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Prolonged swimming performance within the threespine stickleback (*Gasterosteus aculeatus*) adaptive radiation and the effect of dietary restriction

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ABSTRACT

Background: Organisms can be conceptualized as systems in which input energy is allocated to competing needs and on which natural selection acts to maximize one particular need – reproductive output. In large part, this is achieved by minimizing the energy required by other needs. Among the more important competing needs in most fish is the need to swim, and fish musculature, physiology, and shape have evolved to minimize swimming energy use in an environment- and lifestyle-dependent manner.

Question: How have growth and swimming performance evolved during adaptation to different freshwater habitats in the threespine stickleback? Does variation in early life food rations affect performance?

Hypotheses: Based on presumed lifestyles, we predict that oceanic stickleback will show the highest growth rate and swimming performance, and of the freshwater populations, we expect those representing the limnetic ecotype to show stronger swimming performance than populations of the benthic ecotype, but not stronger growth performance. Month-long periods of nutrient deprivation early in life will negatively affect all three ecotypes, with deprivation later in the first growing season (closer to the overwintering period) having the largest effect on both performance variables.

Organism: Eighteen allopatric populations of threespine stickleback, including six ancestral oceanic, six benthic, and six limnetic populations.

Methods: We used an acute swimming assay performed on sub-adult (9-month-old) fish that measures prolonged swimming ability as an indicator of aerobic muscle capacity. We also measured size achieved, because size itself affects swimming performance. We subjected juvenile stickleback to reduced rations (one-half normal) during either their second, third, or fourth month of life to simulate diet variability, which is known to affect growth trajectories and muscle development.

Results: Oceanic stickleback grew faster than freshwater stickleback under control conditions, but comparative growth was more variable when dietary reductions were considered. Diet restriction late in the first fall produced the most significant change in growth. Variations in growth across populations within ecotypes were also observed. Swimming stamina showed

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strong variation across ecotypes, and also modest population-level variation. Dietary restriction had less effect on swimming stamina than on size.

Keywords: ecotype variation, *Gasterosteus aculeatus*, nutrient deprivation, swimming stamina, threespine stickleback.

INTRODUCTION

Perhaps the most important characteristic of the natural habitats of fishes is water movement, such as currents created by gravity, wind, and other animal species (Domenici, 2010). Undoubtedly, across fish systems, swimming ability is a key component for competition success, nutrient foraging, and habitat adaptation. One important mode of locomotion is prolonged swimming, which may last anywhere from 20 seconds to 200 minutes (Beamish, 1978). It has important physiological and behavioural significance in various species of fish, due to its association with burst and glide swimming, which are important for predator avoidance and energy conservation, respectively (Weihs, 1974; Tudorache *et al.*, 2013). Furthermore, the varying natural habitats of fishes in both oceanic and freshwater systems produce substantially different environmental influences (Hiddink *et al.*, 2007), and adaptations for prolonged swimming may favour variations in the overall shape of the body (Walker *et al.*, 2013). Variation in prolonged swimming capacity may even be manifested within a fish species (Reidy *et al.*, 2000), and individual variation in morphological traits is often correlated with swimming capacity in a habitat-specific manner (Robinson and Wilson, 1994; Boily and Magnan, 2002). Distinctions in morphology driven by environment may affect the ability to perform complex manoeuvres, the ability for rapid acceleration, or it may change the energetic cost of prolonged swimming (Webb, 1982; Boily and Magnan, 2002).

Furthermore, swimming performance may be influenced by deviations in nutrient levels and growth trajectories (Sogard and Olla, 2002). For example, in the Atlantic silverside (*Menidia menidia*), fast-growing phenotypes consumed larger meals, inducing greater postprandial oxygen demand and lowering sustained swimming capacity, when the fish were forced to swim near to exhaustion (Arnott *et al.*, 2006). It has also been suggested that dietary shifts can result in changes in muscle structure that linger throughout life (Alvarez and Metcalfe, 2005). In faster than normal growing trout, white muscle fibre diameter decreases and red muscle fibre numbers increase (Valente *et al.*, 1999), resulting in potential swimming performance shifts. Even though anaerobic (white muscle) metabolism may assist during prolonged swimming (Hudson, 1973; Nelson *et al.*, 1996), red muscle provides most of the power in this swimming mode (Rayner and Keenan, 1967; Martinez *et al.*, 2003). Thus, prolonged swimming has been used as a measure of aerobic capacity (Beamish, 1978; Kolok, 1999; Alvarez and Metcalfe, 2005), and therefore it serves as an indicator of physical fitness (Sandvik *et al.*, 1993).

The threespine stickleback (*Gasterosteus aculeatus*) adaptive radiation provides an excellent model system in which to study diversification of prolonged swimming performance. This small (3–8 cm), primitive marine teleost is widespread in Holarctic coastal oceanic waters (Östlund-Nilsson *et al.*, 2006), and during its extensive post-glacial invasion of fresh water it has given rise to hundreds of thousands of separately evolving freshwater isolates. Much of the differentiation in fresh water is along the well-studied benthic–limnetic axis (Schluter and McPhail, 1992; Jones *et al.*, 2012), and is evolutionarily very recent, beginning only with a burst of colonization at the onset of the last glacial recession *c.* 15,000 years ago

(Bell and Foster, 1994). This axis of diversification is particularly relevant in our study because benthic and limnetic ecotypes have markedly different body shapes (Walker, 1997) and use the aquatic environment in very different ways (Rundle *et al.*, 2000). Oceanic stickleback are unlikely to have changed significantly since giving rise to post-glacial freshwater derivatives, and thus can be used as surrogates for the ancestor that gave rise to the freshwater radiation, at least within geographic regions (Taylor, 1999; Hohenlohe *et al.*, 2010; Foster, 2013).

Studies of the divergence of body shape within the stickleback radiation suggest that phenotypic variation in freshwater is strongly associated with habitat-specific foraging and predator-avoidance behaviours (Walker, 1997). Furthermore, oceanic and freshwater stickleback populations inhabit substantially different environments (Foster and Baker, 2004; Wund *et al.*, 2012), likely requiring different specializations; in particular, oceanic stickleback may swim considerably more, and for longer periods, than freshwater stickleback (Quinn and Light, 1989). In addition, most freshwater stickleback live in habitats where strong winters may impact the availability of food, thus reducing annual growth potential (Ab Ghani and Merilä, 2015). In previous work, we showed that during their first growing season, younger stickleback exhibit stronger short-term (within a period of 4 weeks) faster-than-normal growth responses than older fish following exposure to lower-than-normal diets, resulting in a greater capacity to regain structural weight and energy (lipid) content (Reyes and Baker, 2016). The present study extends that work by examining ecotypic- and population-level variation in size achieved, and in prolonged swimming performance, at the sub-adult stage (about 9 months of age), when stickleback in nature would be late in their first overwintering period.

Based on known or inferred lifestyle and habitat, we expect oceanic populations to show stronger prolonged swimming performance, and possibly to be less affected by nutrient deprivation during their first growing season. Furthermore, evidence has also shown strong genetic and environmental contributions to the variations in morphology and swimming performance across freshwater stickleback populations (Sharpe *et al.*, 2008; Hendry *et al.*, 2011), which suggests that prolonged swimming ability in limnetic populations (Blake *et al.*, 2005) will exceed that in benthic populations.

METHODS

Fish husbandry

Adult threespine stickleback were collected early in the spawning seasons of 2012, 2013, and 2014 from 18 allopatric populations in Alaska, USA and British Columbia, Canada, with different populations collected each year (Table 1). Experimental fish included six representatives (populations) of the oceanic ancestor, six of the benthic ecotype, and six of the limnetic ecotype. Populations were classified to ecotype based on the classical benthic–limnetic shape axis (Walker, 1997), and our own unpublished data on food preference: in benthic populations fish eat macroinvertebrates taken largely from the substrate, while in limnetic populations the fish forage principally on plankton in the water column.

Embryos were produced at field sites using standard breeding protocols for stickleback (Cresko *et al.*, 2004), and shipped to Clark University for rearing and testing. Five genetic families (males and females used in only one mating) per population were mixed into a single laboratory ‘population’ and incubated in Petri dishes in custom embryo medium (deionized water with 0.5 ppt Instant Ocean®). Embryo medium was replaced in full every 24 hours. Once fry began exogenous feeding, they were distributed to 1-litre jars (15 fry per

Table 1. The 18 populations of threespine stickleback used in our study, with corresponding geographical regions, coordinates, ecotypes, and year they were collected

Population	Region*	Ecotype	Coordinates		Year	Sample (*N*)
Anchor River	AK	Oceanic	59.7757,	151.8649	2013	85
Buccaneer Marina	BC	Oceanic	49.5323,	123.9548	2014	74
Bush Creek	BC	Oceanic	49.0166,	123.8500	2014	74
Mud Lake	AK	Oceanic	61.5977,	149.3416	2014	61
Rabbit Slough	AK	Oceanic	61.5359,	149.2530	2014	57
Resurrection Bay	AK	Oceanic	61.1257,	149.4209	2013	95
Bear Paw Lake	AK	Benthic	61.6139,	149.7532	2013	108
Beverly Lake	AK	Benthic	61.6150,	149.5656	2013	132
Corcoran Lake	AK	Benthic	61.5745,	149.7252	2012	124
Crystal Lake	BC	Benthic	49.0451,	123.9588	2014	82
Dougan Lake	BC	Benthic	48.7145,	123.6123	2014	98
Willow Lake	AK	Benthic	61.7487,	150.0553	2012	122
Cowichan Lake	BC	Limnetic	48.8875,	124.2905	2014	89
Jean Lake	AK	Limnetic	61.7237,	150.0623	2012	98
Lynne Lake	AK	Limnetic	61.7110,	150.0462	2012	98
Morvro Lake	AK	Limnetic	61.5992,	149.7840	2013	110
South Rolly Lake	AK	Limnetic	61.6672,	150.1384	2013	99
Sproat Lake	BC	Limnetic	49.2673,	125.0389	2014	69

* AK, Alaska; BC, British Columbia.

jar), resulting in five replicate jars per population per dietary treatment (see 'Nutrient deprivation' below). To ensure proper fry health and survival, all the experimental fish were fed *ad libitum* rations of freshly hatched brine shrimp nauplii until the fish reached 1 month of age. Complete water changes were done every 3 days, and uneaten brine shrimp were removed from the jars on a daily basis. For all juvenile and sub-adult fish rearing, water was maintained at 0.5 ppt salt and temperature at a constant 21°C throughout the experimental period.

Nutrient deprivation protocol

For each population, four dietary groups were created: three experimental (age-specific nutrient-deficit) and one control (Fig. 1). The diet manipulation experiments began at 1 month of age, when the youngest experimental group (DPR1) was reduced to a half-ration diet throughout its second month of life. The second experimental group (DPR2) was deprived during the third month, and the third experimental group (DPR3) was deprived during the fourth month. All nutrient-deprived periods lasted 4 weeks, after which all fish were returned to *ad libitum* rations for the duration of the experiment. The control group received *ad libitum* rations throughout the experiment. Once fish reached 5 months of age, they were moved to population- and treatment-specific 38-litre tanks within two larger (120-litre) recirculating aquarium systems, 15 fish per tank, and fed *ad libitum* rations of frozen bloodworms and frozen brine shrimp once daily. The light/dark cycle was changed to mimic high-latitude northern hemisphere winter conditions, with 6 hours of light and 18 hours of

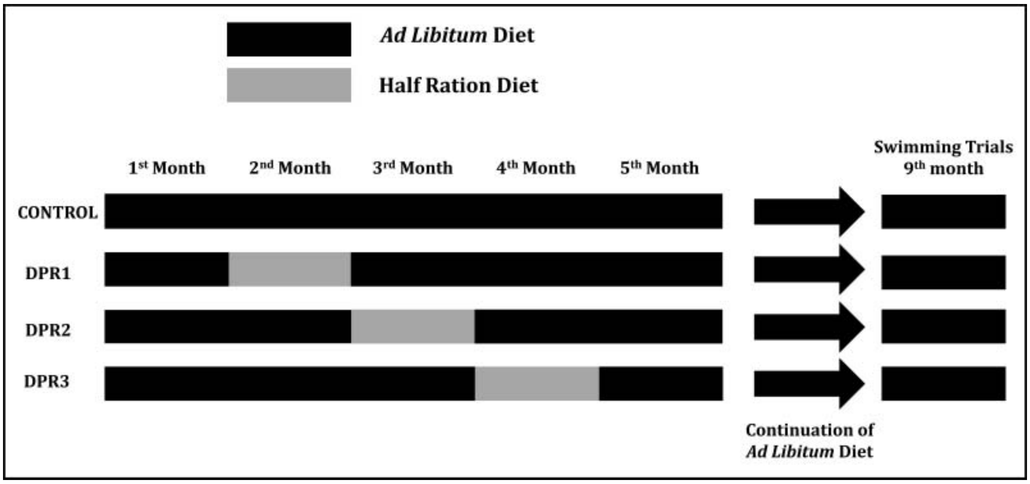

Fig. 1. Experimental protocol. The control fish received *ad libitum* nutrients throughout the experimental period. The three dietary treatment groups (DPR1, DPR2, and DPR3) were exposed to half-ration diets for 4 weeks at the onset of the second, third, and fourth month of age, respectively. Swimming trials were begun once the fish reached 9 months of age.

complete darkness (from a previous exposure of 18 hours of light and 6 hours of darkness). Four months after the end of the nutrient-deficit experiment, when the stickleback were entering their ninth month of life, we began the prolonged swimming stamina assessment.

Determination of swimming stamina

Previous research suggests that fish are able to maintain anaerobic burst swimming for relatively short periods of less than 20 seconds (Kolok, 1999). Swimming performance times longer than this against a continuous flow are defined as prolonged swimming, or swimming stamina (Kolok, 1999; Alvarez and Metcalfe, 2005). Testing was accomplished rapidly, with 30–50 sub-adult fish tested per day over a 10-day period. Approximately 25% of each population tested belonged to each of the four dietary groups. The test apparatus consisted of a specially built water flume (1 m × 0.5 m), constructed of 1-cm thick Plexiglas, with a longitudinal central divider. A swimming chamber (30 × 20 cm) with both ends closed by 1-mm mesh was used to hold test fish. The chamber was immersed at a depth of 27 cm within the water flume on one side of the divider, and a small trolling motor powered by a battery was attached on the opposite side to generate a current. Individual fish were placed in the test chamber where they were exposed to a water current equivalent to one body length per second for a period of 3 minutes. This gentle water current allowed the fish to face the flow and become adjusted to the new conditions. After 3 minutes, the current speed was increased over just a few seconds to eight body lengths per second, until the fish became fatigued. Fatigue was deemed to have occurred when the fish was unable to keep swimming against the current and remained against the rear mesh even when gently probed. All fish showed rapid and complete recovery and displayed normal swimming activity within a couple of minutes. We measured the standard body length (SL, nearest 0.1 mm) and wet body mass (nearest 0.0001 g) of each fish at the time of testing. Because fish may behaviourally cease swimming prior to becoming fatigued, and to avoid swimming behaviour associated entirely with anaerobic processes, only swimming times of 40 seconds or more were used in our analysis. The temperature of the water flume was kept at a constant 21°C during the trials.

Statistical analyses

Standard length, wet body mass, and swimming times were log-transformed to stabilize variances prior to data analysis. The distributions of standard length and wet body mass were visually assessed for any substantial deviation from normality, though none was observed. We analysed swimming times using a multifactorial mixed-model design with population as a random effect nested within ecotype, with ecotype and age-specific treatment as fixed factors, and standard length as a fixed covariate; all interactions were included in the analysis. Wet body mass was analysed similarly, except that no covariate was employed. All statistical analyses were performed using the Statistica v.12.0 software package (StatSoft® 1984–2015).

Partial eta^2 (η_p^2, a model-based effect size metric) was calculated for each effect within each analysis to supplement the statistical results. Cohen's *d*, with 95% confidence limits (Cohen, 1992; Durlak, 2009), was used to explore the scaled mean difference between selected study groups. In contrast to traditional statistical *r* values, metrics such as η_p^2 and Cohen's *d* provide tools to measure the biological importance via magnitudes of effect (LeCroy and Krysik, 2007) and are particularly pertinent in studies that generate large sample sizes, and thus considerable statistical power, such that many or all tests are declared 'significant' (Nakagawa and Cuthill, 2007).

RESULTS

Body mass at 9 months of age

All model effects in the analysis of body mass were statistically significant (Table 2), and via examination of η_p^2 values, inspection of interaction plots (Fig. 2), and targeted pairwise comparisons, important comparative patterns emerged. Stickleback ecotype had the strongest influence on variation in body size, yielding a η_p^2 value (Table 2) nearly four times

Table 2. Results of a multifactorial mixed-model analysis of log body mass (g) at 9 months of age

Effect	SS	d.f.	MS	Error d.f.	Error MS	*F*	*P*	η_p^2
Intercept	664.8	1	664.8	15.171	0.355	1870.6	<0.001	0.992
Ecotype	3.008	2	1.504	15.137	0.359	4.188	0.034	0.356
Population(Ecotype)	5.631	15	0.375	1675	0.0342	10.991	<0.001	0.090
Treatment	1.978	3	0.659	1675	0.0342	19.299	<0.001	0.033
Ecotype × Treatment	0.488	6	0.081	1675	0.0342	2.382	0.027	0.008
Error	57.214	1675	0.034					

Note: Population within ecotype was a random effect; all other effects were fixed. The analytical method employed Satterthwaite's procedure for determining appropriate denominator degrees of freedom and mean squares; this may result in fractional degrees of freedom for some tests.

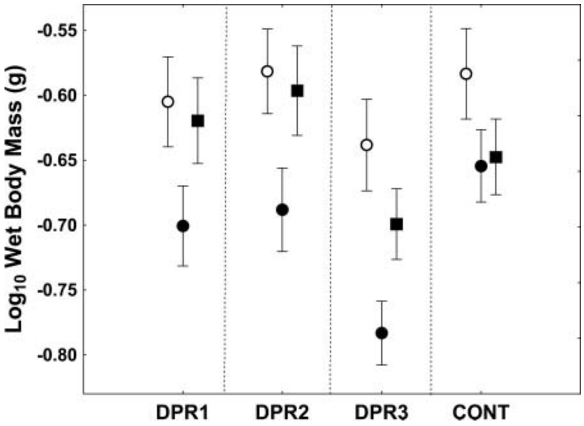

Fig. 2. Size reached at 9 months of age in three ecotypes of threespine stickleback under three dietary reduction treatments (DPR1, DPR2, and DPR3), compared to un-manipulated (CONT) fish. Vertical dashed lines separate experimental groups. ○, oceanic ecotype; ●, benthic ecotype; ■, limnetic ecotype. Data points are means across all populations within each ecotype, with error bars denoting 95% confidence intervals.

that of the next most influential model term (population within ecotype). For un-manipulated fish, oceanic stickleback attained greater sizes than either limnetic or benthic individuals, with the difference between oceanic and pooled freshwater fish being relatively large (16.1%, $d = 0.33 \pm 0.24$). However, dietary reductions altered the comparative growth patterns. In the two earliest diet manipulation groups (DPR1 and DPR2), oceanic and limnetic stickleback grew to more similar sizes, while benthic fish were significantly smaller (Fig. 2). In addition, fish deprived at these two ages exhibited sufficient growth following their return to an *ad libitum* diet to match (oceanic), nearly match (benthic), or even slightly exceed (limnetic) the sizes attained by the un-manipulated fish. In contrast, the dietary reduction imposed latest in the fall (DPR3) caused a dramatic reduction in size at 9 months of age. Oceanic DPR3 fish were 13.1% ($d = 0.34 \pm 0.24$) smaller than their un-manipulated counterparts, limnetic DPR3 fish were 13.5% ($d = 0.31 \pm 0.21$) smaller, and benthic DPR3 fish were 27.6% ($d = 0.63 \pm 0.20$) smaller. Assuming that our diet manipulations approximately reflect typical environmental conditions faced by stickleback juveniles in their first growing season (variable feeding conditions), the average across all treatments may provide the most realistic comparison of ecotypes. Effect size calculations, which scale differences to the underlying variation (expressed as SD), suggested that, overall, oceanic and limnetic sticklebacks did not differ markedly in growth rates ($d = 0.14 \pm 0.25$), while benthic fish had lower growth than both oceanic fish ($d = 0.45 \pm 0.24$) and limnetic fish ($d = 0.31 \pm 0.22$).

Within-ecotype variation (population-level) was also evident (Fig. 3). Based on population means, oceanic populations had a coefficient of variation (CV) of 11%, benthics had a CV of about 18%, and limnetics a CV of 14%. However, with the exception of Willow Lake, benthic populations grew to smaller, and more consistent, sizes generally, while limnetic and oceanic populations were more variable. It is interesting to note that the two British Columbia oceanic populations (Buccaneer Marina and Bush Creek) average somewhat

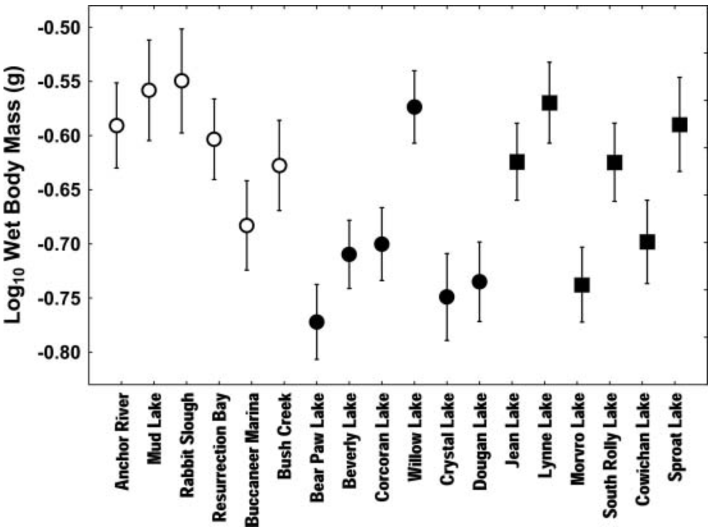

Fig. 3. Pooled (nutrient reduction treatments and un-manipulated fish) body size reached at 9 months of age in each of six populations within three ecotypes of threespine stickleback. ○, oceanic ecotype; ●, benthic ecotype; ■, limnetic ecotype. Data points are means across all treatments within each population, with error bars denoting 95% confidence intervals.

lower growth performance than the four Alaska oceanic populations (18.5% lower; Fig. 3). The British Columbia populations did not stand out within the benthic or limnetic ecotypes, however.

Swimming stamina

A relatively small proportion (1.6%) of fish did not meet our minimum swimming threshold of 40 seconds, so these fish were not included in the analyses. As expected, swimming stamina (time to exhaustion) varied significantly with fish length overall (Table 3), and within each of the three ecotypes (all $P < 0.0001$), with log-log allometric slopes varying from 0.59 to 0.86 across ecotypes. A 25-mm SL oceanic fish would be expected to swim for 125 seconds, and a 45-mm SL fish for 200 seconds. Swimming times for the same size limnetic fish were lower (110 and 160 seconds), and lower still for benthics (100 and 150 seconds). However, even after adjusting for differences in size, there was considerable variation across both ecotypes and populations. Similar to our growth analyses, all swimming stamina model terms were highly significant (Table 3), and again, calculations of standardized effect sizes and inspections of plots were helpful in deriving appropriate inferences from the analysis. As with body mass, the dominant effect shaping swimming performance was ecotype (Table 3 and Fig. 4), with moderate variation among populations within ecotypes (Fig. 5). Oceanic fish performed best, while benthic stickleback showed the least sustained swimming ability, and limnetic stickleback were intermediate. Pooling across manipulated and un-manipulated fish to generate an overall comparison, all three ecotypes differed significantly from each other (Tukey's HSD test, all $P < 0.0001$). Standardized effect sizes were: oceanic vs. limnetic (16.5% difference; $d = 0.53 \pm 0.26$),

Table 3. Results of a multifactorial mixed-model analysis of log swimming stamina (seconds) at 9 months of age

Effect	SS	d.f.	MS	Error d.f.	Error MS	*F*	*P*	η_p^2
Intercept	3.260	1	3.260	1660.59	0.023	143.76	<0.001	0.080
Log SL	1.263	1	1.263	1647	0.023	55.98	<0.001	0.032
Ecotype	3.783	2	1.892	15.30	0.113	16.73	<0.001	0.690
Population(Ecotype)	1.765	15	0.118	1647	0.023	5.22	<0.001	0.045
Treatment	0.259	3	0.086	1647	0.023	3.82	0.009	0.007
Ecotype × Treatment	0.364	6	0.061	1647	0.023	2.69	0.013	0.010
Error	37.162	1647	0.023					

Note: Population within ecotype was a random effect; all other effects were fixed. The analytical method employed Satterthwaite's procedure for determining appropriate denominator degrees of freedom and mean squares; this may result in fractional degrees of freedom for some tests.

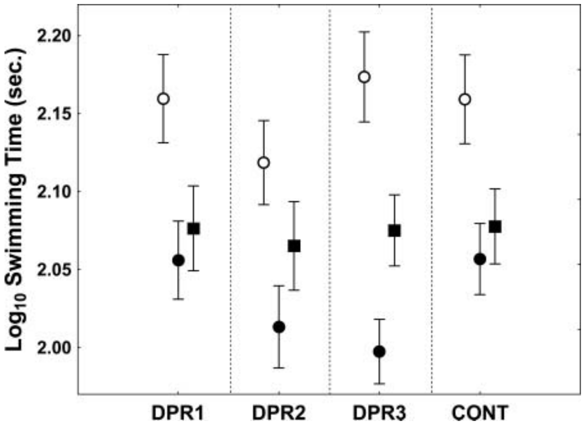

Fig. 4. Swimming stamina at 9 months of age in three ecotypes of threespine stickleback under three dietary reduction treatments (DPR1, DPR2, and DPR3), compared to un-manipulated (CONT) fish. Vertical dashed lines separate experimental groups. ○, oceanic ecotype; ●, benthic ecotype; ■, limnetic ecotype. Data points are means across all populations within each ecotype, with error bars denoting 95% confidence intervals.

oceanic vs. benthic (23.9% difference; $d = 0.76 \pm 0.25$), and benthic vs. limnetic (8.8% difference; $d = 0.23 \pm 0.22$).

The effect of dietary treatment was minor overall, but not without interesting aspects. For oceanic and limnetic ecotypes, there was no substantial treatment effect, as all groups performed similarly (Fig. 4), with the exception of a minor reduction in performance in oceanic fish subjected to the DPR2 treatment. Benthic fish showed a more substantial treatment effect in the DPR2 and especially DPR3 groups; in the latter group, the performance decline was more than 19%, which equated to a standardized difference of 0.5 SD.

Population-level variation was significant (Table 3), with all ecotypes showing variability at this level. Within oceanic stickleback, the two British Columbia populations (Buccaneer

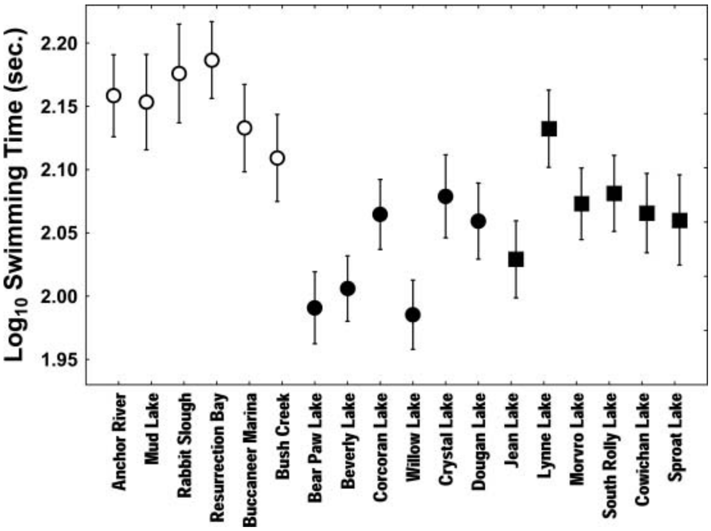

Fig. 5. Pooled (nutrient reduction treatments and un-manipulated fish) prolonged swimming capacity at 9 months of age in each of six populations within three ecotypes of threespine stickleback. ○, oceanic ecotype; ●, benthic ecotype; ■, limnetic ecotype. Data points are means across all treatments within each population, with error bars denoting 95% confidence intervals.

Marina, Bush Creek) again showed lower performance than the four Alaska populations. Among the benthic populations, the two British Columbia populations showed relatively strong swimming performance (Crystal and Dougan Lakes), but among limnetic populations there appeared to be no regional component to the variation.

DISCUSSION

Within the stickleback radiation, freshwater populations can be classified along a benthic (bottom feeding) to limnetic (plankton feeding) ecotypic axis (Foster *et al.*, 2008), with well-defined and distinct morphological characteristics (Kristjánsson, 2005; Wund *et al.*, 2012). One expression of the morphological variation along this axis is in body shape, with mathematical modelling studies indicating that slender fish are better adapted for sustained cruising in open water and feeding on plankton, while deep-bodied fish are better suited for slow-speed manoeuvring in complex habitats (Walker, 1997). Indeed, previous studies have demonstrated that the more slender limnetic fish exhibit greater swimming endurance than the bulkier benthic fish (Blake *et al.*, 2005). Our results are in general agreement; however, our study refines these findings to account for variable first-year feeding experience, and extends them by including the ancestral, oceanic ecotype. In line with our predictions, oceanic fish showed substantially greater prolonged swimming ability than either freshwater ecotype. Research assessing a second aspect of swimming performance also suggests ecotypic-level variation (Dalziel *et al.*, 2012), but for this performance aspect freshwater populations showed greater burst-swimming ability (Taylor and McPhail, 1986) than oceanic populations. A trade-off between these two types of swimming, which depend upon different metabolic and muscular aspects, is expected (Langerhans, 2009).

The comparative results we presented for swimming performance were adjusted to a common size of fish, in order to avoid the confounding effect of intrinsic and experimental variations in growth. However, as we also show, size itself has a pronounced effect on swimming stamina (Ojanguren and Brana, 2003), with larger fish (45 mm SL) outperforming smaller stickleback (25 mm SL) by as much as 60%. The relative growth rate, which clearly varied with diet treatment both early in life (Reyes and Baker, 2016) and subsequently (this study), thus also must be taken into consideration. Periods of low nutrient levels, and subsequent changes in growth trajectories, such as compensatory growth, may have negative effects on muscle development (Johnston *et al.*, 2002), ultimately resulting in a reduced capacity for sustained swimming (Kolok and Oris, 1995; Billerbeck *et al.*, 2001; Killen *et al.*, 2014), suggesting a physiological trade-off between growth rates and swimming capacity. In accordance with a similar study, which showed that abnormal growth diminishes swimming performance (Arnott *et al.*, 2006), our experiment further suggests that growth deviations early in life, which may include faster or slower growth rates, carry long-term consequences for physical performance. Growth-enhanced transgenic salmon have also been shown to be inferior swimmers compared with non-transgenic cohorts of the same size (Sundström *et al.*, 2015).

When fish undergo a period of reduced rations, growth slows down. This may be an unavoidable consequence (non-adaptive plasticity) of inadequate food. Conversely, it could also be an adaptive response, to allocate the limited energy to other needs. Furthermore, once nutrient levels are restored to their optimum, the fish may undergo a compensatory growth response. Any of these effects, individually or in combination, may underlie the variation in sub-adult size and swimming capacity we observed. Although presently we cannot determine this, if indeed our nutrient-deprived fish are exhibiting faster than normal growth, this trade-off between growth rate and swimming capacity may be due to the metabolic scope limitation induced by a fast growth rate, which ultimately reduces energy available for swimming performance (Conover and Schultz, 1997; Billerbeck *et al.*, 2001). At 9 months of age, freshwater stickleback populations within the Arctic region are enduring the overwintering phase. Within this extreme phase, the lakes freeze, reducing the available light and decreasing the ability of the visually feeding stickleback to find nutrients (Guthrie, 1986; Shuter *et al.*, 2012), thus drastically decreasing nutrient intake (Giacomini and Shuter, 2013). Evidence suggests that fish acclimatize to the overwintering phases by lowering energy expenditure and slowing down body metabolism (Ultsch, 1989; Bell, 2012). Although our experimental fish did not undergo a full natural overwintering period, the lower amount of light, mimicking true Arctic winter light conditions, and to which our fish populations were exposed to, may have resulted in lower rates of metabolism.

Additional potential explanations for lower swimming stamina may be weaker muscular composition and relatively high muscular stress acquired earlier in life. Previous work suggests that periods of low nutrients result in muscle fibre composition weakening (Rios *et al.*, 2009), and the subsequent hyperphagia induces detrimental muscular damage (Ali *et al.*, 2003). Furthermore, due to laboratory limitations, all experimental fish were kept at 21°C, which is substantially warmer than their natural habitats, and both threespine and ninespine sticklebacks show deviations in metabolism and susceptibility to disease as a response to thermal acclimation (Bruneaux *et al.*, 2014; Schade *et al.*, 2014), and since such physiological fluctuations may driving lower swimming performance across all our experimental fish, this effect may be more apparent in nutrient-deprived populations.

In our study, all experimental populations were exposed to diets of equal quality – live brine shrimp nauplii (planktonic food) initially, followed by a mix of small and large foods (mixed diet including benthos) later on. Benthic stickleback in nature feed on plankton while young, but shift to snails, fly larvae, dragonfly nymphs, and other larger invertebrates found on the lake bottom, while limnetic fish feed primarily on zooplankton throughout life (Lavin and McPhail, 1986; Schluter, 1993). Interestingly, the nutritional value of typical prey items found in natural benthic and limnetic habitats appears to be similar (Ahlgren *et al.*, 1997); thus, so long as fish acquire an equivalent biomass of food per unit time foraging, the growth rates should be similar – which is what we found for un-manipulated freshwater fish. Although evidence suggests that stickleback fish may show lowered growth rates when they are transplanted between habitats (Schluter, 1995), the only difference in size was among treatment groups. Thus, we suggest that observed body size variability across treatments and ecotypes is a result of the distinct times of nutrient deprivation.

Our study indicates that food deprivation early in life has consequences for both subadult size and physical fitness. Much of the variation in body size and swimming capacity after early-life nutrient deprivation was attributable to differences among populations within ecotypes, showing that populations adapt not just to general benthic or limnetic habitats, but to their specific benthic or limnetic habitat. In turn, this suggests that populations have evolved different abilities in response to low nutrient levels.

In summary, we found substantial ecotypic-level differentiation in growth and prolonged swimming capacity within the stickleback radiation. Oceanic fish generally grew faster, and showed stronger swimming capacity, than either benthic or limnetic freshwater fish. Superimposed on this background differentiation, we also demonstrated that, during the first growing season, body size and swimming performance may be significantly modified by diet fluctuations, with benthic fish being the most affected. Despite this significant ecotypic-level variation, populations showed moderate to large differences, with the differences in swimming performance being particularly pronounced.

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