



Low connectivity between sympatric populations of sunfish ecotypes suggests ecological opportunity contributes to diversification

Will M. C. Jarvis^{1,2} · K. S. Peiman³ · S. J. Cooke³ · B. W. Robinson²

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Abstract

Ecological opportunities, such as access to a novel habitat or the extirpation of a key competitor, can lead to adaptive divergence by exposing populations to diversifying selection. Typically, effects of ecological opportunity on adaptive divergence are inferred from macroevolutionary patterns rather than tested in populations undergoing contemporary divergence. This limits our insight on how ecological conditions contribute to adaptive divergence. Pumpkinseed sunfish (Centrarchidae: *Lepomis gibbosus*) have recently and repeatedly colonized a ‘novel’ pelagic habitat in postglacial lakes, and subsequently undergone phenotypic diversification. We investigated whether ecological opportunity has contributed to diversification in a pumpkinseed population that has diversified between lake habitats. We used a between-year mark-recapture study to evaluate whether (1) the novel pelagic habitat represents an ecological opportunity by supporting a large, high-density population, and (2) connectivity between ecotype populations is restricted by limited adult dispersal. We found that phenotypic variation is spatially structured between habitats, similar to prior studies. Submerged shoals in the pelagic habitat do sustain a large adult population at a density seven times greater than the ancestral littoral habitat. Additionally, body condition and size of pelagic pumpkinseed is similar to littoral pumpkinseed. This suggests the pelagic habitat provides an ecological opportunity to pumpkinseed in the form of abundant, available resources. Furthermore, strong between-year habitat fidelity suggests aspects of the ecological opportunity have reduced adult dispersal and could limit gene flow. In combination with prior evidence indicating diversifying selection between habitats, these results provide an example of how ecological opportunity might contribute to contemporary adaptive divergence.

Keywords Adaptive divergence · Movement · Gene flow · Mark-recapture · Matching habitat choice

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✉ Will M. C. Jarvis
wjjarv030@uottawa.ca

Extended author information available on the last page of the article

Introduction

An ecological opportunity in the form of an abundant and accessible novel resource can initiate adaptive divergence by allowing a population to expand its habitat or resource use (Schluter 2000; Simpson 1953; Stroud and Losos 2016; Yoder et al. 2010), creating the potential for diversifying selection (Wellborn and Langerhans 2015). Barriers to connectivity between populations can further promote adaptive divergence when they limit gene flow (Schluter 2009; Yoder et al. 2010). However, the effects of the ecological opportunity on adaptive divergence are seldom directly verified, and more often their effects are inferred from the macroevolutionary outcome of adaptive radiation, which may not represent the initial ecological conditions because the ecology of a lineage can evolve for a variety of reasons (Stroud and Losos 2016). Here, we evaluate the case for an ecological opportunity in a population currently undergoing adaptive divergence.

Ecological opportunity can arise through *de novo* access to a resource or habitat, the loss of a predator or competitor, or the evolution of a key innovation (Simpson 1953; Wellborn and Langerhans 2015; Yoder et al. 2010). The ecological opportunity initially relaxes stabilizing selection on a previously optimal phenotype permitting expanded phenotypic variation, resource or habitat use, and population size presumably driven by intraspecific competition (Yoder et al. 2010). In the context of adaptive divergence, an ecological opportunity represents a vacant fitness peak in the local adaptive landscape (Martin and Wainwright 2013; Stroud and Losos 2016). Persistent occupancy of an ecologically distinct ‘novel’ niche can then generate diversifying selection that drives adaptive divergence, providing there is sufficient genetic variation and minimal constraints (Wellborn and Langerhans 2015). Diversifying selection has been repeatedly implicated in cases of divergent ecotypes and species of fishes in lakes, such as benthic and limnetic populations of stickleback (McKinnon and Rundle 2002; Schluter and Mcphail 1992), cichlids (Seehausen and Wagner 2014; Sturmbauer 1998) and whitefish (Doenz et al. 2018). Persistence in a novel habitat, particularly when replicated, is a hallmark of an ecological opportunity because a viable population strongly implies the availability of sufficient resources and opportunities for reproduction (Robinson and Wilson 1994; Wellborn and Langerhans 2015). The existence of secondary fitness peaks strongly implies that ecological opportunity could drive diversification (Schluter and Grant 1984; Stroud and Losos 2016). By supporting a viable population that can come under selection, ecological opportunity can facilitate adaptive divergence.

For ecological opportunity to lead to adaptive divergence, other conditions must also be met. Adaptive divergence is more likely where mechanisms arise that promote assortative mating and reproductive isolation (Hendry and Taylor 2004; Lenormand 2002; Nosil and Crespi 2004), since linkage disequilibrium among adaptive alleles can otherwise be broken down by gene flow (Bolnick and Fitzpatrick 2007). Furthermore, theory indicates that the spatial scale of environmental heterogeneity relative to dispersal distance should influence whether selection favors adaptive divergence, phenotypic plasticity, or some intermediate response strategy (Baythavong 2011). Hence, many of the best examples of ecological opportunity leading to adaptive divergence involve spatially isolated habitats where population connectivity and gene flow are already reduced, such as on islands (Givnish et al. 2009; Lovette et al. 2002; Roderick and Gillespie 1998) and inland lakes (Robinson and Wilson 1994; Seehausen and Wagner 2014; Sturmbauer 1998).

Gene flow consists of two events that are strongly influenced by ecology: successful movement of individuals between populations (i.e. population connectivity), and after

movement occurs, the successful establishment of immigrant alleles into a population through survival and sexual reproduction (Mallet 2001). Since connectivity reflects the movement of individuals between populations, reductions in connectivity that increase assortative mating may subsequently restrict gene flow. However, our understanding of connectivity among populations undergoing contemporary adaptive divergence is narrow, for example focusing on host-shifts by short-lived insects where reproductive isolation is rapidly established (Berlocher and Feder 2002; Bolnick and Fitzpatrick 2007; Dres and Mallet 2002) or between populations that are nearly completely reproductively isolated (McKinnon and Rundle 2002; Seehausen and Wagner 2014; Sturmbauer 1998), leaving much of the sympatric divergence continuum unobserved.

Despite a substantial theoretical framework for why ecological opportunity should promote adaptive divergence (Bolnick and Fitzpatrick 2007; Wellborn and Langerhans 2015) and numerous studies pointing to historic effects of ecological opportunity on adaptive divergence, there are few studies of contemporary populations exploiting ecological opportunities, particularly in sympatry (Stroud and Losos 2016; Yoder et al. 2010). Here, we investigated the case for ecological opportunity in the adaptive divergence of ‘polyphenic’ pumpkinseed ecotypes that coexist by inhabiting different lake habitats. We define a polyphenic population as one with high continuous phenotypic variation, as opposed to discrete phenotypes. Polyphenic pumpkinseed sunfish (Centrarchidae: *Lepomis gibbosus*, Linnaeus 1758) have expanded phenotypic variation to exploit a ‘novel’ pelagic niche in a pattern repeated in multiple postglacial lakes (Robinson et al. 1993; Weese et al. 2012; Robinson et al. 2000).

Ancestrally, pumpkinseed are inshore shallow-water specialists, with functional traits for feeding on larger hard-bodied prey like snails and insect larvae (Parsons and Robinson 2006; Wainwright 1991; Werner and Hall 1977). However, in nutrient-poor post-glacial lakes with low fish diversity, multiple populations have been found to expand their habitat use by colonizing the deep-water pelagic habitat where all life stages feed extensively on zooplankton prey such as *Daphnia* (Robinson et al. 2000; Weese et al. 2012), a niche that is typically occupied by a competitor, the bluegill sunfish (Centrarchidae: *Lepomis macrochirus*, Rafinesque 1819) (Robinson et al. 2000, 1993). This pattern of diversification is similar to other well-studied systems of sympatric divergence between littoral and pelagic niches in freshwater fish species including stickleback (Schluter 1993), arctic char (Gislason et al. 1999), and whitefish (Doenz et al. 2018; Lu and Bernatchez 1999). Key ecological differences between littoral (shallow, near-shore) and pelagic (open-water) habitats involve structural characteristics and available prey (Jastrebski and Robinson 2004). The shallow littoral habitat is limited to the shoreline, tends to be sheltered, structurally complex due to vegetation, rocky substrate, and coarse woody debris, and host to a wide array of benthic macroinvertebrate prey and spawning habitat. The deeper pelagic habitat is a structurally simple but open water environment punctuated by submerged rocky shoals, with access to abundant zooplankton prey while providing refuge from predators and spawning habitat.

Phenotypic variation in polyphenic pumpkinseed populations is expanded and spatially structured (Berchtold et al. 2015; Colborne et al. 2016; Robinson et al. 2000; Weese et al. 2012), indicating some habitat specialization by ecotypes. Ecotypes consistently differ in ecologically relevant traits after accounting for body size. The pelagic ecotype has smaller oral jaws, pharyngeal jaws, and brain size (Jarvis et al. 2017; Robinsin, unpublished result; Axelrod et al. 2018), but longer gill rakers compared to the littoral ecotype (Jastrebski and Robinson 2004). The body form of the pelagic ecotype is more ‘tear-drop’ in shape than the littoral type due to a smaller head and more forward placement of pectoral fins (Colborne

et al. 2016; Gillespie and Fox 2003; Jastrebski and Robinson 2004; Weese et al. 2012). For all traits, variation is continuous rather than discrete and intermediate forms occur in both habitats. Diversifying selection likely acts between ecotypes because foraging performance on habitat-specific prey types is related to phenotype in the lab (Parsons and Robinson 2007), and phenotype predicts growth and body condition in lake habitats (Colborne et al. 2016; Jastrebski and Robinson 2004; Robinson et al. 1996). Evidence from population genetic studies of polyphenic pumpkinseed indicates that they are unlikely the result of multiple colonization events, and instead are examples of repeated within-lake divergence (Weese et al. 2012). One reciprocal transplant common garden study revealed that phenotypic plasticity and canalized genetic effects accounted for 53 and 14% of variation in body form respectively between sunfish ecotypes (Robinson and Wilson 1996), and further study revealed an interaction effect that suggests plastic responses have diverged between ecotypes (Parsons and Robinson 2006).

Two reproductive isolating mechanisms have already been identified between pumpkinseed ecotypes: assortative mating based on isolation between littoral and pelagic habitat during the spawning season, and mate choice (Colborne et al. 2016; Jarvis et al. 2017). However, these mechanisms may be insufficient barriers to gene flow between ecotypes, particularly if there is considerable dispersal of pumpkinseed between habitats. Adult pumpkinseed express high site fidelity (i.e., low habitat connectivity) over the 4-month summer growing season when reproduction and the majority of foraging occurs. For example, a mark-recapture study found a 97 and 98% probability of remaining in the same site during the summer for pelagic and littoral pumpkinseed, respectively (McCairns and Fox 2004). Additionally, experimental transplants revealed greater fidelity by littoral origin than pelagic origin pumpkinseed with 93 and 74% probability of returning within a summer to their initial capture site respectively (McCairns and Fox 2004). However, pumpkinseeds can live up to 10 years in the wild (Fox 1994), which allows for individuals to disperse over longer time scales. For example, each fall adult pumpkinseed in northern temperate lakes leave their summer feeding sites to overwinter at a greater depth before migrating back to more productive shallower summer sites the following spring (personal observation). High between-year habitat connectivity due to active switching or accidental straying could potentially generate gene flow between ecotypes that limits adaptive divergence.

We used a between-year mark-recapture study to evaluate two hypotheses. First, that the pelagic habitat represents an ecological opportunity for pumpkinseed. We compared population abundance and density, as well as body size and condition, of pumpkinseed between the ancestral littoral and novel pelagic habitats. If an ecological opportunity exists in the pelagic habitat, then we would expect to see at least equivalent population size, density, and performance there relative to pumpkinseed in the littoral habitat. Second, we tested for reduced connectivity between littoral and pelagic populations between years. We assessed individual movement between years, both between habitats and among sites within habitats. If connectivity between habitats is limited, we would expect to find lower rates of movement between habitats than among sites within habitats. Our work tests key predictions of the ecological opportunity hypothesis in a population undergoing early divergence and increases our understanding of how ecological opportunities and connectivity can interact to drive adaptive divergence.

Methods

Study location

The pumpkinseed of Ashby Lake, ON (45.092 N, 77.351 W) are representative of the pumpkinseed trophic polyphenism (Jastrebski and Robinson 2004; Weese et al. 2012) and have been the focus of continuing study since 2000. Ashby is an oligotrophic Canadian Shield lake composed of three basins with a combined surface area of 2.6 km² and a maximum depth of 36.6 m. The dominant habitat by area is a deep open water pelagic zone punctuated by islands (all <0.04 km²) and 11 submerged rocky shoals. Productive littoral habitat with soft organic substrates and vegetation is restricted to sheltered bays separated by segments of rocky shorelines with a narrow littoral strip exposed to prevailing winds (Jastrebski and Robinson 2004). Submerged rocky shoals in the pelagic habitat provide access to zooplankton prey, physical shelter from predators, and suitable sites for pumpkinseed reproduction (Colborne et al. 2016; Jastrebski and Robinson 2004). The distances among shoreline littoral sites and among pelagic shoal sites often exceed the distances between littoral and pelagic sites (Fig. 1), so habitats are not isolated by distance alone. Fish diversity is low (seven species and no other Lepomids; Jastrebski and Robinson 2004), and the only potential competitor for pelagic zooplankton resources are lake trout (Salmonidae: *Salvelinus namaycush*, Walbaum 1792) which are restricted to deep, cold water during the summer season.

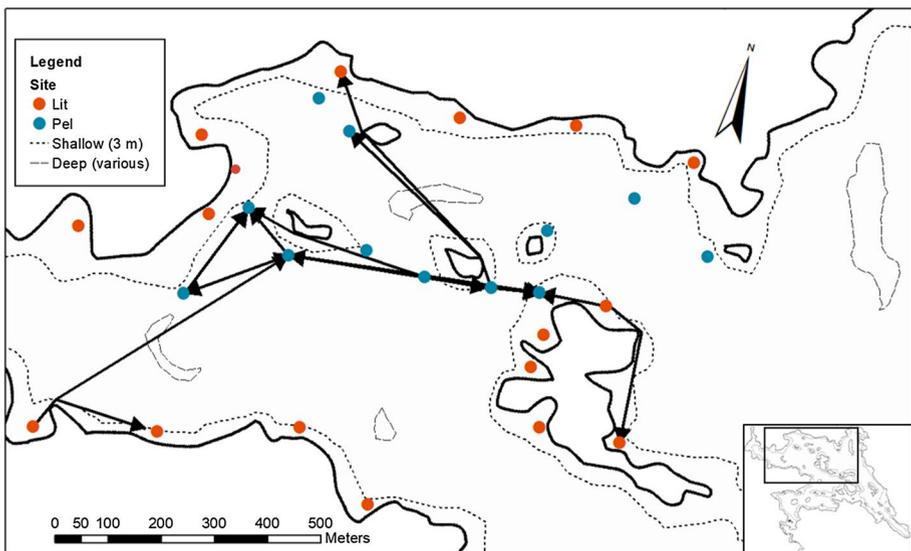


Fig. 1 Map of the north-west basin of Ashby Lake showing all recorded pumpkinseed movements within and among sites from the summer of 2016 to the summer of 2017. Circle symbols indicate mark and recapture sites (pelagic = blue, littoral = red). Arrows indicate the direction of an individual's movement. The dotted line represents the 3 m depth contour along shorelines. The smaller map indicates the area of Ashby Lake (45.092 N, 77.351 W) enlarged

Tagging and recapture

To estimate relative population sizes and quantify rates of movement between and within littoral and pelagic habitat sites, we employed a mark-recapture study of pumpkinseed from summer 2016 to summer 2017. Between June 24th and July 19th, 2016, we collected 516 pumpkinseed from littoral (L) and 469 from pelagic (P) habitats in Ashby Lake through a combination of angling (L: $n=235$; P: $n=209$) and trapping (L: $n=281$; P: $n=260$) to limit possible behavioural biases due to capture method. We sampled exclusively in the north-west basin (0.8 km² surface area) because of its separation from the southern and eastern basins by deep, narrow channels. This restricted spatial focus allowed us to maximize the efficiency of our recapture effort. We sampled 12 pelagic sites, consisting of 11 rocky shoals and 1 small islet, each defined as the area < 3 m deep around the shoal/island where pelagic pumpkinseed spend the majority of their time (personal observations). We sampled 17 littoral sites either in sheltered bays ($n=11$) or along 100 m lengths of shoreline ($n=6$), each defined as the area < 3 m deep, and roughly corresponding to the home range size of pumpkinseed (~3000 m²) (Fish and Savitz 1983). We anesthetized pumpkinseed on capture using immersion in clove oil solution (0.01%) for 3 min, measured individual wet weight (g) and external oral jaw width (mm), and took a side view photograph against a calibrated scale for measurement of standard length (mm). We then marked each fish with a unique visually coded wire tag (CWT: 1.1 mm Length × 0.25 mm Dia., Northwest Marine Technologies, Inc.) inserted into dermal tissue at the posterior base of the anal fin (Brennan et al. 2005). We clipped the 4th dorsal spine and the posterior portion of the dorsal fin to help visually identify marked fish during recapture and to assess tag loss rates. We released all marked pumpkinseed at their site of capture.

In summer 2017, we recaptured pumpkinseed through a combination of angling and trapping between June 6th and August 24th. We attempted to evenly distribute recapture effort between angling and trapping, but angling proved substantially more successful (76.1% of fish by angling vs. 23.9% trapping). We sampled at the 29 sites where pumpkinseed had previously been captured and released. We captured 4874 pumpkinseed (L: $n=1956$; P: $n=2918$), visually identified marked individuals by clipped dorsal spines, and additionally checked all pumpkinseed with a magnetic tag detector (Northwest Marine Technologies, Inc.). Of 75 pumpkinseed with clipped dorsal spines, only 46 had tags, indicating 38.7% tag loss. Tag loss was independent of body size ($Z_{74} = -0.22$; $p=0.83$) and we have no reason to expect tag loss to differ between habitats. We euthanized marked pumpkinseed by immersion in clove oil solution (anesthetized at 0.01% solution for 5 min and euthanized at 0.2% for 5 min), extracted CWTs by dissection for identification, and preserved the fish in 10% buffered formalin.

Pumpkinseed phenotype, performance, and habitat use

To evaluate the spatial structure of phenotypic variation, we compared the phenotype of a randomly selected subset ($n=30$) of pumpkinseed from each of the littoral and pelagic habitats collected during the marking phase in 2016. A subset was used to time constraints and were selected using a random number generator. These samples were distributed across 4 littoral sites ($n=6, 6, 7, 11$) and 6 pelagic sites ($n=3, 3, 4, 5, 7, 8$). We assessed phenotype of the subset based on oral jaw size, a trophic trait positively related to consumption of benthic macroinvertebrates and capable of distinguishing ecotypes here (Jarvis et al. 2017) and stickleback

species elsewhere (Schluter and Mcphail 1992). Oral jaw size was measured as the lateral distance between maxillaries using digital calipers. We tested for the effect of habitat on oral jaw size with ANCOVA, treating body size (standard length, mm) as a covariate, habitat as a factorial variable, and initially including their interaction (which was subsequently removed as non-significant, $t_{59} = -1.66$, $p = 0.10$). We also tested for differences in mean body size (standard length, mm) and body condition between habitats with ANOVA. Body condition, a proxy for fat reserves, is expected to reflect performance in fishes where fat reserves are critical for winter survival (Peig and Green 2010). Body condition was estimated using the Scaled Mass Index, which performs better than either mass to length ratios or residuals from an OLS regression (Peig and Green 2010). Individuals < 75 mm standard length were not used to estimate the Scaled Mass Index because small pumpkinseed have different growth trajectories that generate outliers (Osenberg et al. 1988), leaving 57 pumpkinseed to compare body condition between habitats ($n_{\text{Littoral}} = 27$, $n_{\text{Pelagic}} = 30$). The size range included large juveniles and sexually mature individuals (Gillespie and Fox 2003). Sex was not determined for individuals collected in 2016 as it can only be reliably determined by dissection of dead specimens and that would preclude release for our mark-recapture study.

We assessed the sex, phenotype, body condition, and habitat use of the 46 recaptured tagged pumpkinseed to investigate what factors might influence individual movement. In the lab, we identified individuals from their tags and determined sex by visual examination of gonads under a dissecting microscope. We used measurements of oral jaw width (mm), mass (g), and standard length (mm) at capture (2016) in order to test their influence on subsequent movement. To increase the accuracy of size corrected trait estimates, we used a larger data set combining the recaptured pumpkinseed with the subset of randomly selected (see above) marked (but not recaptured) pumpkinseed from each habitat ($n_{\text{total}} = 106$). We assessed phenotype for this combined group with the residuals from an OLS regression of external oral jaw width (mm) against standard length (mm). We estimated body condition using the Scaled Mass Index (Peig and Green 2010), excluding individuals with standard length < 75 mm (Osenberg et al. 1988), leaving 41 recaptured pumpkinseed with an estimate of body condition ($n_{\text{Littoral}} = 18$, $n_{\text{Pelagic}} = 23$). Lastly, we assessed long-term habitat use of recaptured pumpkinseed from the number of external parasites that infest pumpkinseed via intermediate snail hosts typically found in the littoral habitat (McCairns and Fox 2004; Robinson et al. 2000). Infection occurs when a free-swimming larva released from the snail contacts a fish host (Wilson et al. 1996). We counted the number of trematode ‘blackspot’ parasites (*Neascus* spp.) encysted in the skin and fins on each individual recaptured in 2017. To compare long-term habitat use between littoral and pelagic pumpkinseed, parasite counts were modeled using a generalized linear model with a Poisson distribution and log link function (O’Hara and Kotze 2010), with standard length (proxy for age) as a covariate, habitat as a factorial variable, and including their interaction. The data was not over dispersed and therefore did not violate Poisson distribution assumptions.

Population size and density

To assess if the novel pelagic habitat supports a viable local population, we estimated total population sizes of pumpkinseed separately in the littoral and pelagic habitats in the study area using the modified Lincoln-Peterson estimator (Seber 1982):

$$N = [M(C + 1)] / (R + 1) \quad (1)$$

where N is the size of the population at time of marking, M is the number of individuals marked in the first sample, C is the total number of individuals captured in the second sample, and R is the number of marked individuals in the second sample. The number of marked individuals in the second sample (R) included all those recaptured with clipped dorsal spines regardless of whether they still had their wire tag because this reflected real recaptured marks despite lacking individual identity. This estimator is nearly unbiased with 7 or more recaptures (Seber 1982). It is unlikely that this is a closed population because the year between marking and recapture permits deaths, births, immigration, and emigration to alter the relative number of marks in the population. However, dispersal between habitats was low as shown below. Additionally, compared to the original marked sample (2016), our recapture sample (2017) was biased towards individuals collected by angling (76% of recaptures vs. 45% of originally marked), and towards larger individuals (mean = 39.5 g vs. mean = 28.5 g). However, these recapture biases did not differ between littoral and pelagic habitats and so should not influence comparisons between habitats (Seber 1982). Lastly, during the recapture phase, some unmarked individuals were likely captured multiple times since these were released at the end of each collection trip. These violations tend to overestimate absolute population size but there is no reason to expect this to differ between habitats and so influence relative sizes between habitats. Furthermore, absolute population size was likely underestimated because juvenile pumpkinseed (< 60 mm) were excluded as they were too small to be marked. We calculated conservative 95% confidence intervals for population size estimates using the Poisson distribution to account for reduced precision (Seber 1982).

Mean population density was estimated in each habitat by dividing the estimated population size by the total surface area of the habitat type in the north-west basin (ArcGIS 10.6, ESRI 2011). Location data was collected from Google Maps (2018). Total littoral habitat was measured as the area between the shoreline and the 3 m depth contour (Fish and Savitz 1983). Pelagic habitat was measured as the total area shallower than 3 m around each rock shoal or island. We choose the 3 m depth contour because extensive observation on this population since 2000 reveals that pumpkinseed spend the majority of their time at this depth or shallower during the summer season. The pelagic shoal area was estimated at 37 240 m², an order of magnitude smaller than littoral area at 253 500 m². We did not estimate population density at individual sites because while local capture effort varied this data was not available. However, total recapture effort (sampling duration) was approximately equal between habitats.

Connectivity analyses

We assessed between-year connectivity at two spatial scales: among sites within a habitat, and between littoral and pelagic habitats. We calculated binomial proportion confidence intervals for each estimate (Clopper and Pearson 1934). The level of connectivity between habitats was assessed by comparing the frequency of movement among sites within a habitat to the frequency of movement between habitats using Fisher's exact test. We also compared the frequency of movement among sites within a habitat between littoral and pelagic habitats using Fisher's exact tests.

Lastly, we investigated individual movement at each spatial scale. Only three individuals switched between habitats (see results) precluding statistical tests of factors associated with movement at this larger scale. A moderate number of individuals moved among sites within habitats, especially within the pelagic habitat (see results), allowing us to test

whether morphology, body condition, or parasite load predicted movement among sites within a habitat. We used a binomial generalized linear model with a logit link function to estimate the odds of moving between sites within a habitat using oral jaw size, body size, body condition, sex, and parasite load as fixed effects. All analyses were performed in R 3.5.0. (R Development Core Team 2018).

Results

Pumpkinseed phenotype, performance, and habitat use

We found evidence of spatially structured phenotypic variation consistent with previous studies (Gillespie and Fox 2003; Jarvis et al. 2017; Jastrebski and Robinson 2004; Robinson et al. 2000). Oral jaw width, a key trophic trait, differed between pumpkinseed from littoral and pelagic habitats in Ashby Lake. In the 2016 subset of initially marked pumpkinseed, those collected from the littoral habitat had oral jaws 8.8% larger than those from pelagic habitat after accounting for body size ($t_{59} = -3.91$, $p = 0.00027$; Fig. 2; Table S1). Furthermore, the oral jaws of recaptured tagged pumpkinseed collected in 2017 from littoral sites were 5.6% larger than those collected from pelagic sites after accounting for effects of sex and body size ($t_{42} = 2.71$, $p = 0.010$; Table S2).

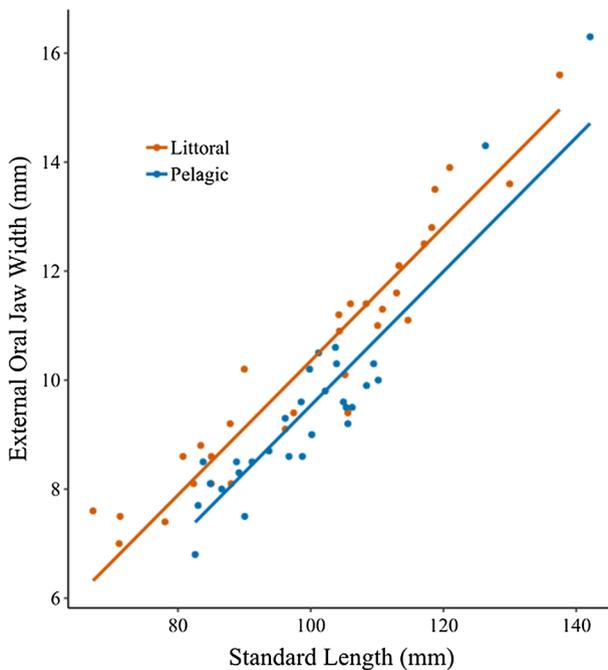


Fig. 2 Effect of standard length (body size) and initial capture habitat on oral jaw size for pumpkinseed collected in 2016, using an ANCOVA model. Pumpkinseed sampled from the littoral habitat had consistently wider oral jaws than those sampled from the pelagic habitat ($t_{59} = 3.91$, $p = 0.00027$) after accounting for the effect of body size

There was no evidence of differences in performance among pumpkinseed from littoral and pelagic habitats, at least with respect to body condition. Mean pumpkinseed body condition did not differ between habitats in the dataset of initial captures in 2016 ($F_{1,52} = -1.28, p = 0.21$; Table S3) or in the 2017 dataset of recaptured pumpkinseed ($t_{40} = 0.23, p = 0.82$; Table S4). Body size did not differ between marked fish sampled from littoral and pelagic habitats in the 2016 subset ($t_{59} = -0.61, p = 0.55$; Table S5). Recaptures in 2017 collected from pelagic sites were 12.0% larger than those from littoral sites ($t_{43} = 2.86, p = 0.0065$; Fig. 3; Table S6). Lastly, there was some evidence of differentiation in long-term habitat use by individuals that was related to size. At smaller body sizes, littoral pumpkinseed recaptured in 2017 had higher parasite loads than those from pelagic habitat sites ($z = -6.96, p < 0.0001$; Fig. 4; Table S7). However, this difference disappeared at larger body sizes above about 110 mm SL (habitat and body size interaction: $z = 6.039, p < 0.0001$; Fig. 4).

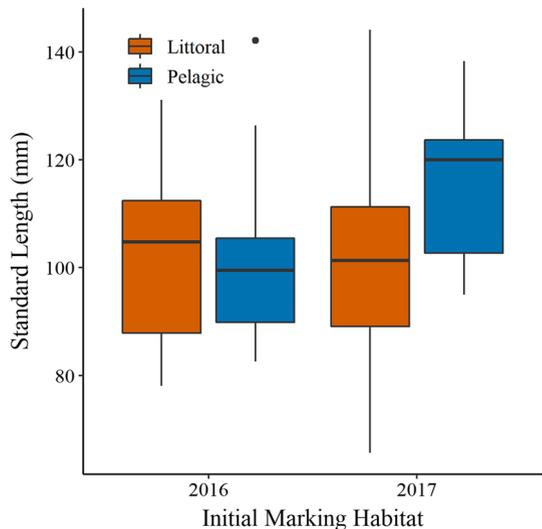
Population size and density

Absolute population size estimates (individuals ≥ 60 mm standard length) were similar between littoral and pelagic habitats in the north-west basin of Ashby Lake, however density in the pelagic habitat was 7.1 times higher than in the littoral habitat because of the smaller extent of the pelagic area around shoals. We estimated a population size of 29 760 (CI 21 520, 42 580) pumpkinseed in the littoral habitat, at a density of 0.12 (CI 0.085, 0.17) individuals/m², and 31 770 (CI 23 970, 43 140) pumpkinseed in the pelagic habitat, at a density of 0.85 (CI 0.64, 1.16) individuals/m².

Habitat and site connectivity

Pumpkinseed were 6.2 times more likely to move among sites within a habitat than to move between littoral and pelagic habitats (*odds ratio* = 6.15, $p = 0.0061$). Out of the 46 recaptured

Fig. 3 Boxplots of standard length (body size) by initial capture habitat for pumpkinseed measured in 2016 and 2017. Boxes show the mean value, and the upper (75th) and lower (25th) quartiles. Tails indicate the extent of the data. Pumpkinseed from the pelagic habitat had 12.0% larger mean body size than those from the littoral habitat in 2017 ($t_{43} = 2.86, p = 0.0065$), but there was no difference between samples collected in 2016 ($t_{59} = -0.61, p = 0.55$)



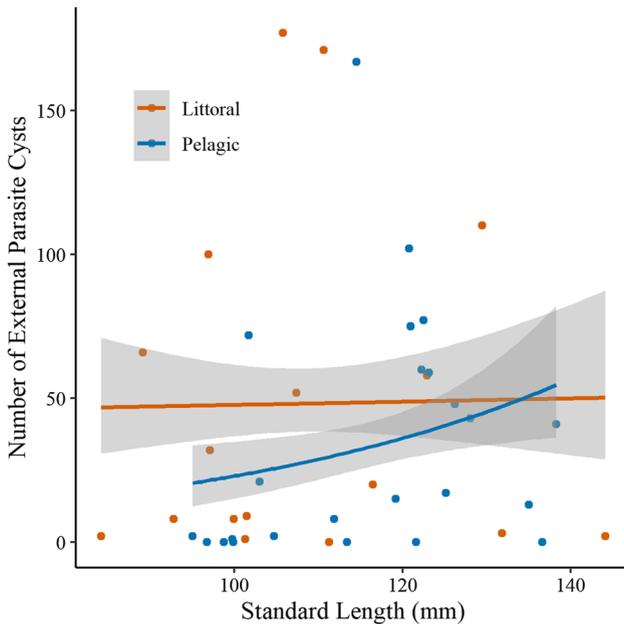


Fig. 4 Effects of standard length (body size) and collection habitat on the frequency of external ‘blackspot’ parasites (*Neascus* spp.) for pumpkinseed marked in 2016 and recaptured. Shaded area reflects the estimated 95% confidence interval from the generalized linear model. Pumpkinseed from the littoral habitat tended to have higher parasite loads on average than those from the pelagic habitat ($z = -6.96$, $p < 0.0001$), although this difference disappeared at the largest body sizes

individuals that retained their tag, 17 were recovered at sites different from their original capture location (37.0%; CI 23.2%, 52.5%; Fig. 1, 5). Of those, only 3 individuals were recovered in a habitat different from that of their original capture (6.5%; CI 1.4%, 17.9%; Fig. 1, 5). Within habitats, pumpkinseed were 8.1 times (*odds ratio* = 8.08, $p = 0.0087$) more likely to move among sites in the pelagic habitat (12/24; 50.0%; CI 29.1%, 70.9%) than among sites in the littoral habitat (2/19; 10.5%; CI 1.3%, 33.1%), despite almost twice as many littoral than pelagic sites sampled in the study.

The low frequency of movement between habitats ($n = 3$, 6.5%) precluded analysis of the effects of phenotype, body condition, or habitat use on individual movement. Within the pelagic habitat, the odds of moving among sites were predicted by body size, with smaller individuals more likely to have moved between years than larger individuals ($z = 2.04$, $p = 0.042$; Fig. 5b). Jaw size, body condition, sex, and parasite load did not predict individual movement among pelagic sites (all $p > 0.1$). The low frequency of movement among sites in the littoral habitat ($n = 2$, 10.5%) precluded further analysis of these effects.

Discussion

Rather than inferring the historic effect of an ecological opportunity on adaptive divergence, we have found demographic evidence of a contemporary ecological opportunity, as well as evidence of reduced connectivity between ecotypes, in a population at an early

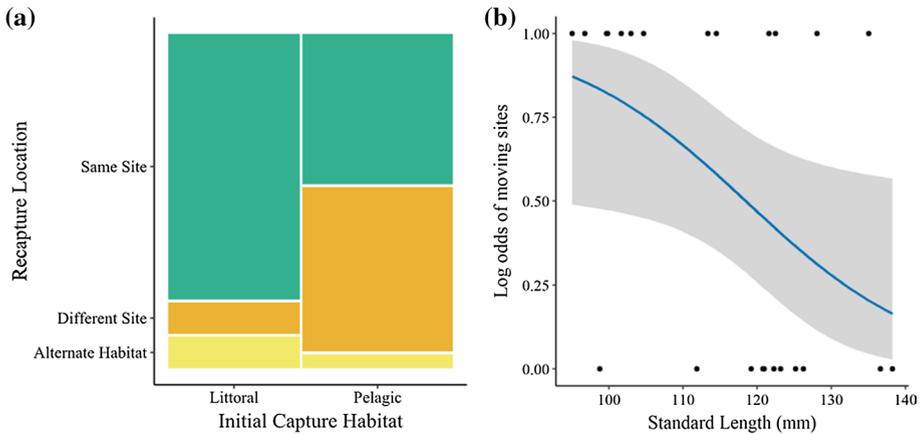


Fig. 5 **a** Proportions of individuals recaptured in 2017 in each recapture location by initial capture habitat. Colours indicate locations of individuals recaptured in 2017: in the alternate habitat type (yellow), in a different site of the same habitat (orange), or the same site (green). There is no difference in the proportion of individuals captured in the alternate habitat (*odds ratio* = 0.42, $p = 0.59$), but the proportion of individuals that moved among sites within habitat was greater in the pelagic than in the littoral habitat (*odds ratio* = 8.08, $p = 0.0087$). **b** Negative effect of standard length on the probability of moving among pelagic sites between years ($z = 2.04$, $p = 0.042$). Shaded area reflects the 95% confidence interval

stage of sympatric divergence. The pelagic habitat sustains a large population of pumpkinseed at a higher density than the ancestral littoral habitat, and these individuals suffer no apparent cost to performance. This suggests that a secondary fitness peak exists in the pelagic habitat (Robinson and Wilson 1994) and implies that ecological opportunity is driving diversification (Martin and Wainwright 2013; Stroud and Losos 2016). Oral jaw size, a trophic trait diverging between benthic and limnetic stickleback species (Schluter and Mcphail 1992), also differs between pumpkinseed from the two habitats in this and other lake populations (Gillespie and Fox 2003; Jarvis et al. 2017; Robinson and Schluter 2000; Robinson et al. 1996). This suggests that these habitat conditions favor specialized trophic ecotypes. The ecological opportunity afforded by the pelagic habitat supports an adult pumpkinseed population presumably through access to substantial zooplankton resources that are largely unavailable in the littoral habitat (Jastrebski and Robinson 2004; Robinson et al. 2000; Weese et al. 2012). We also found that the between-year connectivity of populations in littoral and pelagic habitats was greatly reduced compared to connectivity observed among sites within habitats, indicating that fidelity to habitat was strong. Reduced connectivity between pumpkinseed in these habitats may contribute to stronger assortative mating (Colborne et al. 2016), and in concert with mate choice (Jarvis et al. 2017), could potentially reduce gene flow and promote adaptive divergence of pumpkinseed ecotypes here.

Ecological character release has been implicated in initiating pumpkinseed divergence (Robinson et al. 2000), similar to other species with sympatric adaptive divergence such as stickleback (Schluter and Mcphail 1992), arctic char (Gislason et al. 1999), and whitefish (Lu and Bernatchez 1999). The primary focus of prior research has been on factors such as intraspecific competition that drive individuals to exploit a novel niche, and the sources and targets of diversifying selection, rather than on how characteristics of the novel niche affect population viability. For character release to lead to adaptive divergence, the

newly exploited niche must provide an ecological opportunity by supporting a viable local population. Ashby Lake is a nutrient-poor lake with limited soft substrate for macrophytic plants. Littoral productivity is low, and a large portion of the productivity may be concentrated in the pelagic habitat, where the epilimnion supports densities of zooplankton in June as high as 4.73 mg/m^3 (Robinson unpublished results). Interspecific competition for zooplankton is expected to be weak in Ashby Lake as the only other zooplanktivorous fish are small-bodied lake trout (*Salvelinus namaycush*), a cold-water species with limited access to the warm epilimnion during the productive summer period. Thus, our first goal was to evaluate the extent that the pelagic rocky shoal habitat represents an ecological opportunity by supporting a viable local population.

Here we show that the pelagic habitat appears to provide sufficient resources to sustain a viable population because the pumpkinseed population density there was seven times greater than in the littoral habitat. High densities of pumpkinseed have been observed each summer season at these pelagic shoals since this population was first sampled in 1999 (Jastrebski and Robinson 2004), indicating that the current population size estimates are likely not outliers. Diversifying selection is more likely to promote adaptive divergence when diverging populations are stable (Stroud and Losos 2016; Wellborn and Langerhans 2015; Yoder et al. 2010). Phenotype influences habitat-specific performance in pumpkinseed, consistent with diversifying selection. Morphology is related to prey-specific feeding performance in the lab for both ecotypes (Parsons and Robinson 2007) and to annual growth rate and lipid levels especially in the pelagic habitat (Robinson et al. 1996). When pelagic resources are underexploited, such as in lakes with few zooplankton competitors, pumpkinseed populations expand in phenotypic variation and habitat use in a pattern replicated across multiple populations (Robinson et al. 2000; Weese et al. 2012) that is consistent with character release (Robinson and Wilson 1994; Yoder et al. 2010).

Despite high population densities in the pelagic habitat, there was no evidence of a cost to growth performance, as pelagic pumpkinseed had equivalent mean body condition to littoral pumpkinseed, which is consistent with other trophic polyphenisms (Robinson and Wilson 1994). Furthermore, mean body size which can be linked to fitness in this species (Danylchuk and Fox 1994; Murphy et al. 2012) was not consistently different between habitats. The standard length of pumpkinseed recaptured in the pelagic habitat in 2017 was 12% larger than littoral pumpkinseed. However, size differences were not found in the 2016 sample, nor between adult ecotypes in three other polyphenic pumpkinseed populations in this region (Gillespie and Fox 2003). Similarities between pumpkinseed ecotypes in mean individual body condition and size indicate that resources are readily available in the pelagic habitat. Successful annual spawning activity and juvenile recruitment also occur at pelagic shoals (personal observations). Hence, many of the requirements for local population viability are in place in the pelagic habitat of Ashby Lake.

We may have overestimated total adult population abundance in the north-west basin of Ashby Lake since several model assumptions were violated (see Methods: *Population size and density*), however, the confidence interval method used here was conservative in order to address this issue (Seber 1982). More precise estimates of population size are possible with multiple recaptures of individuals (Grimm et al. 2014). These methods were not possible here because removal of the coded wire tags required euthanizing fish on their first recapture. Our recapture rate after 12 months was also lower than most mark-recapture studies of pumpkinseed (McCairns and Fox 2004; Wilson and Godin 2009), although the higher rates of recapture in those studies likely occurred because of a much shorter 4-month re-sampling interval. The number of individuals initially marked (985) and captured during resampling (4874) were reasonable for this large a population (Seber 1982;

Robson and Regier 1964). Nevertheless, we have no reason to expect that uncertainty in the absolute population size would affect our critical observation that littoral and pelagic habitats support similar relative population sizes, and hence our conclusion about population expansion and ecological opportunity afforded by the pelagic habitat in this lake.

Theory suggests that the evolution of divergent habitat or resource preferences are a critical component of adaptive divergence in sympatry (Ravigne et al. 2009; Scheiner 2016). Preferences that reduce connectivity can limit the opportunity for gene flow that otherwise constrains local adaptation (Nosil et al. 2006). Pumpkinseed express considerable habitat fidelity in Ashby Lake as demonstrated by several key observations. Only 6.5% of recaptured pumpkinseed switched between littoral and pelagic habitats from 2016 to 2017. This is an order of magnitude lower than the rate of movement among sites within the pelagic habitat (50%). Despite this high movement within the pelagic habitat, there was almost no movement from pelagic sites to accessible littoral sites at the same spatial scale (Fig. 1). This suggests some form of habitat fidelity as otherwise we would expect similar rates of movement among sites within the pelagic habitat, and between pelagic sites and geographically accessible littoral sites, which we did not find. Our findings replicate evidence of habitat fidelity from another polyphenic pumpkinseed population that was observed over the 4-month summer season (McCairns and Fox 2004), while also demonstrating that this pattern extends over longer time scales. This suggests that habitat fidelity may be a common feature of the trophic polyphenism in pumpkinseed. Both of these studies only observed pumpkinseed during the summer season, so it is possible that pumpkinseed populations mix overwinter (Suski and Ridgway 2009). However overwinter mixing is unlikely to affect assortative mating and gene flow since pumpkinseed only spawn during the summer after migrating back to summer seasons sites (Danylchuk and Fox 1994).

The restricted habitat connectivity found here may be indicative of long-term habitat segregation since we found that smaller pumpkinseed (less than 110 mm) from the littoral habitat tended to have a higher frequency of blackspot (*Neascus* spp.) parasites than those from the pelagic habitat. This is supported by previous findings that parasite loads in this species are related to long-term habitat use (McCairns and Fox 2004; Robinson et al. 2000). Juvenile sunfish are probably less likely to travel through open water than larger fish because of risk from lake trout and loon predators in Ashby Lake (Matsumura et al. 2010; Osenberg et al. 1992; Osenberg et al. 1988). However, the frequency of parasites on larger fish converged between habitats, indicating that pelagic ecotypes are exposed to *Neascus* later in life (Allison et al. 1977). Habitat connectivity may fluctuate over time, perhaps depending on the resource profitability of littoral relative to pelagic habitat, driving some larger pelagic pumpkinseed to disperse to the littoral habitat when pelagic zooplankton resources become limiting. Alternatively, adult pumpkinseed may migrate to littoral habitat during spawning season if nest sites are limited in the pelagic habitat. Pumpkinseed males typically excavate nests in soft gravel substrate (Scott and Crossman 1973) that is rare on the rocky pelagic shoals. These short-term movements could increase habitat connectivity that contributes to gene flow, although we have no direct evidence these migrations are taking place.

Although we found evidence of reduced connectivity, the observed rate of movement may be sufficiently high to allow gene flow to homogenize neutral genetic differences if individuals that switch habitats survive and reproduce. Neutral genetic population structure (F_{st}) approaches 0 when the effective population size (N_e) multiplied by the migration rate (m) is greater than 1 (Mallet 2001). We have no estimates of the reproducing proportion in either population, but the effective population size (N_e) is likely a small fraction of total adult abundance because nest locations are likely limiting (personal observation).

However, the relatively large size of both populations means that genetic drift likely has only minor effects (Mallet 2001). Even if only 1% of the population reproduced each year, then $N_e m$ would still be much greater than 1. For neutral alleles to diverge among populations in Ashby Lake, either the migration rate must be further reduced, immigrants must have poor survival in a new habitat, or mate choice must limit their reproductive success. At least over short time scales transplanted adult pumpkinseed appear to have high survival (McCairns and Fox 2004), but survival over longer time scales requires further study. Jarvis et al. (2017) found evidence of mate choice in this population that could reduce the reproductive success of immigrants, although this varied between years. There is some evidence that neutral alleles have diverged between pumpkinseed ecotypes in other polyphenic populations (Weese et al. 2012), however not here (Colborne et al. 2016). Gene flow persists in the Ashby Lake population because adult habitat preferences, immigrant mortality, or mate choice are all still too weak to generate complete reproductive isolation among pumpkinseed ecotypes, or because larval drift (Dettmers et al. 2005; Houde 1994) between habitats dominates gene flow rather than adult movement.

Some degree of gene flow does not preclude the segregation of adaptive alleles between sympatric populations in theory. Phenotypic differences between ecotypes have persisted in this and other polyphenic pumpkinseed populations (Berchtold et al. 2015; Robinson et al. 2000; Weese et al. 2012) despite gene flow. Although plasticity certainly plays a role in generating phenotypic differences between ecotypes, evidence from reciprocal transplant common garden studies also suggest that genetic differences exist between ecotypes in trophic traits and in plastic developmental responses to environmental conditions (Parsons and Robinson 2006; Robinson and Wilson 1996). Thus, it is possible that phenotypic population structure could reflect underlying adaptive genetic population structure despite gene flow, as for example in cichlids (Fruciano et al. 2016; Henning et al. 2017) and stickleback (Ravinet et al. 2018).

One critical uncertainty in this pumpkinseed population is whether the few individuals that change habitats constitute random or directed gene flow (Edelaar and Bolnick 2012; Edelaar et al. 2008). Theory strongly indicates that phenotypically biased gene flow, for example through phenotype-matching habitat choice, facilitates adaptive divergence by sorting functional alleles into their optimal habitat (Ravigne et al. 2009; Scheiner 2016). Theory also suggests that intermediate levels of random gene flow can increase phenotypic variance and thereby increase the opportunity for selection (Kirkpatrick 2000; Nosil et al. 2003), and favor adaptive phenotypic plasticity instead of divergent local trait adaptations (Baythavong 2011). Thus, the effects of individual movement on reproductive isolation, gene flow, and evolutionary responses are not solely a function of the numbers of individuals dispersing but also of their type (Edelaar and Bolnick 2012).

Unfortunately, with only three individuals recovered that switched habitats, we could not test for phenotype-matching habitat choice. This reveals a challenging paradox for studies focusing on factors that limit individual movement in spatially structured populations; high habitat fidelity makes it difficult to test the causes of natural movement even with significant tagging effort. There was evidence that movement among sites within the pelagic habitat was biased towards smaller individuals, although this was not predicted by oral jaw size which is unsurprising given that zooplankton resources are common to all shoals (Jastrebski and Robinson 2004). Nor was pelagic site movement predicted by body condition, a common proxy for individual performance, as is the case in some other species (Bowler and Benton 2004). Instead, smaller pumpkinseed were more likely to move between pelagic shoals than larger pumpkinseed, perhaps because they face competition from larger individuals for reproductive sites. By comparison,

movement among sites in the littoral habitat was rare, indicating that factors regulating individual pumpkinseed movement may differ between habitats. Manipulative studies involving experimental displacements of individuals with known phenotypes and observing their subsequent habitat selectivity (e.g. McCairns and Fox 2004) may better reveal the causes of individual movement.

Gaining mechanistic insights into how ecological opportunity contributes to diversification is a key goal of the ecological theory of adaptive radiation. Recent research has focused on evaluating the availability of secondary peaks in a local adaptive landscape by testing for diversifying selection (reviewed in Stroud and Losos 2016). But this leaves open the effects of ecological opportunity on population viability (Yoder et al. 2010) and gene flow (Wellborn and Langerhans 2015), each of which can also affect diversification potential. We provide robust demographic evidence that an ecological opportunity in the form of abundant resources is available in the pelagic habitat. This opportunity effectively doubles the total pumpkinseed population in Ashby Lake, without any notable cost to individual growth performance. These findings support previous evidence that exploitation of the novel pelagic habitat has repeatedly led to recent character release in pumpkinseed (Jastrebski and Robinson 2004; Parsons and Robinson 2006; Robinson et al. 2000; Weese et al. 2012) and other fish populations in post-glacial lakes (Robinson and Wilson 1994; Seehausen and Wagner 2014). Furthermore, the connectivity between pumpkinseed ecotype subpopulations is quite limited at short timescales within summer seasons (McCairns and Fox 2004) as well as among years, suggesting that gene flow could be constrained by ecological properties that distinguish littoral from pelagic habitats (Colborne et al. 2016; Jarvis et al. 2017; McCairns and Fox 2004). A key uncertainty is whether adult movement that occurs between habitats is random or not with respect to type. Predicting the chances of further divergence by these sunfish ecotypes or any other set of diverging populations requires a more detailed mechanistic understanding of the consequences of ecological opportunity on population dynamics and individual movement as populations diverge, in addition to effects on the shape of the local adaptive landscape.

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Author contributions WJ, KP, SC, and BR conceived the ideas and designed methodology; WJ, KP, and BR collected the data; WJ and BR analyzed the data; WJ and BR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility The data for this study has been included in online supplementary material. We intend to archive our data with Dryad Digital Repositories <https://datadryad.org/>

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest

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Affiliations

Will M. C. Jarvis^{1,2}  · K. S. Peiman³ · S. J. Cooke³ · B. W. Robinson²

¹ Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada

² Department of Integrative Biology, University of Guelph, Guelph, ON N1G 2W1, Canada

³ Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, ON K1S 5B6, Canada