr from other locations as an expansion towards the consumption of greater profundal benthic resources (Knudsen *et al.*, 2006), we consider expansion towards the consumption of limnetic resources, or zooplanktivory. Zooplanktivory is common in European Arctic charr (McCarthy, 2007), but appears to occur as a result of displacement from benthic zones by co-occurring species, as Arctic charr eat considerably more benthic foods,

including fish, when in isolation (Langeland *et al.*, 1991; Halvorsen *et al.*, 1997; Forseth *et al.*, 2003; Klemetsen *et al.*, 2003). Therefore, an increase in zooplanktivory due to niche expansion may only be detectable in regions where co-occurring species are few, such as Iceland. This hypothesis would be supported by an association between a greater or more widespread consumption of zooplankton and greater incidence of polymorphism or divergence of morphs.

Finally, if polymorphism is based on increased vulnerability to predation rather than frequency dependence at intermediate trait values (hypothesis 3), then greater predation on Arctic charr should occur in lakes with polymorphism present (Abrams, 2000; Vamosi and Schluter, 2002; Rundle *et al.*, 2003; Doucette *et al.*, 2004; Langerhans *et al.*, 2004). This could be detected as an association between higher frequencies of polymorphism or greater divergence and higher predator densities (i.e. brown trout). Predation due to cannibalism could not be tested in this study, as Icelandic Arctic charr are rarely cannibalistic according to extensive gut content data in the ESIL database (<1% of ≈3500 records, unpublished data).

The three expected hypotheses are not mutually exclusive, but one would not expect the first and third to apply simultaneously due to their differing expected relationships with brown trout (i.e. polymorphism should occur with low brown trout density in hypothesis 1 versus high brown trout density in hypothesis 3). However, hypothesis 2 is compatible with hypotheses 1 and 3.

MATERIALS AND METHODS

Detection of polymorphism

Data on length-at-age, morphology, maturity, and diet of Arctic charr populations from 51 lakes in an Ecological Survey of Icelandic Lakes (August to September in 1992–2004) were used to detect polymorphism in our companion paper (Woods *et al.*, 2012, this issue). (For details on sampling and methods for detecting polymorphism, see the Methods section and Table 1 therein.) Results of that study indicated the presence of resource polymorphism in 16 lakes at $P = 0.5$ when (1) mixture models indicated more than one group, (2) differences between groups were strong enough to be detected in a *post-hoc* analysis of variance (ANOVA) and could not be attributed to sexual dimorphism, and (3) there were differences in diet between groups. A measure related to effect size from an ANOVA, termed 'Differentiation', was also used to describe the extent of divergence between morphs for populations with only two morphs present.

This three-step confirmation process ensures that the analyses presented in this study are based on relatively strong polymorphism that is related to resource use, although the nature of this polymorphism may vary among lakes (i.e. differences in morphological characteristics and resource use may not be consistent among lakes). Analyses in our companion paper also did not control for *P*-value inflation due to repeating statistical tests across many lakes. As the final step of the confirmation process involved repeating tests at $P = 0.05$ across 27 lakes, we can expect that 1–2 of the 16 confirmed lakes may be misclassified. If a *P*-value correction were applied to the 27 tests, however, this would likely inflate our type II error rate to a greater extent than our type I error rate is affected under no *P*-value correction. Therefore, we decided to use all 16 confirmed lakes for further analyses with the understanding that the presence of 1–2 misclassified lakes reduces the power of further tests.

Patterns of resource consumption in the presence of polymorphism

To first confirm that niche expansion most likely occurs via zooplankton consumption, rather than the consumption of other resources, consumption of the prey taxa analysed by Woods *et al.* (2012, this issue) were compared between lakes with monomorphic and polymorphic Arctic charr. We chose these taxa to represent the most prominent prey in a study of habitat-related feeding categories (Woods *et al.*, in press) (see also the Appendix). Woods *et al.* (2012, this issue) analysed the cladocerans *Bosmina* spp., *Daphnia* spp., *Alona* spp., and *Eurycercus* spp.; the snail *Radix peregra*; the tadpole shrimp *Lepidurus arcticus*; chironomid pupae; chironomid larvae; pea clams *Pisidium* spp.; and the threespine stickleback (*Gasterosteus aculeatus*). In that study, differences between morphs in the consumption of chironomid larvae, pea clams, and tadpole shrimp (i.e. axis 2 of the decentralized correspondence analysis) were always associated with differences in other taxa (i.e. axes 1, 3, or 4 of the decentralized correspondence analysis). Therefore, these three taxa (i.e. chironomid larvae, pea clams, and tadpole shrimp) were uninformative for distinguishing morphs and we did not consider them further in this study.

An expansion of the range in food consumed is expected by the niche expansion hypothesis; however, in practice, this increase in range or variance can be difficult to define in multivariate diet data. Therefore, as a simple proxy for range of food consumed within populations, we (1) defined a subset of prey more often consumed within polymorphic populations using GLMs, and (2) compared the proportion of individuals that consumed zooplankton within polymorphic populations to the proportion expected from monomorphic populations. To accomplish the first step, each of the prey taxa considered were summed across individual gut contents within a lake, and generalized linear models (GLMs) with negative binomial error were used to predict these counts using the presence of confirmed resource polymorphism as a categorical predictor (K), brown trout abundance as a continuous variable (BTA), and their interaction (K*BTA). The interaction was removed when not significant. Brown trout abundances were included in the model as an attempt to control for the greater consumption of zooplankton via behavioural displacement (Langeland *et al.*, 1991; Halvorsen *et al.*, 1997; Forseth *et al.*, 2003; Klemetsen *et al.*, 2003), which would confound support for the niche expansion hypothesis. Model result *P*-values were then adjusted for seven tests using a sequential Bonferroni correction.

For the second step, the mean proportion of individuals that consumed zooplankton in monomorphic populations was then used as the expected probability of succeeding trials to define a null distribution in a binomial model. The probability of observing the actual number of succeeding trials (i.e. individuals consuming zooplankton) within each polymorphic population given this binomial model was then used to calculate a *P*-value, testing whether this proportion differed significantly in that polymorphic population. For comparison with an extremely conservative null hypothesis, the maximum proportions instead of the mean proportions were used from the monomorphic populations as the expected probability, and tests were repeated. Tests were done separately for subsets of lakes that had brown trout present versus absent, but a sequential Bonferroni correction was used to adjust *P*-values across all polymorphic populations.

Predicting polymorphism using environmental variables

To examine hypotheses 1–3, we evaluated (1) the presence of polymorphism (categorical variable) using random forest models, and (2) the continuous measure of polymorphism ('Differentiation') using multiple regression. Environmental data were not available for all lakes, so analyses were restricted to the 41 of 51 lakes that had complete data, including 14 lakes with polymorphic Arctic charr. Variables included physical, chemical, and biotic variables taken or calculated from the ESIL database (Malmquist *et al.*, 2000; Karst-Riddoch *et al.*, 2009; Kristjánsson *et al.*, 2011; Woods *et al.*, in press). Physical and chemical variables included altitude (ALT, m), surface temperature (ST, °C), mean depth (MD, m), pH (PH), conductivity (COND, $\mu S \cdot cm^{-1}$), alkalinity (ALK, $meq \cdot L^{-1}$), total phosphorus (TP, $\mu g \cdot L^{-1}$), total nitrogen (TN, $\mu g \cdot L^{-1}$), total organic carbon (TOC, $mg \cdot L^{-1}$), and silicon dioxide (SiO_2, $mg \cdot L^{-1}$). Chemical concentrations were obtained from one-litre unfiltered samples taken from 20 to 40 cm under the lake surface. To correct for skewness, these variables were all log-transformed, except for SiO_2 which was square-root transformed. Biotic variables included Arctic charr abundance (ACA, # per gillnet per hour) and brown trout abundance (BTA, # per gillnet per hour), the presence/absence of threespine stickleback (*Gasterosteus aculeatus*), detected using minnow traps (SP), and prey abundances listed below (Z, C, S, T, P) as well as a Shannon index of diversity for invertebrates collected from stones (SH). Prey abundances from ESIL invertebrate surveys were calculated within prey categories defined by Woods *et al.* (in press) (see also the Appendix). Each category was named by its dominant prey items but included a wide variety of taxa ('zooplankton' Z, 'chironomid pupae' C, 'snails' S, 'tadpole shrimp' T, and 'pea clams' P). In the ESIL survey, invertebrates were collected and counted from the shallow (0.2–0.5 m), rocky, littoral habitat, the offshore sediment habitat, and the pelagic habitat (for details, see Malmquist *et al.*, 2000; Karst-Riddoch *et al.*, 2009; Woods *et al.*, in press). Counts within each category were converted to biomass (mg) by multiplying a weight representative of the order of magnitude for each species and then summing within categories (for details, see Woods *et al.*, in press) (see also the Appendix). Volumetric zooplankton densities were multiplied by lake mean depth to yield m^{-2} areal measures comparable to those of benthic invertebrates, and all were transformed by $\log(x + 1)$. Fish abundances (BTA and ACA) were transformed by $\log(x + 1)$, but SH was normally distributed and not transformed. Atlantic salmon (*Salmo salar*) were rarely caught in lakes so were excluded from analyses. A correlation matrix of all environmental variables is given to aid the interpretation of possible colinearity (Table 1).

To predict the presence of polymorphism, we used random forest categorization models. Random forest models are machine learning methods that compare favourably with other methods for modelling classification data (Breiman, 2001; Prasad *et al.*, 2006). With this method, a random forest is 'grown' by collecting a number of classification trees formed by fitting a classification tree to a bootstrap sample of the data. For each classification tree, a set of explanatory variables are tested to determine which one best splits the data into possible classes (i.e. lakes with monomorphic versus polymorphic Arctic charr populations) with the least number of misclassified data points. Each split leads to two nodes, with data points predicted to occur as a certain class within that node. Final classifications for each data point are then aggregated across individual trees to yield a collection of votes (1 per tree) for an overall prediction based on the proportion of votes from the forest. We used bootstrapping without replacement to reduce bias in importance measures of explanatory variables (Strobl *et al.*, 2007). The overall error rate of the model was calculated by forming

Table 1. Pearson correlation coefficient matrix of environmental variables used in the analyses

	ALT	ST	MD	PH	COND	ALK	TP	TN	TOC	SiO_2	ACA	BTA	Z	C	S	T	P
ALT																	
ST	−0.19																
MD	0.07	0.23															
PH	0.11	−0.20	−0.18														
COND	− 0.65	− 0.16	−0.20	0.15													
ALK	−0.17	0.12	−0.26	0.46	0.36												
TP	0.03	−0.33	−0.12	0.07	0.26	0.28											
TN	−0.27	−0.20	−0.61	0.24	**0.50**	0.46	**0.53**										
TOC	−0.39	−0.03	− 0.56	0.20	**0.54**	0.42	0.19	**0.85**									
SiO_2	0.14	0.40	0.40	0.03	−0.27	0.11	−0.13	−0.46	−0.45								
ACA	0.31	−0.24	−0.29	0.13	−0.08	0.07	0.08	0.08	0.00	0.10							
BTA	−0.24	0.34	−0.15	−0.25	−0.05	0.07	−0.31	−0.05	0.08	0.05	−0.23						
Z	−0.28	0.24	0.11	−0.30	0.17	0.07	−0.27	−0.35	−0.07	0.32	0.15	0.17					
C	−0.07	0.25	0.15	0.04	−0.04	0.11	−0.04	−0.25	−0.11	0.46	0.07	0.02	**0.56**				
S	0.28	−0.10	−0.04	0.26	−0.13	0.10	0.00	−0.16	−0.27	0.41	0.23	−0.15	0.03	0.28			
T	0.16	0.02	−0.02	−0.22	−0.05	0.03	0.00	−0.14	−0.05	0.31	0.14	−0.16	0.47	0.44	0.18		
P	0.06	0.18	−0.09	−0.05	0.02	0.22	−0.09	−0.22	−0.12	0.40	0.15	−0.03	**0.62**	**0.69**	0.42	**0.72**	
SH	−0.37	0.18	−0.32	0.07	0.31	0.14	−0.08	0.31	0.49	0.12	0.05	0.39	0.17	0.32	0.15	0.08	0.20

Note: Environmental variables included altitude (ALT), surface temperature (ST), mean depth (MD), pH (PH), conductivity (COND), alkalinity (ALK), total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC), silicon dioxide (SiO_2), Arctic charr abundance (ACA), brown trout abundance (BTA), Shannon index of stone invertebrate diversity (SH), and the prey abundance categories zooplankton (Z), chironomid pupae (C), snails (S), tadpole shrimp (T), and pea clams (P). Correlations in **bold** are considered strong ($P > 0.5$).

predictions for the data that were not collected in the bootstrap sample (i.e. 'out-of-bag' data), and calculating a misclassification rate over all data points (OOB error rate). Preliminary analyses of single classification trees indicated that the maximum number of explanatory variables within single trees is not likely to be high. Therefore, the maximum number of nodes was set to 8, and the number of trees grown was set to 100,000. The package randomForest v.4.6-2 (Liaw and Wiener, 2002) was used within the statistical software R v.2.13.0 (R Development Core Team, 2011).

Because random forest models simply detect whether a splitting point in the predictor variable is adequate to classify groups, they can detect effects of predictor variables even if they are non-linear or have complex interactions with other explanatory variables. However, the flexibility of this framework may also lead to the incorporation of improper variables in over-parameterized models (Eustace *et al.*, 2011). Therefore, we used a step-wise model reduction method to remove non-significant variables (Diaz-Uriarte and Alvarez de Anderes, 2006; Genuer *et al.*, 2010; Sethi, 2010). We performed an initial model fit including all explanatory variables, and then ordered predictors according to importance using the decrease in node impurity as measured by the Gini Index, which characterizes the explanatory variable's ability to distinguish classes at a given split. This index is more stable than the Mean Decrease in Accuracy for small sample sizes (Breiman, 2002; Liaw and Wiener, 2002; Strobl *et al.*, 2007). The least important test variables were then successively deleted if their inclusion in the model did not reduce the OOB error rate.

Because model results may change depending on (1) the number of explanatory variables subsampled and (2) the threshold proportion of votes for prediction (e.g. default majority vote, 0.5), these were also varied for each combination of explanatory variables included in the model. Thresholds were tested at 0.4, 0.5, and 0.6 of votes necessary for classification, whereas the bootstrap subset size of predictor variables ranged from 1 to 8 (or the maximum number of variables included in the model if <8). Our sample of 41 lakes is rather small for evaluating the model based on cross-validation methods beyond those provided by OOB, so we instead used a receiver operating characteristic (ROC) curve (Zou *et al.*, 2007) of the fitted values as an indicator of model performance with ROCR package v. 1.0-4 (Sing *et al.*, 2005) in the R statistical software. The area underneath ROC curves (AUC) reflects the trade-off in model performance between a true-positive and false-positive classification: 0.5 reflects a model no better than chance whereas 1.0 reflects perfect correspondence between predictions and data. Partial dependence plots were used to show the marginal effect of successful variables on the classification probability of each variable retained in the reduced model. For example, a high partial dependence value at a high value of a successful variable indicates that, after accounting for all other variables in the model, it is more likely for polymorphism to occur at high values of that variable.

Multiple regression was used to determine which environmental variables best fitted how different forms were from each other within a population (as indicated by Differentiation). Differentiation values for lakes with two morphs confirmed as exhibiting resource polymorphism (see Tables 1 and 2 in Woods *et al.*, 2012, this issue) were $\log(x + 1)$ transformed and standardized to yield standard coefficients in the multiple regression. Only 11 of the 16 confirmed lakes had polymorphic populations with only two morphs and sufficient data to be included. Due to the reduction in sample size, a reduced set of environmental variables reflecting patterns listed in the Introduction was used in forward and backward selection by AICc. The model was chosen with the minimum AICc, although all models with AICc values < 4 above the minimum were shown for comparison. The reduced set of

environmental variables included altitude, mean depth, conductivity, surface temperature, total nitrogen concentration, total phosphorus concentration, silicon dioxide concentration, brown trout abundance, Arctic charr abundance, and zooplankton abundance.

RESULTS

Patterns of resource consumption in the presence of polymorphism

Using the 41 lakes with complete data, generalized linear models with negative binomial error indicated that the presence of polymorphism, brown trout abundance, and their interaction had a significant effect on the consumption of *Bosmina* spp. and *Daphnia* spp. (Table 2). *Eurycercus* spp. also showed significance in the main effects only; however, they were consumed more often in monomorphic rather than polymorphic populations.

Proportions of individuals within lakes consuming either *Bosmina* or *Daphnia* spp. were then used as a proxy for range in zooplankton consumption. The mean proportion of individuals observed to consume *Bosmina* or *Daphnia* spp. (i.e. succeeding trials) among monomorphic populations with brown trout present was 0.15, and the maximum

Table 2. Results of generalized linear models with negative binomial error distribution used to predict the counts of each of the indicated dietary items summed across individual gut contents within lakes

		Est.	S.E.	*Z*	*P*	%DE
Bosmina spp.	K	2.7729	0.0140	197.3838	<**0.0001**	26.12
	BTA	0.3636	0.0110	33.0259	<**0.0001**	
	K*BTA	−0.6872	0.0128	−53.5541	<**0.0001**	
Daphnia spp.	K	0.4001	0.0125	32.0704	<**0.0001**	41.54
	BTA	−0.2753	0.0090	−30.6854	<**0.0001**	
	K*BTA	1.6672	0.0110	151.8219	<**0.0001**	
Alona spp.	K	−3.0054	1.2940	−2.3226	0.2222	11.52
	BTA	2.0919	0.6654	3.1439	**0.0167**	
Eurycercus spp.	K	−1.1132	0.0182	−61.2193	<**0.0001**	12.34
	BTA	−0.9180	0.0145	−63.4081	<**0.0001**	
Radix peregra	K	−0.1413	0.6750	−0.2094	1.0000	4.23
	BTA	0.4771	0.3472	1.3741	1.0000	
Chironomid pupae	K	−0.3919	0.5771	−0.6791	1.0000	1.79
	BTA	−0.2446	0.2970	−0.8237	1.0000	
Gasterosteus aculeatus	K	−0.0767	0.7874	−0.0974	1.0000	12.24
	BTA	−1.3418	0.4252	−3.1558	**0.0144**	

Note: The coefficient estimates (Est.) and standard errors (S.E.) are for the effect of the presence of polymorphism in the lake as a categorical factor (K), the abundance of brown trout (BTA), and their interaction when significant (K*BTA). Coefficient *Z*-scores, coefficient *P*-values, and the percent of deviance explained by the model (%DE) are shown. Forty-one lakes were included in each model. *P*-values were adjusted using a sequential Bonferroni correction for the 16 tests shown. *P*-values in **bold** are significant ($P > 0.5$).

proportion among these was 0.70. The mean proportion for a succeeding trial among monomorphic populations with no brown trout present was 0.019, and the maximum was 0.067. These proportions were then used as expected probabilities to test the probability of observing the number of succeeding trials exhibited in polymorphic populations. For lakes devoid of brown trout, four of the ten polymorphic populations (in lakes 9, 17, 20, and 65) showed a greater occurrence than expected of individuals consuming *Bosmina* or *Daphnia* spp. These occurrences within the four populations were still significantly more frequent when maximum proportion in monomorphic populations was used instead as the expected probability. When diets of Arctic charr in the other six lakes (lakes 4, 22, 28, 32, 37, and 47) were analysed in our companion study (see Woods *et al.*, 2012, this issue), differences in diet between morphs were attributable either to fish or zooplankton versus snail consumption (lakes 4, 22, 28, and 37) or *Eurycercus* sp. versus chironomid pupae consumption (lakes 32 and 47). In lakes with brown trout, two of the four populations showed more individuals consuming *Bosmina* spp. or *Daphnia* spp. (lakes 19 and 58) than the mean of monomorphic populations. In the two other lakes, there was dietary differentiation between morphs in consumption of *Eurycercus* vs. chironomid pupae, but not zooplankton consumption (lakes 10 and 23) (Woods *et al.*, 2012, this issue). When the maximum proportion from monomorphic populations was instead used as the expected probability, all polymorphic populations had comparatively less frequent zooplankton consumption (Table 3, Fig. 1).

Table 3. Results of binomial tests to determine whether *Bosmina* spp. or *Daphnia* spp. were consumed more frequently among individuals in polymorphic populations than expected based on the null probabilities calculated from monomorphic populations

Lake	Actual proportion	Mean null *P*-value		Max. null *P*-value	
Brown trout present					
10	0.0000	0.0085	<	<0.0001	<
19	0.4000	<**0.0001**	>	<0.0001	<
23	0.0286	0.1274	<	<0.0001	<
58	0.5053	<**0.0001**	>	0.0001	<
Brown trout absent					
4	0.0000	1.0000	<	1.0000	<
9	0.2456	<**0.0001**	>	**0.0001**	>
17	0.1930	<**0.0001**	>	**0.0085**	>
20	0.3382	<**0.0001**	>	<**0.0001**	>
22	0.0313	1.0000	>	1.0000	<
28	0.0000	1.0000	<	0.3981	<
32	0.0000	1.0000	<	1.0000	<
37	0.0000	1.0000	<	0.0365	<
47	0.0000	1.0000	<	0.0713	<
65	0.2826	<**0.0001**	>	<**0.0001**	>

Note: The mean null proportion when brown trout were present was 0.15 and 0.019 when brown trout were absent; the maximum null proportion when brown trout were present was 0.70 and 0.067 when brown trout were absent. Next to the *P*-value is an indicator of whether the proportion found in the polymorphic population (Actual proportion) was larger (>) or smaller (<) than the proportion from monomorphic populations (mean or maximum) used to form the expected probability. *P*-values were corrected using a sequential Bonferroni adjustment and are shown in **bold** when significant and proportions were larger than expected. Lake numbers correspond with those used by Woods *et al.* (2012, this issue) (see Table 1 therein).

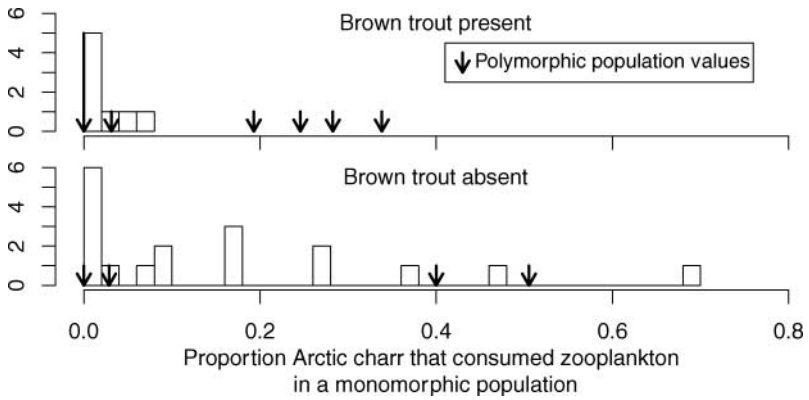

Fig. 1. Histograms of the number of monomorphic populations (y-axis) with the observed proportions of Arctic charr individuals that consumed *Bosmina* spp. or *Daphnia* spp. Arrows indicate values for proportions calculated from polymorphic populations, with the length of the arrows indicating the number of lakes with that value.

Predicting polymorphism using environmental variables

The random forest model performed best when the number of subset explanatory variables was set to 3, and the best threshold proportion of votes for prediction was 0.5. Seven explanatory variables were included according to the stepwise selection procedure in the best model (Diaz-Uriarte and Alvarez de Andres, 2006; Genuer *et al.*, 2010; Sethi, 2010): Z, SiO_2, MD, ST, ACA, BTA, and ALT (in order of Gini index). The OOB error rate was 34.15%, with 22 of 27 monomorphic and 5 of 14 polymorphic lakes correctly classified. The model generally had a low AUC of 0.586, indicating moderate to low explanatory ability.

When interpreting partial dependence plots, it is important to note the rug plots along the x-axis. These indicate how well the trends were supported within the data range observed (Fig. 2). The greater partial dependence of resource polymorphism at low values of zooplankton abundance and surface temperature was well supported by lakes that span the data range, indicating that resource polymorphism was more likely to occur in cooler lakes with low zooplankton abundance. In partial dependence plots, polymorphism was more likely at low and high values of brown trout abundance and silicon dioxide concentration, but less likely at intermediate values. However, closer inspection showed that the greater probability at higher values of these variables depended on only a single lake for each plot; therefore, the right side of these graphs beyond the data range should be disregarded. The association of resource polymorphism with low brown trout abundance contradicts hypothesis 3, that greater predator density causes polymorphism, and supports pattern 1b, that polymorphism should be found where there are few interspecific competitors. The trends with Arctic charr abundance and altitude were rather weak and variable, so they are not interpreted further. Deeper lakes were also more likely to contain polymorphic populations (Fig. 2).

Multiple regressions by AICc backward and forward selection yielded a set of four models with a change in AICc (δAICc) values < 2 (Table 4). Because the AICc values for these models differed by less than 2 from the minimum AICc value, they are considered

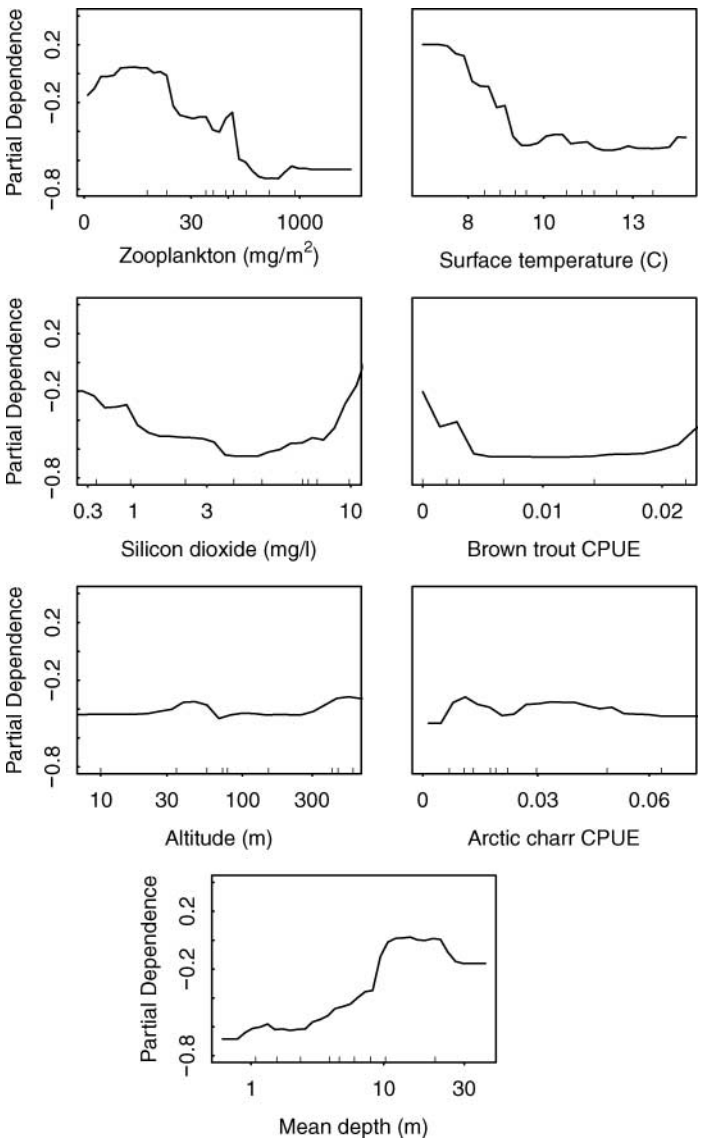

Fig. 2. Partial dependence plots showing marginal effects of ecological variables on probability of a polymorphic classification in the best random forest model.

equivalent (Bolker, 2008). Therefore, total nitrogen, total phosphorus, brown trout abundance, and silicon dioxide can all be thought of as having negative effects on differentiation, so tests for significance and partial R^2 values are given for each variable within each of the four models (Fig. 3). Total nitrogen and total phosphorus were highly correlated, but none of the predictors within each of the four models were strongly correlated (Table 1).

Table 4. Subset of best multiple regression models to predict Differentiation using the environmental variables indicated in equation form (separated by +)

Predictor variables	AICc	δAICc
Z + TP	−10.25	0.00
Z + TP + BTA	−9.58	0.67
Z + TN	−9.24	1.01
Z + TN + SiO_2	−9.06	1.19
Z + TP + MD	−8.00	2.24
Z + TP + BTA + ST	−7.75	2.50
Z + TP + ST	−7.71	2.53
Z + TP + ACA	−7.14	3.11
Z + TP + ALT	−6.38	3.87
Z + TP + SiO_2	−6.33	3.92

Note: Models above the dashed line are those that have a δAICc (i.e. difference between AICc and minimum AICc) < 2, indicating models that are equivalent. All predictors included within the subset of equivalent models had negative effects (Fig. 3). Model predictors include zooplankton abundance (Z), total phosphorus concentration (TP), brown trout abundance (BTA), total nitrogen concentration (TN), silicon dioxide concentration (SiO_2), mean depth (MD), surface temperature (ST), Arctic charr abundance (ACA), and altitude (ALT).

DISCUSSION

Our results relating Arctic charr polymorphism to lake ecology across Iceland were consistent with the first two hypotheses: frequency-dependent selection and niche expansion. These hypotheses are not mutually exclusive, so many of the observed trends may be co-dependent and require experimentation to disentangle. No support was found for the third hypothesis, suggesting that polymorphism in Iceland was not related to predation levels on Arctic charr.

Support of niche expansion through diet analyses

Niche expansion was detected by two analyses. First, greater total quantities of zooplankton were consumed in lakes with polymorphic Arctic charr populations according to generalized linear models (GLMs). Second, in roughly half the lakes with polymorphic populations, individuals consumed zooplankton more frequently, based on binomial tests. Both GLMs and binomial tests also indicated the importance of brown trout abundance in increased use of zooplankton resources. Therefore, the detection of polymorphism via niche expansion was confounded whenever brown trout were present. Furthermore, the relative strength of zooplanktivory in Arctic charr likely differs by lake.

We interpret increased consumption of zooplankton in lakes with polymorphic populations as niche expansion, but polymorphic lakes lacking detectable increased zooplankton consumption do not necessarily lack niche expansion. If a morph is highly efficient at consuming a secondary resource (e.g. zooplankton) or the abundance of that

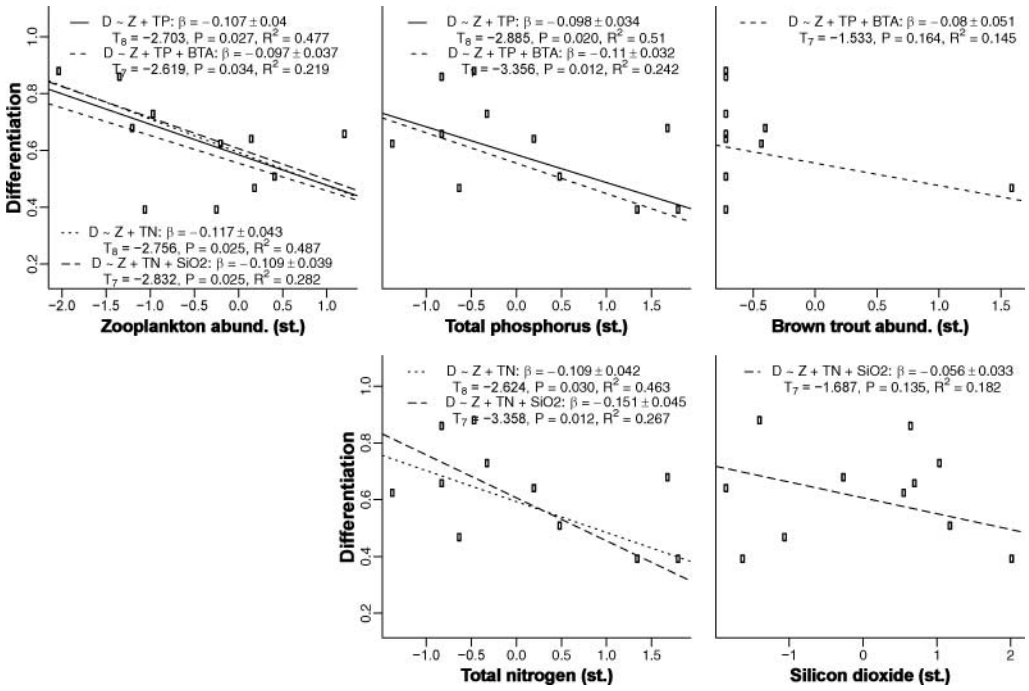

Fig. 3. Differentiation among morphs was greater in lakes with lower standardized values (st.) of zooplankton abundance (Z), total phosphorus (TP), brown trout abundance (BTA), total nitrogen (TN), and silicon dioxide (SiO_2) in multiple regression models. Correlations were similar among the four models that were indistinguishable by AIC (Table 4), and are shown collectively: each trend line within a panel represents the effect of a predictor within one of the four models. Legends within each panel show which trend line type corresponds with which model in the form of D ~ predictors (separated by +). Fewer than four trend lines indicates that the predictor was not found in all models. Models are followed by the coefficient values for the panel x-variable (β ± standard error); a *t*-statistic (T) with subscript degrees of freedom used to determine whether coefficient values differed significantly from 0; *P*-values associated with these tests; and partial R^2 values.

resource is low relative to others, then the zooplanktivorous morph could likewise be expected to occur at lower densities. Therefore, low zooplankton consumption within a population could simply result from low relative abundances of the zooplanktivorous morph, and this pattern could be exacerbated by sampling methods biased towards capturing other morphs.

Closer examination of the six lakes with polymorphic populations that did not show greater zooplankton consumption (and had no brown trout) indicated that four of these lakes (4, 22, 28, and 37) had a second morph that consumed either fish, zooplankton, or a combination of these, more often than snails [based on ANOVAs of diet axes shown by Woods *et al.* (2012, this issue)]. If zooplankton were consumed, possibly in combination with fish, this could constitute a form of partial niche expansion undetectable by our methods. Piscivory, on the other hand, likely represents the use of an additional resource as it becomes available, as fish are usually consumed by Arctic charr in both monomorphic and polymorphic populations when prey fish are available [such as lake 4 (Jónsson, 2002)].

For all other polymorphic populations that showed low zooplankton consumption (32 and 47 with no brown trout present; 10 and 23 with brown trout present), diet differences were instead based on chironomid pupae versus *Eurycercus* spp. consumption (Woods *et al.*, 2012, this issue). As *Eurycercus* spp. were consumed less and chironomid pupae were not consumed more in polymorphic populations (GLMs), our data suggest a partitioning of the ancestral morph between these resources, rather than niche expansion. However, this was based on few lakes and further comparison of chironomid pupae consumption in monomorphic versus polymorphic populations is warranted. Perhaps this also constitutes a rarer form of niche expansion, as chironomid pupae may be consumed in limnetic zones as they emerge, and are thought to be an important food source for zooplanktivorous forms during periods of peak chironomid pupae abundance (Malmquist *et al.*, 1992; Woods *et al.*, in press). In any case, this form of resource segregation is rarely mentioned in the literature, yet appears to be as common in our dataset as the well-known existence of piscivorous Arctic charr forms.

Environmental characteristics of lakes containing polymorphic populations

In fitting polymorphism to environmental variables, we note that many of the expected trends within the three broad hypotheses cannot be separated due to natural correlations among landscape features. For example, considering the frequency dependence hypothesis, patterns 1b and 1c (see Introduction) are linked in a manner that would be difficult to disentangle: high altitude and recently deglaciated regions naturally have fewer species (Bernatchez and Wilson, 1998; Robinson and Schluter, 2000; Griffiths, 2006). In addition, monomorphic populations in lower elevations may experience more gene flow if there are fewer barriers to migration. Pattern 1a may be further correlated with these conditions because higher conspecific Arctic charr densities are expected under low competitor density, i.e. fewer brown trout (Langeland *et al.*, 1991; Hesthagen *et al.*, 1997; Jansen *et al.*, 2002; Helland *et al.*, 2011). Therefore, the patterns presented here should be taken as broadly encompassing many facets, as many environmental variables showed strong correlations among each other (Table 1).

In support of hypothesis 1, lakes were more likely to contain polymorphic Arctic charr populations if they were deep, had few competitors, low silicon dioxide concentrations, and low zooplankton abundance. A similar connection to zooplankton scarcity, low nutrient values (i.e. total nitrogen, total phosphorus, and silicon dioxide), and low brown trout abundances was found in multiple regressions to predict greater differentiation (although the effects of silicon dioxide and brown trout abundance were quite low). Upon first glance, the results of GLMs appear contradictory: the negative binomial models indicated that zooplankton (i.e. *Bosmina* spp. and *Daphnia* spp.) were consumed more frequently by polymorphic populations, but random forest and multiple regression model results suggest that the presence of polymorphism was associated with low abundances of zooplankton. As low total nutrient levels likely indicated both low dissolved and particulate nutrients (including phytoplankton standing stocks), we offer two explanations for low zooplankton abundances and low nutrient levels: (1) general productivity and nutrient levels were low, or (2) productivity was high, but biomass turnover rates, via predation and natural mortality, were so fast that biomass levels of all but the top predator (Arctic charr) were low.

For the first explanation for the 'low nutrient, low zooplankton abundance, high zooplankton consumption' pattern, Arctic charr would be more likely to consume zooplankton when low zooplankton abundance results from low nutrient loading. Low nutrient loading

is not uncommon in Icelandic lakes, as studies of nutrient flux (Jónasson *et al.*, 1992; Einarsson *et al.*, 2004; Gíslason *et al.*, 2004), diatom assemblages (Dickman *et al.*, 1993; Karst-Riddoch *et al.*, 2009), experimental nitrogen and phosphorus additions (Friberg *et al.*, 2009; Guðmundsdóttir *et al.*, 2011), and terrestrial vegetation succession (Cutler, 2011) all suggest that Icelandic lakes and streams, especially in geologically young volcanic zones, tend to be nitrogen-limited, and sometimes phosphorus-limited. Nitrogen limitation is not uncommon for high-altitude or -latitude lakes due to reduced leaching of limiting nutrients from terrestrial vegetative areas (Pienitz *et al.*, 1997; Edmundson and Mazumder, 2002; LaPerriere *et al.*, 2003). If nutrient loading and productivity are generally low, total food availability is likely limiting and Arctic charr populations may diverge to use alternative resources according to their relative availability. This explanation seems likely, given the generalist feeding habits of Arctic charr (Langeland *et al.*, 1991; Halvorsen *et al.*, 1997; Forseth *et al.*, 2003; Klemetsen *et al.*, 2003; McCarthy, 2007).

On the other hand, co-occurring low total phosphorus and nitrogen levels in our data may indicate that enough nitrogen fixation has occurred to approach co-limitation (Jónasson *et al.*, 1992; Einarsson *et al.*, 2004). This idea is supported by the high correlation between nitrogen and phosphorus in our data (Table 1). Nutrients would then be limited due to high uptake rates, and phytoplankton and zooplankton production may be higher than our data suggest. This pattern is supported by exceedingly high estimates of productivity found in two relatively large oligotrophic Icelandic lakes [Thingvallavatn and Mývatn (Jónasson *et al.*, 1992)]. This pattern has been interpreted as resulting from high phytoplankton activity per unit biomass due to the dominance of a small-bodied, fast-growing phytoplankton species (Jónasson *et al.*, 1992). In this case, an association of polymorphic populations with low nutrient levels may signal that the presence of a second morph is facilitated by fast nutrient and energy cycling in the water column.

Although the mechanism behind the 'low nutrient, low zooplankton abundance, high zooplankton consumption' pattern cannot be inferred from our study, this pattern is also not uncommon. Siwertsson *et al.* (2010) found a similar pattern of greater lake whitefish divergence towards zooplankton consumption in lakes with low nutrient values. Landry *et al.* (2007) also showed that lake whitefish differentiation towards greater zooplankton consumption was positively correlated with an indicator of productivity and low zooplankton abundance. Our results also support past studies indicating the importance of lake depth in the development of polymorphism (Bolnick and Lau, 2008; Vonlanthen *et al.*, 2009). Bolnick and Lau (2008) found that lakes of intermediate depth, rather than shallow or deep lakes, were more likely to contain polymorphic threespine stickleback, potentially indicating the importance of evenly balanced resource availability (Bolnick and Lau, 2008). Therefore, a prime area for future research would be to understand how low nutrient levels and lake depths affect relative resource abundances, thereby allowing for the development of polymorphism.

Overall, the random forest model used to fit the probability of polymorphism based on environmental variables had a relatively low predictive ability, especially for polymorphic populations. Such results may occur either because the environmental conditions surrounding similar polymorphic lakes are highly variable, or because the nature of the polymorphism defining these lakes is highly variable, or both. For example, perhaps polymorphic lakes should be further categorized by patterns in resource differentiation to obtain clearer links with the environmental variables. The consumption of *Daphnia* spp. vs. zoobenthos could be analysed separately from the consumption of *Bosmina* spp. vs. zoobenthos, as the dominance of these taxa may hint at differences in zooplankton

community, possibly as a result of environmental differences. Rarer morphs, such as those that consumed mainly fish or chironomid pupae, could also be defined as separate polymorphism types. Unfortunately, to follow through with this fine-scale categorization environmental analyses would require more data than available even in the extensive database used for this study.

In summary, this study has indicated that patterns in resource polymorphism in Arctic charr across Icelandic lakes likely result from frequency-dependent selection, not predation risk. Niche expansion towards the use of zooplankton appears to occur in approximately half the cases of polymorphism, although less common morphs that consume fish or chironomid pupae were also detected. Polymorphic populations were more likely to occur or become more greatly differentiated in lakes with low nutrient concentrations, low zooplankton abundances, and greater depths. However, the mechanisms inducing frequency-dependent selection under these environmental characteristics remain unknown. Future studies should focus on elucidating measures of within-lake relative prey abundance that better link how frequency dependence and niche expansion develop within specific polymorphism types, and how this is influenced by nutrient dynamics or lake depth.

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APPENDIX

Indicator biomass conversions for prey taxa are given along with the frequency found in capture habitat based on invertebrate surveys. Biomass indicators are based on the order of magnitude of dry weights found for individual taxa in the literature (see Woods *et al.*, in press, for details). Capture habitats include stone (S), fine-grained sediment (from Kajak cores, mud: M), or the water column (from plankton nets, W). Prey categories included snail (S), tadpole shrimp (T), pea clam (P), zooplankton (Z), chironomid pupae (C), and fish (F). Capture habitats are listed in order of importance, with backslashes indicating similar frequencies (20–80%). Parentheses indicate that expert knowledge was used to fill information for taxa absent in invertebrate surveys. Adapted from Woods *et al.* (in press).

Dietary taxa	Common name	Biomass (mg)	Capture Habitat	Prey Cat.
Gammarus sp.	amphipod	1	S	0
Limnephilus spp. L	caddisfly larvae	1	M/S	S
Apatania zonella L	caddisfly larvae	1	M/S	S
Radix peregra	snail	1	M/S	S
Daphnia spp.	cladoceran	0.01	W, M	T
Cyclops spp.	copepod	0.01	M, W	T
Ostracoda	ostracod	0.001	M, W	T
Macrothrix sp.	cladoceran	0.01	M, W	T
Alona spp.	cladoceran	0.001	M, W	T
Lepidurus arcticus	tadpole shrimp	10	M, W	T
Coleoptera	beetle	1	S	T
Other flies	other flies	0.1	S	T
Eurycercus sp.	cladoceran	0.1	M, W	P
Polyphemus sp.	cladoceran	0.001	(M, W)	P
Simocephalus vetulus	cladoceran	0.001	M, W	P
Chironomidae L	fly larvae	0.1	M/S	P
Pisidium casertanum	pea clam	1	M, W	P
Annelida	worm	0.1	M, W	P
Diaptomus spp.	copepod	0.01	W, M	Z
Bosmina sp.	cladoceran	0.01	W, M	Z
Copepoda	copepod	0.01	(W/M)	Z
Trichoptera A	caddisfly	0.1	(S)	C
Chironomidae A	fly	0.1	S	C
Hemiptera	true bug	0.1	(WC/M)	C
Hydracarina sp.	water mite	0.1	WC/M	C
Chironomidae P	fly pupae	0.1	M/S	C
Small fish	small fish	10	M/S	F