

# Contemporary phenotypic divergence of an introduced predatory freshwater fish, the northern pike (*Esox lucius*)

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## ABSTRACT

**Background:** Northern pike (*Esox lucius*) were introduced to southcentral Alaska in the 1950s and have subsequently colonized multiple lakes and rivers. These populations serve as an opportunistic natural experiment to understand the rate and form of phenotypic divergence.

**Question:** How have pike diverged in their native and introduced range and how quickly? Do morphological differences correlate with diet-mediated growth or characteristic lake or river habitat attributes?

**Hypotheses:** Given the known time since colonization, populations that have been separated for longer are likely to have diverged further from their source population than populations that have been separated for less time. Morphology will be at least in part shaped by growth and major (riverine vs. lake) habitat differences.

**Analytical methods:** We studied phenotypic divergence among nine putative populations of northern pike with a principal component analysis of individual measurements of body dimensions as well as counts of pigmentation (i.e. spotting pattern). Size-at-age was used as a proxy for growth rate and tested for associations with morphology. Evolutionary rates (haldanes) for the phenotypic divergence of each population from the putative source population were calculated and compared to a data set of ~2700 rates from different taxa to put the observed divergence into context.

**Results:** Morphological differences were detected between native and invasive groups. Invasive populations were significantly deeper bodied and had shorter heads than native populations. Body shape variation could only partially be explained by age, length, and weight (growth proxy), with values of 27% and 24% for PCI and PCII, respectively. Habitat type (river vs. lake) was significantly associated with body shape in native populations, but data limitations preclude a comparison among invasive populations. The detected phenotypic divergence estimates fall near the median of previous evolutionary rate estimates.

**Keywords:** morphometrics, northern pike, invasion, haldane, phenotypic divergence, shape variation.

## INTRODUCTION

Despite the pressing conservation implications of invasive species, the introduction of non-native species to novel environments provides for unplanned large-scale experiments to explore ecological and evolutionary change occurring in real time (Sax and Brown, 2000; Sax *et al.*, 2007; Fausch, 2008; Westley, 2011). Theory predicts that the success of invasion will hinge, in part, on the match between the phenotype of the invader and site-specific selection pressures of the recipient ecosystem (Facon *et al.*, 2006). Within-population phenotypic variation is the basic template on which natural selection can act, and thus understanding this variation is of vital importance for assessing the potential for adaptive divergence (Schluter, 1996; Draghi and Whitlock, 2012). To persist and avoid immediate extinction in a new habitat, adaptation or adaptive phenotypic plasticity must be sufficient in magnitude and speed (Sax and Brown, 2000; Ghalambor *et al.*, 2007) in changing environmental conditions such as during an invasion. Specifically, when predators are confronted with new selective landscapes, selection on standing phenotypic variation and/or plastic responses are vital for persistence in novel environments. The strength of selection is predicted and observed to be increased during periods of environmental change, including in the case of invasion (Schluter, 1996; Kingsolver and Pfennig, 2007). Consequently, the time-frame in which adaptive phenotypic divergence occurs can be short in invasive species, compared with the gradual adaptation species often undergo in other selective contexts (Sax *et al.*, 2007; Kinnison *et al.*, 2008; Westley, 2011). Therefore, invasions offer opportunistic natural examples to explore divergence in fitness-related traits in contemporary time.

Northern pike (*Esox lucius*) are a circumpolar apex freshwater predator native to North America and Eurasia (Mecklenburg *et al.*, 2002). Their morphology largely reflects their ambush feeding strategy: a fusiform body shape and distally positioned dorsal and anal fins allow for rapid acceleration of the body from a resting state to capture prey (Pierce, 2012; Skov and Nilsson, 2018). Northern pike are proficient colonizers of new habitats, not only because of their high tolerance to environmental factors, such as low oxygen levels, but also because of their trophic flexibility (Chapman *et al.*, 1989; Beaudoin *et al.*, 1999; Skov and Nilsson, 2018). Their dietary plasticity to various types of prey likely facilitates their persistence even after eradication of a primarily targeted prey species (Chapman *et al.*, 1989; Sepulveda *et al.*, 2013; Cathcart *et al.*, 2019), which also complicates plans for restoration efforts of prey items such as Pacific salmon (genus *Oncorhynchus*). Furthermore, northern pike are coveted by anglers and known to effectively restructure ecosystems. Intentional introductions and stockings into many habitats within the US and Europe presumably occurred due to the piscatorial popularity of the fish and its functionality as an ecosystem engineer (Berg *et al.*, 1997).

In Alaska, northern pike are native to habitats north and west of the Alaska Range mountains, but since the 1950s have been found in lakes and rivers in the Anchorage area (where approximately half of the state's 750,000 residents reside) and on the Kenai peninsula (Haught and von Hippel 2011). Anecdotal evidence indicates that an illegal introduction in the 1950s from a primary native source population near Fairbanks, Alaska, facilitated the establishment of new populations and fuelled the subsequent and on-going spread to new areas (Rutz, 1999; Pierce, 2012, ch. 14; Sepulveda *et al.*, 2013). Multiple sightings and catches indicate that northern pike are thriving in hundreds of these newly encountered habitats. In addition to the rising numbers, northern pike tend to prioritize juvenile salmon as prey (Sepulveda *et al.*, 2013; Courtney *et al.*, 2018; Cathcart *et al.*, 2019) and have driven the extinction of a rare, weakly armoured morph of threespine stickleback (*Gasterosteus aculeatus*) (Patankar *et al.*, 2006) in at least one lake. Although substantial efforts have been devoted to suppression and eradication, existing research is largely limited to diet analyses among invaded sites and the consequences for salmon consumption, and little is currently known about functional morphology of the invaders.

In this paper, we examine to what extent phenotypic variation exists among established northern pike populations and how much populations have diverged in novel habitats. Additionally, we seek to identify potential factors that may play into potential phenotypic divergence. In line with literature, we expect to see less variation among the comparably young and newly established invasive populations compared with well-established, presumably highly genetically diverse native populations (Westley, 2011). Previous research suggests a common correlation between habitat and morphology of fish (Nosil, 2012; Westley *et al.*, 2012; Senay *et al.*, 2017), which alludes to similar natural selection and evolutionary pressures in similar habitats. To the extent that this is true, a parallel adaptive divergence is to be expected in similar habitats. Indeed, Senay *et al.* (2017) recently found a correlation between habitat and phenotype in northern pike within their native range. In addition to these correlations, other factors may influence the expression of phenotypic traits. For example, environmental differences in available resources can have a plastic influence on growth and growth-related trait expression (Devlin *et al.*, 2012). To examine these effects in northern pike in Alaska, we conducted a morphometric study in varying habitats in the native and the invaded range in Alaska.

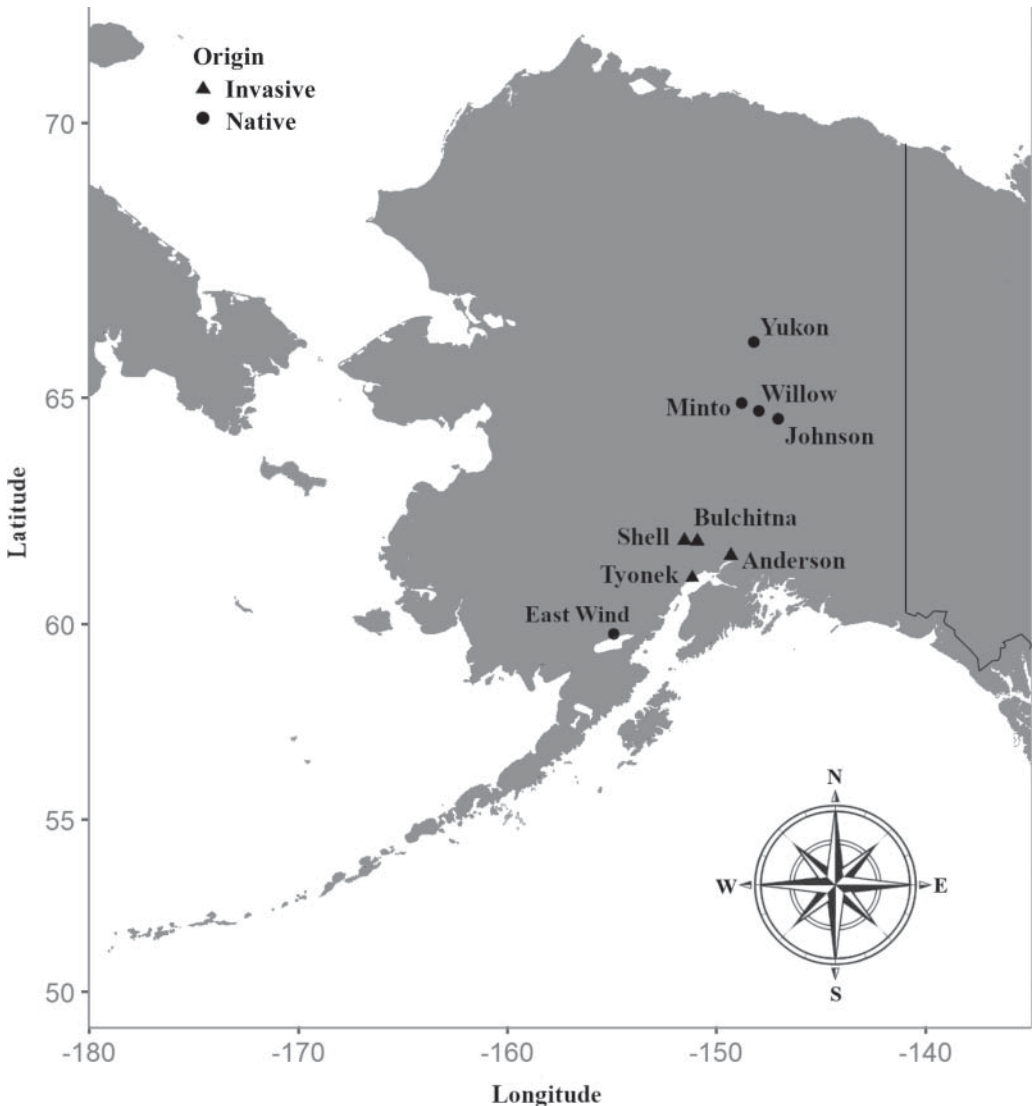
Specifically, we wished to quantify the extent of phenotypic divergence and the proposed resulting evolutionary rate (haldanes) using a synchronic comparison approach (Hendry and Kinnison, 1999). Our objectives were to: (1) quantify among-population variation in traits, such as body shape, eye size, head size, and cryptic coloration; (2) correlate phenotypic variation with habitat characteristics (e.g. river vs. lake habitat); (3) explore the potential influence of growth and diet compositions on phenotypes; and (4) put in perspective the rates of observed divergence by comparisons to an existing database of evolutionary rates (Hendry *et al.*, 2008; Westley, 2011).

## METHODS

### Study sites and sampling

We aimed to capture a minimum of 25 individual northern pike from each sampling site, where sites are interpreted as putative biological populations. The sites were chosen dependent on a combination of environmental factors and logistical considerations of access in the native range of northern pike in interior Alaska, as well as in the invaded range in southcentral Alaska in the Susitna River Basin (Fig. 1). In the invaded range, four sites were sampled, all within the Matanuska-Susitna Valley (Fig. 1): Bulchitna Lake, which is thought to be the initial introduction site (Sepulveda *et al.*, 2013), together with Tyonek and Anderson lakes, were all sampled in the summer of 2017; while for Shell Lake, samples were available from 2015 suppression efforts (Courtney *et al.*, 2018). We obtained our target sample size in all but one location in the invaded range – Bulchitna Lake, where only seven fish were caught. We included this site despite concerns over the underestimation of the pooled standard deviation in the following evolutionary rate analysis and the consequential inflation of the calculated rate, due to the relative importance of the site. As Bulchitna Lake is hypothesized to be the initial introduction site of northern pike south of the Alaska Range, it therefore represents the population with the longest time since colonization. The small sample size from Bulchitna Lake likely reflects low local abundance and the large body size of pike present there.

In the native range, we sampled fish from five sites: the Minto Lake complex near Fairbanks (the putative ancestral source), Johnson Lakes (Tanana River Drainage near Fairbanks), East Wind Lake (Bristol Bay), Old Lost Creek (Yukon River), and Willow Creek (Tanana River). We were only able to capture 16 individuals from the Johnson Lakes and seven from Old Lost



**Fig. 1.** Sampling map of Alaska. Triangles indicate sampling sites in the invaded range of northern pike, circles indicate sampling sites within the native range.

Creek, a tributary of the Yukon River at the southern edge of the Arctic Circle (Old Lost Creek will hereafter be labelled 'Yukon' in each plot). Like Bulchitna Lake, samples from Old Lost Creek were included in the following analyses, despite the negative implications for the reliability of the evolutionary rates calculated similar to the Bulchitna Lake fish, owing to the importance of having a population that is further north than the other populations for the reasons mentioned above. East Wind Lake, the Minto Flats, and Willow Creek – a clear water slough that drains into the Tanana River near Fairbanks – yielded at least 25 samples. All sampling sites are shown in Fig. 1.

Habitats differed qualitatively, with habitats in the invaded range relatively more similar to each other than habitats in the native range. The Minto Flats show similar habitat features to a highly vegetated shallow lake, however, the area is an open system that connects to the Tanana River system and therefore also allows for the passage of salmonids. Willow Creek is a clear water, slow-flowing tributary with large amounts of logs and woody debris in it, that drains into the Tanana River and therefore also connects to salmonid habitat. Old Lost Creek is a tributary of the Yukon and therefore is also in close proximity to salmonid habitat. These three habitats are examples where interactions between salmonids and northern pike are to some extent expected. The remaining two native range habitats are the Johnson Lakes close to Salcha, which are stocked with hatchery-reared rainbow trout by the Alaska Department of Fish and Game (Alaska Department of Fish and Game, 2012), and East Wind Lake, which is located close to the city of Iliamna in the Bristol Bay Region and not known to be connected to any river system. All sampling habitats were categorized into riverine or lake-type habitats. Only two populations within the native range (Willow Creek and Old Lost Creek/Yukon) were categorized as rivers.

Fish, except samples from Shell Lake obtained in 2015, were captured in the summer of 2017 with 20-metre gill nets (20 mm mesh size), as well as by angling. Northern pike from Shell Lake were obtained by gill netting as part of suppression efforts described by Courtney *et al.* (2018). Captured fish were euthanized, chilled on ice to the extent possible in the field, and subsequently frozen and stored at 0°F until examination and processing in the laboratory.

After thawing of each specimen, photographs were taken at  $3456 \times 5184$  pixels resolution (Canon Eos rebel T5i) in a standardized orientation on the left side after being placed as straight and flat as possible. The camera was mounted in a fixed position, after adjusting it to the largest specimen.

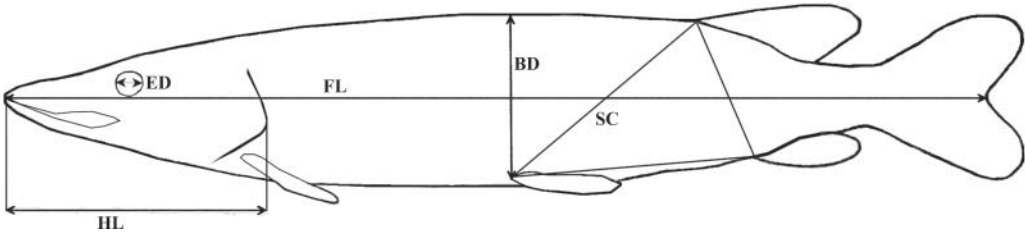
In addition, the length from the tip of the snout to the fork of the caudal fin, the circumference at the insertion of the pelvic fin, head width, and the wet weight of each specimen were recorded. Cleithra and stomachs were extracted for subsequent ageing and a diet composition analysis. Sex was determined and recorded by internal dissection.

The cleithra were aged according to a method described in Casselman (1990). Age was estimated by annulus counts in the calcified structures that are attributable to seasonal growth (Casselman, 1990). The mean of three independent estimates by trained readers was taken as the best approximation of fish age at capture.

Diet analyses followed the approach and categories outlined by Cathcart *et al.* (2019). Briefly, after removing the stomach contents from the digestive tract, their constituents were identified to two categories of taxa. Vertebrate prey were weighed individually, while invertebrate prey were weighed together.

### Direct linear measures of traits for functional shape analysis

Fiji, a version of the image-processing software ImageJ for scientific image analysis (Schindelin *et al.*, 2012), was used to take linear measurements in millimetres of body depth, head length, eye diameter, fork length, and a designated triangular area on each fish's side. Body depth was measured from the pelvic fin's insertion to the perpendicular dorsal side of the fish (Fig. 2). Head length was measured from the tip of the snout to the most posterior portion of the operculum, while eye diameter was measured horizontally across the visible part of the eye. To verify the accuracy of the digital measuring method, fork length was re-measured on the physical specimen and subsequently compared. We counted the number of pigmentation spots within a triangular area on the side of each specimen defined by lines drawn between the anal fin insertion, the dorsal



**Fig. 2.** Standardized depiction of a northern pike. Linear measurements and triangle used for analyses were taken according to the depiction. ED = eye diameter, FL = fork length, HL = head length, BD = body depth, SC = spot count within triangular area.

fin insertion, and the pelvic fin insertion (Fig. 2). This area was chosen to ensure consistency among specimens varying in size. Spots that were connected to the white of the ventral area, but clearly reached into the darker coloration, were counted as well as spots that fell on the marked outlines of the area. Due to scarring or other skin damage, 10 individuals were not quantified for pigmentation. To include this trait in our analysis despite missing data, a linear regression for spots at size across all populations was conducted and the missing ten samples were allotted predicted values based on the best fit line and the measured length of the respective sample ( $y = -6.65 * 1.62x$ ,  $P < 2.2e^{-16}$ ,  $R^2 = 0.76$ ). Mean values for each population are shown in Table 1.

### Statistical analysis

In order to control for allometric effects of size on body shape, we plotted all logarithmically transformed morphological traits against logarithmically transformed body length, and residuals of each measurement were used in a principal components analysis. We determined the number of components to retain for interpretation with a visual examination of a scree plot and a broken stick analysis (Jackson, 1993). The first two components, hereafter PCI and PCII, were retained. Factor loadings above 0.3 and below  $-0.3$  were considered to be statistically significant and

**Table 1.** Overview of population means of phenotypic traits and age for northern pike in Alaska

Population	Length (cm)	Spots (cm)	Body depth (cm)	Head length (cm)	Eye diameter (cm)	Weight (g)	Circumference (cm)	Head width (cm)	Age (years)
Anderson	433	22.8	6.4	11.4	1.32	796	18.3	3.95	3.6
Bulchitna	685	36.9	10.1	17.5	1.67	3436	30.1	7.04	4.9
Shell	308	13.9	4.9	8.4	0.94	337	13.6	2.89	2.6
Tyonek	490	26.6	7.6	13.1	1.36	1496	21.7	4.81	3.8
Johnson	334	17.8	4.7	9.1	1.25	273	13.3	2.78	4.3
Minto	599	48.7	9.1	16.3	1.74	1765	25.3	5.73	5.4
East Wind	511	39.6	6.7	12.9	1.68	883	19.5	4.28	7.2
Willow R	484	32.1	7.3	13.0	1.48	936	20.5	4.76	4.4
Yukon R	703	47.0	10.8	19.6	1.77	2979	30.7	6.53	8.2

*Note:* Riverine habitats are denoted with an R after the site name.



interpreted to load significantly on each component (Table 2). One-way analysis of variance (ANOVA) and Tukey *post-hoc* analyses on principal component scores were used to test for significant phenotypic divergence among native and invasive groups and among habitat types. A comparison of lake-type habitat populations testing for the significance of origin as an explanatory variable for populations of similar habitat type was conducted to ensure observed differences between native and invasive populations could not be explained by major habitat differences.

Given that growth rate, independent of body size, can influence shape variation (e.g. Devlin *et al.*, 2012), we also examined the effect of weight and length at age (growth proxy) on phenotypic variation by conducting linear regressions on PC scores vs. weight, length, age, and origin.

Diet-composition ratios were computed based on the proportion of vertebrate-to-invertebrate weight in grams in the stomach contents sampled. Empty stomachs were excluded from the analysis. Means for each population were computed and tested for significance as predictors of the mean PC scores (shape variables) for each population. Additionally, the mean vertebrate-ratio in diets of each population was tested for a significant association with mean length.

### Haldane rates

To quantify the observed divergence, we calculated haldanes (Gingerich, 1993, 2001) using PC scores. Haldane rates were calculated using the following expression:

$$h = \frac{\left( \frac{x_2}{s_p} \right) - \left( \frac{x_1}{s_p} \right)}{g}$$

Here,  $x_2$  and  $x_1$  represent the mean trait values (PC scores) of two populations or groups being compared with one another, and  $s_p$  is the pooled standard deviation for PC scores from the dataset. We determined  $g$ , the number of generations since separation, by estimating the age at maturity for northern pike in Alaska at 4 years. Miller and Kennedy (1948) established a length of 390–450 mm at maturity for northern pike in Great Bear Lake in Canada. We compared this estimate (Miller and Kennedy, 1948) to our length measurements and concluded a high probability of maturity at age 4 with the mean length mostly exceeding the minimum length of 390 mm.

Based on the literature (Sepulveda *et al.*, 2013; Jalbert, 2018), we considered Bulchitna Lake to be the original site of introduction of northern pike into watersheds of the Matanuska-Susitna Valley. For this reason, we compared each native and invasive population to the Bulchitna Lake population. There are two alternative hypotheses that could explain contrary outcomes: (1) northern pike were transported to Bulchitna Lake from the Minto Flats, hence there will be little phenotypic variation between the Minto Flats and Bulchitna Lake populations; or (2) the opposite could be the case – since northern pike from Bulchitna Lake have presumably been separated the longest, the greatest phenotypic divergence could be expected between the same two populations. Since the spread of northern pike into other habitats is likely to have occurred from Bulchitna Lake, we hypothesized that the remaining invasive sites would show less marked phenotypic divergence than native populations. Finally, the obtained intraspecies comparisons for this project were compared to a set of evolutionary rates (haldanes) that were previously collected and catalogued by Hendry *et al.* (2008) and updated by Westley (2011).

## RESULTS

### Quantifying between-population variation in traits

We detected significant phenotypic variation among northern pike populations based on the retention of two principal components on size-corrected traits. Aspects of body size, such as body depth, weight, head width and circumference, loaded heavily on PCI and explained ~51% of the variation, whereas PCII explained ~17% of the variation and was interpreted as effects of eye diameter, head length, and spot count (Table 2). PCI therefore describes traits that correlate to the body condition of an individual (e.g. condition factor). Low PCI scores are positively correlated with weight, circumference, body depth, and head width, whereas higher PCII scores are correlated with smaller eye size, head length, and pigmentation counts (Fig. 3).

Populations differed significantly in mean principal component scores based on analysis of variance (ANOVA) with PCI and PCII scores as separate response variables and population and origin (native or invasive) as a fixed categorical factor (ANOVA, PCI scores ~ population:  $P < 0.0001$ ; PCII scores ~ population:  $P < 0.0001$ ; PCI scores ~ origin:  $P < 0.0001$ ; PCII scores ~ origin:  $P < 0.0001$ ) (Fig. 3).

We detected a clear separation of native vs. invasive populations in phenotypic space (Fig. 3). Native populations tended to cluster in the bottom part of the plot (low PCII scores, larger eyes, longer heads, and more spots) and spread further across the PCI axis. The invasive populations grouped in the top of the plot with high PCII scores (smaller eyes, shorter heads, and fewer spots) and showed relatively little variation in low PCI scores. Mean PC scores and standard deviations are listed in Table 3.

### Correlating phenotypic variation with lake vs. stream habitats

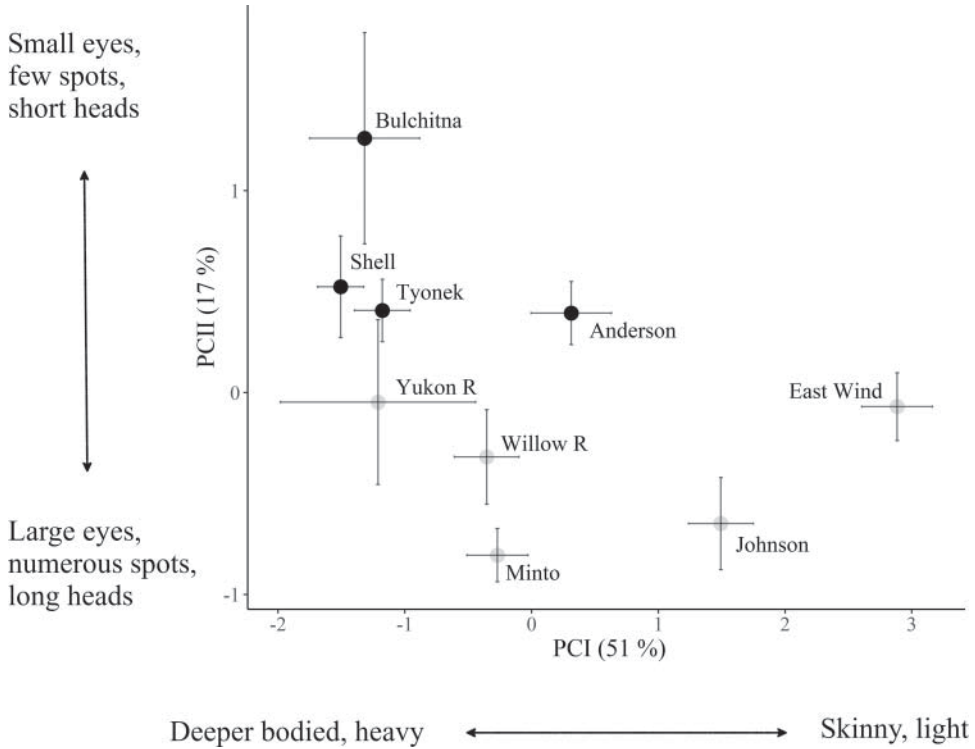
The invasive populations sampled were all from lake-type habitat, and thus comparisons to riverine populations within the introduced range were not possible. However, the native populations were sampled in both riverine and lake habitats and a one-way ANOVA showed a significant difference between habitat types for PCI ( $P < 0.0001$ ), and for PCII ( $P < 0.0001$ ) where the river fish were significantly deeper bodied than fish from lake-type habitats. The stronger the current (i.e. the more the habitat resembles riverine characteristics), the deeper bodied the northern pike. However, to ensure habitat was not a confounding effect for the origin variable (e.g. invasive vs. native), we dropped river-type populations from a one-way ANOVA and were able to detect a significant difference between lake-type invasive and lake-type native populations ( $P < 0.01$ ), justifying further investigation of other potential influences on shape variants.

**Table 2.** Results of principal component analysis

Trait	PCI	PCII	Variation explained
Body depth	<b>-0.46</b>	-0.21	51%
Head width	<b>-0.37</b>	0.00	
Circumference	<b>-0.50</b>	-0.06	17%
Weight	<b>-0.47</b>	-0.08	
Spot count	0.21	<b>-0.64</b>	
Eye diameter	0.24	<b>-0.61</b>	
Head length	-0.28	<b>-0.42</b>	

*Note:* Values above 0.3 or below -0.3 are considered statistically significant and indicated in **bold**.





**Fig. 3.** Comparative plot of mean PCI and PCII scores for native (grey) and invasive (black) populations of northern pike. Error bars indicate standard error.

**Table 3.** Mean principal component scores (PCI and PCII) and standard deviation by population

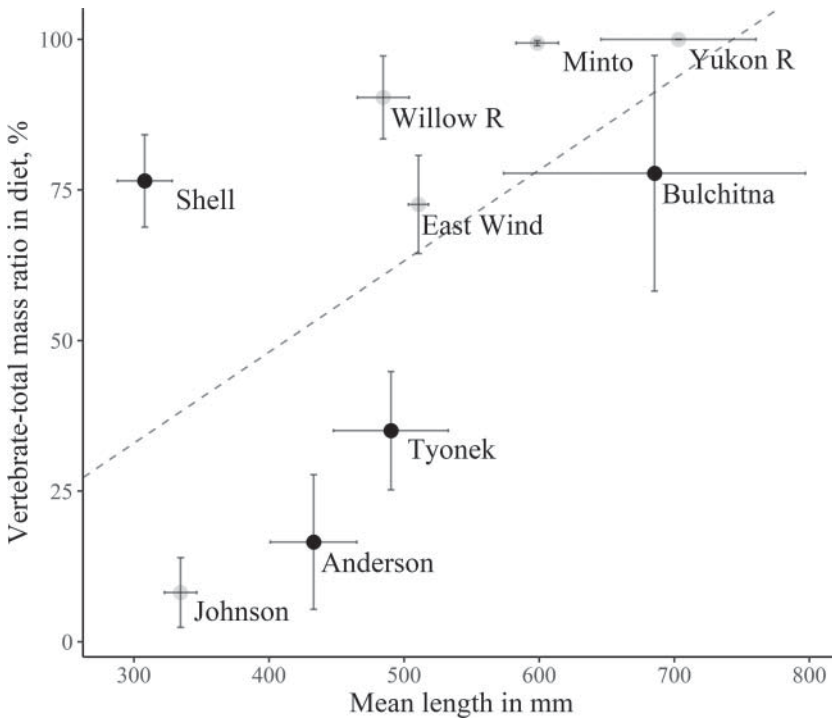
Population	Origin	PCI		PCII	
		Mean	SD	Mean	SD
Anderson	Invasive	0.31	1.62	0.39	0.80
Bulchitna	Invasive	-1.32	1.15	1.26	1.38
Shell	Invasive	-1.51	0.93	0.52	1.28
Tyonek	Invasive	-1.18	1.12	0.41	0.79
Johnson	Native	1.49	1.02	-0.65	0.91
Minto	Native	-0.27	1.28	-0.81	0.70
East Wind	Native	2.88	1.40	-0.07	0.84
Willow R	Native	-0.35	1.30	-0.32	1.19
Yukon R	Native	-1.21	2.04	-0.05	1.08

*Note:* Riverine habitats are denoted with an R after the site name.

### Potential influences of growth-mediated plasticity on morphology

To assess the influence of growth on morphological traits, we conducted a stepwise multiple linear regression of PC scores vs. age, length, weight, and origin. No interactions between age and length or weight were detected for PCI. However, age and weight were statistically significantly associated with PCI for native and invasive populations ( $R^2 = 0.27$ , age:  $P < 0.0001$ , weight:  $P < 0.0001$ , origin:  $P < 0.01$ ). PCII scores were weakly but statistically significantly related with age ( $P < 0.01$ ) and an interaction between length and age ( $P < 0.01$ ) and native or invasive origin ( $P < 0.001$ ), respectively ( $R^2 = 0.24$ ).

Diet composition based on the ratio between vertebrate and invertebrate prey was similar for native and invasive populations (Fig. 4). Within the native range, 47% of pike stomachs contained vertebrates, compared to 44% of fish from the invasive range. Average vertebrate composition of stomach contents (by mass) ranged from 8% to 100% in native populations and 17% to 78% in invasive populations. There was no statistically significant relationship between vertebrate-ratio (vertebrate mass/total mass of stomach contents) and previously obtained shape variables both for PCI ( $P = 0.38$ ,  $R^2 = 0.11$ ) and PCII ( $P = 0.89$ ,  $R^2 = 0.00$ ). This was also true for a comparison of vertebrate-ratio in diets of northern pike and mean length (Fig. 4,  $P = 0.09$ ,  $R^2 = 0.27$ ).



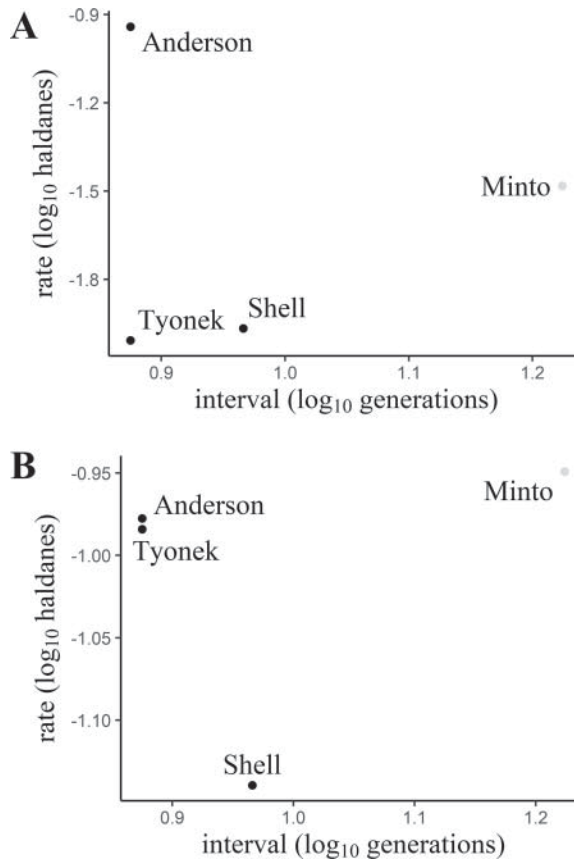
**Fig. 4.** Vertebrate-to-total-mass ratio in diet of northern pike vs. mean length in millimetres. Black dots indicate invasive populations, grey dots indicate native populations. Populations denoted with R resemble riverine habitats, all other populations are lake-type habitat. No statistically significant relationship was detected between vertebrate-to-total-mass ratio in diet and mean length ( $P = 0.09$ ,  $R^2 = 0.27$ ).

### Evolutionary rate of phenotypic divergence

We calculated rates of phenotypic divergence in haldanes for PCI and PCII of previously acquired phenotypic trait measurements (Table 4). Figure 5 shows the rate of divergence detected in shape variation (in  $\log_{10}$ (haldanes)) between Bulchitna Lake (first invaded population) and the

**Table 4.** Calculated haldane rates for invasive range populations and putative source, Bulchitna Lake

Population	Origin	Absolute haldane rate for PCI	Absolute haldane rate for PCII	Generations since separation
Anderson	Invasive	0.115	0.105	7.5
Shell	Invasive	0.0107	0.0725	9.25
Tyonek	Invasive	0.0098	0.104	7.5
Minto	Native	0.033	0.112	16.75



**Fig. 5.** Haldane rates and time intervals relative to Bulchitna Lake are expressed as  $\log_{10}$  values for consistency with previous literature (Gingerich, 1993; Hendry and Kinnison, 1999) and visualization of (A) PCI scores and (B) PCII scores in native (grey) and invasive (black) populations of Northern pike.

Minto Flats (ancestral source) as well as between Bulchitna Lake and the remaining invaded sites. For consistency with previous literature (Hendry and Kinnison, 1999), absolute haldane rates and generations since separation [appropriated to the presumed introduction events (Alaska Department of Fish and Game 2018)] were  $\log(\log_{10})$  transformed. The hypothesized 'site zero' for the introduction of northern pike into southcentral Alaska, Bulchitna Lake, showed little divergence in body condition (PCI scores) from its presumable source Minto Flats ( $h_{\text{PCI}} = 0.03$ ). However, Bulchitna Lake individuals showed considerable divergence for PCII (spot count, head length, and eye diameter,  $h_{\text{PCII}} = 0.11$ ) compared to Minto Flats individuals.

The least divergence in body condition (PCI scores) from Bulchitna Lake was observed for Tyonek ( $h_{\text{PCI}} = 0.0098$ ), followed by Shell Lake ( $h_{\text{PCI}} = 0.0107$ ), while Anderson Lake showed most divergence ( $h_{\text{PCI}} = 0.07$ ) and the lowest body condition of all invaded sites (highest PCI scores). For PCII scores, the least divergence was observed between Shell Lake and Bulchitna Lake ( $h_{\text{PCII}} = 0.07$ ), with comparable PCII divergence rates for all other sites (Anderson:  $h_{\text{PCII}} = 0.105$ ; Minto:  $h_{\text{PCII}} = 0.112$ ; Tyonek:  $h_{\text{PCII}} = 0.104$ ).

### Putting divergence into perspective

Compared to 2569 evolutionary rates obtained from Hendry *et al.* (2008) and updated in Westley (2011), the phenotypic changes observed are modest relative to other taxa. The largest detected divergence in this study was a haldane rate of 0.112 between Bulchitna Lake and Minto Flats for PCII scores (eye size, spot count, head length).

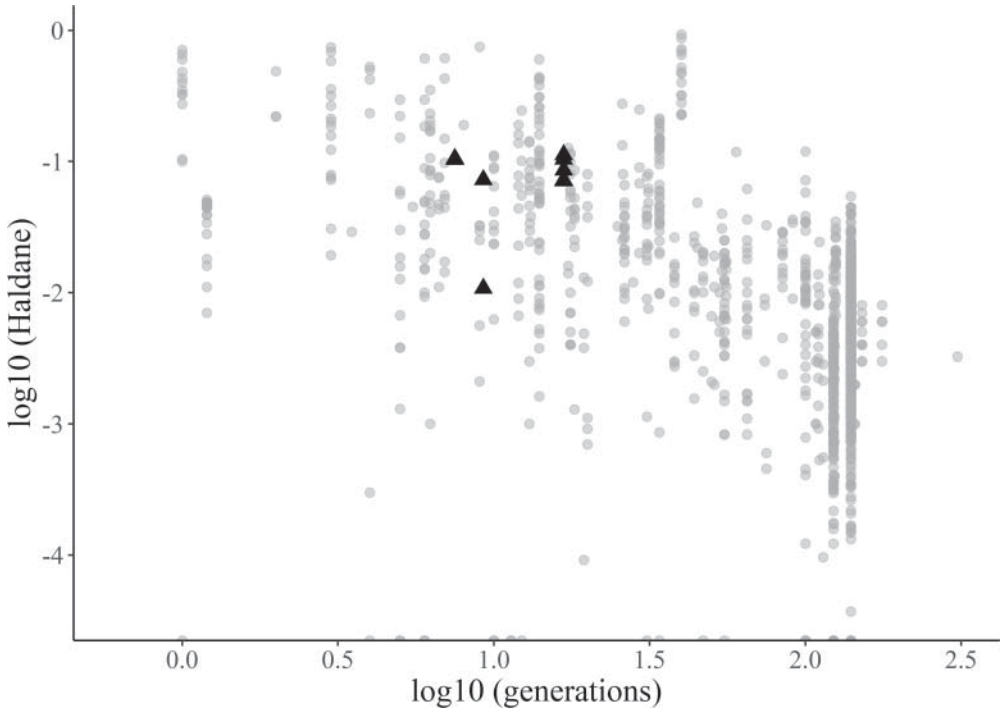
Of all recorded haldane rates, 442 described experiments with generation times of 20 or less. Of these 442, 132 exceeded our observed rate of 0.112, i.e. ~30% of previously recorded rates with a comparable time interval since separation exceeded our highest observed rate of divergence.

## DISCUSSION

Biological invasions are routinely used as opportunistic natural experiments to explore evolutionary responses to abrupt environmental change. Commonly, phenotypic divergence in the context of invasions is reported to be rapid and large in magnitude. In contrast, we found that notwithstanding the detected significant phenotypic divergence between invasive and native populations of predatory northern pike in Alaska, the observed rates of change (haldanes) were modest. Patterns of phenotypic divergence revealed invasive populations are, on average, deeper bodied, heavier and tend to have smaller eyes, shorter heads, and less cryptic coloration spots than native populations. We also detected greater variation in phenotypic traits between native populations, which likely reflects a combination of environmentally induced plasticity and greater genetic variation, consistent with potential genetic bottlenecks during the invasion process. Simple growth-mediated plasticity is unlikely to be the sole cause of the detected morphological expression, because only part of the observed phenotypic divergence could be explained by an association with an effect of age. Taken as a whole, our study of northern pike invasion to southcentral Alaska demonstrates moderate phenotypic divergence after establishment in new sites that is not fully explained by faster growth rates and the availability of higher-quality prey.

### Phenotypic variation among populations of northern pike

Overall, invasive populations tended to have deeper bodies, larger body circumferences and head widths, presumably as a result of larger body weights and increased energy content (lower PCI



**Fig. 6.** Visualization of haldane rates calculated in the present study (black triangles) vs. ~2700 rates of divergence recorded by Hendry *et al.* (2008). Shown are haldane rates vs. time intervals (generations) expressed as  $\log_{10}$  values for consistency with previous literature (Gingerich, 1993; Hendry and Kinnison, 1999).

scores,  $P < 0.001$ ). On the other hand, invasive populations tended to have short heads and small eyes for their size compared to native populations (higher PCII scores,  $P < 0.000$ ). These findings roughly correlate with growth rates in salmonids reported by Devlin *et al.* (2012) and generally support the broad pattern of deep-bodied fish tending to be fast growing (Wringe *et al.*, 2016). Bulkier bodies (i.e. better body condition) also suggest greater prey availability that may induce increased body depths at younger ages to accommodate an increase in energy storage.

### Potential influences of life-history variation on phenotypes

Growth-induced plasticity, by itself, does not explain the differences observed, as we detected a significant relationship between age, weight and PCI, and length and age and PCII, but only 27% and 24% of the observed variation, respectively, could be explained by the best fit models. These results suggest additional mechanisms, such as founder effects or adaptive divergence, to be involved in the expression of the observed phenotypic variations in combination with a weak correlation between deeper-bodied fish with smaller heads and faster growth rates, similar to Devlin *et al.* (2012). Surprisingly, an analysis of diet composition did not yield a significant relationship between the vertebrate-ratio and shape variants (PCI:  $P = 0.38$ ,  $R^2 = 0.11$ ; PCII:  $P = 0.89$ ,  $R^2 = 0.00$ ). Only 11% of the shape variation attributed to PCI was explained by the vertebrate-ratio of diets (0% for PCII). The analyses of age, length, and weight (growth proxies) as explanatory variables for shape variation yielded statistically significant relationships, but

were only able to explain 27% (PCI) and 24% (PCII) of the detected shape variation, respectively. These findings are especially surprising in the context of significant reductions to soft-fin-rayed fishes that were previously correlated with the introduction of northern pike (Sepulveda *et al.*, 2015; Cathcart *et al.*, 2019). The results of this study may, however, be limited by small sample sizes (not all sampled northern pike had stomach contents) and the resulting restricted ability to detect the previously described trends. Another possibility is the presence of other prevailing effects that influence body shape. The influence of temperature on growth, for example, is currently unknown and was not collected across the sites sampled.

We acknowledge our explicit assumptions regarding the interpretation of phenotypic divergence: that sites represent putative biological populations, our sample sizes of sites and numbers of individuals were small compared to the known range in the native and invasive range, and that the individuals sampled from each population reflect the true phenotypic mean and variance of those sites. While we think it unlikely that sampling bias alone drove the patterns we detected, it is prudent to consider this analysis as a first step towards a more intensive phenotypic census across the landscape. Overall, we suggest our results are conservative, especially given the rate of divergence observed (see below).

### Correlating phenotypic variation with habitat types

Because invasive species tend to originate from small founder populations, these populations often tend to have less genetic variation or phenotypic variation (Sax and Brown, 2000), and recent work has similarly revealed markedly reduced genetic variation in invasive northern pike populations compared to their native counterparts (Jalbert, 2018). Consistent with this, we observed larger trait ranges in body shape (PCI) in native range populations than in invasive populations (Fig. 3).

To test for another potential influence on phenotypic variation, we examined habitat differences in the native populations. All sampling sites in the invaded range resemble lake-type habitats and so functional differentiation was not possible. In the native range, however, we sampled two riverine habitats (Willow Creek, Yukon/Old Lost Creek) and three lake-type habitats (Johnson Lakes, Minto Flats, East Wind Lake). We hypothesized that this strong variation in habitat would influence the body shape of northern pike similar to the findings of Senay *et al.* (2017), who report body shape variation in northern pike to be linked to flow regimes of habitats. Specifically, northern pike from a hydropeaking (and therefore more variable) river were significantly deeper bodied than congeners from a river with a more stable flow (Senay *et al.*, 2017). In the present analysis, a significant difference in PCI and PCII for native populations from different habitat-types was observed. Northern pike from riverine habitats showed lower PCI and higher PCII scores, reflecting higher body depth, circumference, weight and head width, and shorter heads, smaller eyes and lower spot count than northern pike from lake-type habitats (Fig. 3).

Again, our findings are consistent with Senay *et al.* (2017), who report a correlation of deeper bodies in northern pike in habitats with stronger water flow. A potential explanation could be that a larger body translates to more muscle mass to navigate and hold position in strong currents for sustained and burst swimming in riverine habitats, whereas a shallow body type may be advantageous, or larger muscle mass less advantageous, in shallow lake habitats. However, habitat differences did not entirely explain phenotypic differences: lake-type fish from native and invasive populations were statistically significantly different from each other, suggesting additional influences of perhaps prey quantity or quality on phenotypic expression.



Coloration and cryptic colour patterns in fishes are influenced by a number of factors: prey type, sediment or habitat type, and social interactions between congeners may influence colour phenotypes to a varying degree (Leclercq *et al.*, 2010; Nilsson Sköld *et al.*, 2013). Controlled by complex genetic and environmental systems that are difficult to examine, disentangling the underlying mechanisms that produce an observed coloration and pattern is therefore complicated (Hubbard *et al.*, 2010). In sculpin, for example, coloration and pattern were shown to be connected to phenotypic plasticity rather than genetic expression (Whiteley *et al.*, 2009). Despite not knowing the underlying influences on the expression of coloration in northern pike, we observed a significant difference in the number of spots (pattern) between invasive and native populations (loaded heavily onto PCII). Native populations tend to have more spots than invasive populations. This difference may be attributable to habitat factors that make a smaller number of spots in invaded habitats favourable by increasing the effectiveness of crypsis, and consequently increasing predatory success. Furthermore, northern pike generally do not express rapid changes in coloration, and a stronger genetic component may be the cause. Because we only examined phenotypic traits, a clear statement as to whether cryptic coloration differences are grounded in genetic (e.g. founder effects) or phenotypic plasticity is not possible.

### Rates of divergence

Despite the significant phenotypic divergence observed among populations, haldane rates remained modest and generally in line with other examples in nature. Indeed, compared to approximately 360 other species that diverged over 17 generations or less, rates of divergence in northern pike grouped at or below the 66th percentile.

Body condition (PCI) showed the greatest variability across populations. Fish from Tyonek ( $h_{\text{PCI}} = 0.0098$ ,  $h_{\text{PCII}} = 0.104$ ) and Shell Lake ( $h_{\text{PCI}} = 0.0107$ ,  $h_{\text{PCII}} = 0.0725$ ) showed the least divergence from Bulchitna Lake overall, while Anderson Lake showed the greatest divergence in PCI ( $h_{\text{PCI}} = 0.115$ ). Anderson Lake fish appeared to be of substantially different physical condition than Bulchitna Lake fish (Fig. 3), while divergence for PCII ( $h_{\text{PCII}} = 0.105$ ) appears similar to other sites. The difference in body condition (PCI) may be attributable to the closed nature of the system compared to the at least seasonally connected system of Bulchitna Lake.

While differences in vertebrate-ratio in diet did not yield significant results for body condition, it is possible that increased access to prey is responsible for the increase in body condition in northern pike from Bulchitna Lake. Observed divergence for PCII from Bulchitna Lake was very similar for all populations despite presumed differences in time since separation. Shell Lake showed the least divergence from Bulchitna Lake in both measures and geographical proximity suggests either gene flow or recent introductory events from Bulchitna Lake into Shell Lake. However, the similarity between these habitats may explain the relatively limited divergence. Tyonek fish showed similarly small divergence from Bulchitna Lake in PCI, despite larger divergence in PCII. Divergence from Bulchitna Lake was surprisingly large in PCII for all populations irrespective of time of separation.

These outcomes suggest that Bulchitna Lake with its small sample size may be the outlier, and future research should aim to collect more samples from there to reduce the confounding effect. A genetic examination may additionally clarify the distinct pedigree of the invasive populations and serve to illuminate the causal association between genotype and phenotype. The extent to which the modest phenotypic divergence reflects contemporary adaptation to novel environments remains to be tested and would be a fruitful area of research in this model system.

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