

**Matching habitat choice influences movement decisions of sympatric ecotypes  
of pumpkinseed sunfish (*Lepomis gibbosus*)**

by

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## ABSTRACT

### MATCHING HABITAT CHOICE INFLUENCES MOVEMENT DECISIONS OF SYMPATRIC ECOTYPES OF PUMPKINSEED SUNFISH (*LEPOMIS GIBBOSUS*)

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Local adaptation to different environmental conditions can cause associations between phenotype and environments that are reduced when individuals disperse randomly but can be enhanced when dispersal is non-random with respect to phenotype. Matching habitat choice (MHC) is a process where individuals select habitats to maximize performance based on phenotype which can facilitate local adaptation and adaptive divergence. Empirical evidence of MHC is limited because it is difficult to isolate from other mechanisms that form phenotype-habitat associations. I overcome this issue by transplanting pumpkinseed sunfish (*Lepomis gibbosus*) ecotypes across habitats and compared explicit models related to movement behaviour. MHC strongly affected habitat selection and potentially homing behaviour but was less important in leaving decisions. The non-random movement likely enhances phenotype-habitat associations that contribute to ecotype divergence.

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## **LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE**

AH – Alternate Habitat treatment, release at a different site in the alternate habitat type as the initial capture habitat

AIC – Akaike Information Criterion

H – Home treatment, release at initial capture site

MHC – Matching habitat choice

NHP – Natal habitat preference

OJW / ROJW – Oral jaw width / Residual oral jaw width (adjusted for body size)

SF – Site fidelity

SH – Same Habitat treatment, release at a different site in the same habitat

SMI – Scaled Mass Index

TL – Total length (mm)

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# **Matching habitat choice influences movement decisions of sympatric ecotypes of pumpkinseed sunfish (*Lepomis gibbosus*)**

## **Introduction**

Individuals can alter the frequency of observed phenotypes in a population through movement and by doing so alter the mean and variance of population fitness (Matthysen 2012). Movement is also linked to local adaptation through dispersal when populations occupy heterogeneous environments. Dispersal is the movement of an individual from some location to a new locale where it will subsequently breed, and is a principle mechanism that regulates gene flow (Cayuela et al. 2018). Gene flow reflects the movement of alleles among populations, and is influenced by landscape connectivity (Robertson et al. 2018) and animal behaviour (Armansin et al. 2019), making gene flow fundamentally an ecological process. Gene flow has important consequences for the maintenance of genetic variation and the adaptive potential of populations (Clobert et al. 2012). Quantifying the mechanisms that govern individual movement in populations will then help us better understand the factors that influence phenotypic diversity and adaptive population divergence.

Movement can facilitate or degrade the development of phenotype-habitat associations depending on mechanism and context. Diversifying selection is a mechanism that favours different optimal phenotypes among habitats in heterogeneous environments and under ideal conditions, can result in local adaptation. However, local adaptation can be resisted by incoming genetic variation brought by individuals that disperse from other habitats where they may have different adaptations (Cayuela et al. 2018; Nicolaus and Edelaar 2018). Alternatively, gene flow

can act as a source of variation that increases the opportunity for selection especially in small, isolated populations. Models of local adaptation expect that the degree of local adaptation reflects a balance between the strength of local selection and the rate of arrival of maladaptive alleles, referred to as the migration load. Typically migration load has been assumed to reflect movement that is random with respect to important ecological conditions (Bolnick et al. 2009). However, animal movement is seldom random in nature, in which case mechanisms of non-random movement (here defined as directed movement) become important because of their potential to alter the balance between local adaptation and gene flow, also referred to as the migration-selection balance (Holt 1987; Bolnick and Otto 2013; Edelaar et al. 2019). For example, homing, defined as the ability of a displaced individual to return to a previously occupied location, is one form of non-random movement that is expected to favour local adaptation (and thus development of phenotype-habitat associations) by reducing dispersal (Bolnick et al. 2009). Another possible form of animal movement that could contribute to phenotype-habitat associations is one where individuals express phenotype-dependent habitat choice as they disperse, referred to as matching habitat choice (MHC).

Habitat selection by MHC provides a behavioural mechanism of phenotypic ‘sorting’ of individuals among habitats that spatially structures phenotypic variance with potentially important evolutionary consequences (Edelaar and Bolnick 2012; Berner and Thibert-Plante 2015; Jacob et al. 2017; Jacobson et al. 2017). Individuals accomplish this through selecting habitats where their phenotype will function best based on self-assessment of performance (Edelaar et al. 2008). Assuming heritability of functionally important phenotypic traits, the sorting of phenotypes to suitable habitats rapidly increases rates of genotype-environment

correlations with potent effects both on local adaptation as well as on the adaptive divergence of populations between habitats depending on the strength of diversifying selection and scale of habitat heterogeneity (Edelaar et al. 2008). Recent evolutionary theory expects that local adaptation and adaptive population divergence are facilitated by MHC through the movement of adaptive alleles into habitats in which they perform best, highlighting that not all gene flow among diverging populations is detrimental to local adaptation (Armsworth and Roughgarden 2005; Edelaar et al. 2008; Bolnick and Otto 2013; Nicolaus and Edelaar 2018). For example, an experimental study using ciliates demonstrated how local adaptation was favored by individuals with habitat matching behaviour compared to situations where individuals were randomly dispersed (Jacob et al. 2017). Despite these emerging theoretical perspectives and limited experimental evidence involving laboratory organisms, it is surprising that few studies have tested for MHC in natural populations (Camacho and Hendry 2020). Part of this is due to the difficulty in isolating MHC from other mechanisms that generate phenotype-habitat associations (Edelaar et al. 2008). Given few empirical tests of MHC, little is known how MHC functions in nature or contributes to the evolution of diverging populations. Hence, a key uncertainty is how matching habitat choice contributes to the spatial structuring of phenotypic variation, especially for natural populations potentially under diversifying selection between different environmental conditions (Edelaar et al. 2008; Camacho and Hendry 2020).

An obvious ecological effect of MHC is its contribution to structuring phenotypic variation among populations in heterogenous environments, suggesting that such patterns can be indicative for MHC. However, these spatial patterns can also arise as a result of phenotypic plasticity and natural selection (Edelaar et al. 2008). For example, highly plastic individuals can

match local environmental conditions by changing their phenotype to increase their performance. Similarly, persistent or strong selection can also drive a local population to evolve towards a locally optimal phenotype (subject to limits imposed by heritable variation and maladaptive gene flow). Hence, observations of phenotype-habitat associations are insufficient to infer presence of MHC behaviour unless the confounding effects of plasticity and selection are rejected through other tests (Edelaar et al. 2019; Camacho and Hendry 2020). One alternative is to test expected predictions of MHC behavior in addition to other forms of individual movement during dispersal.

Dispersal can be conceptualized as a set of sequential decisions by an individual as it moves through the environment, with each decisions potentially influenced by MHC behaviour. These decisions are: emigration, the decision to leave a location; transfer, the movement to a new location; and immigration, settlement into a new location (Clobert et al. 2012). There is a history in behavioural ecology that has considered dispersal through a lens of phenotype-dependent or condition-dependent factors where individual phenotype (morphology, behaviour, life history etc.) and environmental conditions (competition, habitat quality, social status etc.) interact to influence dispersal decisions (Jones 1982; Stamps 2006; Matthysen 2012; Moore and Whiteman 2016). The relative importance of these factors may also vary over these different dispersal steps. For example, once an individual has dispersed from a natal habitat, sequential movement may be more influenced by other factors such as environmental information gained through transit or endurance (Matthysen 2012). MHC behaviour is expected to only influence emigration and immigration decisions where reliable information about local conditions can be obtained and assessed through performance (Jacob et al. 2015). It is also possible that MHC behaviour may

only act through emigration decisions, where leaving a location occurs as a result of poor phenotypic performance, while immigration decisions are governed through some other mechanism, or vice versa. Hence, the effects of MHC behaviour may vary in importance between emigration and immigration steps, suggesting separate tests of MHC behaviour for each step (Jacob et al. 2015).

MHC can also be a mechanism that may contribute to residency or homing behaviour. Homing to a site (a location within a habitat, here referred to as site fidelity) or to a habitat (an ecologically definable space in an ecosystem, here referred to as habitat preference) can also be influenced by MHC. For example, given strong MHC behaviour, we expect that a sampled individual initially phenotypically matched to its local environment that is returned to that location will display residency behaviour consistent with site fidelity and habitat preference. Whereas an individual mismatched to its local environment and returned there will disperse, exhibiting minimal site or habitat fidelity. Similarly, an individual taken from a 'matched' environment and placed in a new location where it is phenotypically 'mismatched' is more likely to disperse and may return to its original site or habitat, where MHC shares the same pattern of movement with site and/or habitat homing. However, there are other advantages for an individual to return to a site or habitat independent of MHC that have been considered, including social (Armansin et al. 2019), feeding (Merkle et al. 2014), movement efficiency (López et al. 2001), and predator avoidance (Brown 2001) strategies that are obtained primarily through learning and experience in that habitat. The benefits of habitat fidelity may also cause individuals who disperse from a natal site to select new sites that resemble their natal habitat, termed natal habitat preference, although this depends on the quality of experience obtained through survival

(Stamps and Swaisgood 2007; Mabry and Stamps 2008; Stamps et al. 2009; Sanz-Pérez et al. 2018). Importantly, natal habitat preference is a homing mechanism expected to contribute to habitat rather than site fidelity. Therefore, contributions to homing and resident behaviour by site fidelity and natal habitat preference likely also occur independent of MHC behaviour. Few studies have distinguished among different mechanisms that can cause homing and I attempt to do that here.

Testing predictions of MHC requires experimental manipulations that isolate the effects of phenotype from other mechanisms that generate phenotype-habitat associations and homing behaviors. There are two approaches: (1) manipulating phenotypes or (2) manipulating phenotype-habitat associations. For example, Edelaar et al. (2019) experimentally altered body colour to test for effects on behavioural microhabitat choice in azure sand grasshoppers (*Sphingonotus azurescens*). Remarkably, the manipulated individuals matched their new body colour to microhabitat to enhance crypsis in the field and in the lab, thereby demonstrating strong MHC behaviour (Edelaar et al. 2019). Manipulating phenotype provides a strong test of MHC behaviour, but the logistics of phenotypic manipulations can limit the utility of this approach (Edelaar et al. 2008). Alternatively, one can manipulate phenotype-habitat associations by reciprocally transplanting individuals among habitats, and then assess whether subsequent movement decisions are predicted by phenotype (Edelaar et al. 2008; Bolnick et al. 2009), provided that the pre-existing phenotypic variation is related to ecological performance trade-offs across habitats. Under the MHC hypothesis, individuals are predicted to settle in habitats to which they are phenotypically matched and depart habitats where they are phenotypically mismatched. I test predictions of the MHC hypothesis by manipulating phenotype-habitat

associations through experimental translocation of individuals. More specifically I test for phenotypic effects on decisions during emigration and immigration stages of dispersal by translocating individuals while simultaneously accounting for homing.

Phenotypic polymorphism related to resources or trophic position (Bolnick et al. 2009; Jacobson et al. 2017), predation risk (Edelaar et al. 2019), or thermal environment (Jones 1982; Jacob et al. 2017) provide some of the best opportunities to test for MHC, especially when phenotype-related performance trade-offs exist across ecological conditions (Edelaar et al. 2008). High levels of phenotypic variation enhance tests of MHC behaviour because movement is expected to increase in importance with increased costs resulting from mismatches between phenotype and habitat (Camacho and Hendry 2020). MHC is also more likely when phenotypic traits that are functionally linked to resource use generate trade-offs in performance across ecological conditions (Edelaar et al. 2017; Camacho and Hendry 2020). Strong trade-offs can occur in resource polymorphisms especially when a generalist feeding strategy is less beneficial than specializing on different prey/resource types (Smith and Skúlason 1996; Robinson 2000; Parsons and Robinson 2007). Intraspecific studies of MHC behaviour also allows us to study the contribution of MHC behaviour to adaptive diversification with gene flow, which is not as possible with interspecific studies because MHC behaviour may evolve after ecologically specialized species have evolved and where gene flow is already constrained.

I evaluate MHC behaviour using a polymorphic lake population of pumpkinseed sunfish (*Lepomis gibbosus*) composed of ecotypes that differ in functional trophic traits across two distinct habitats. Because there is a demonstrated phenotypic gradient across and within habitats, identified performance trade-offs of phenotype, and evidence of phenotype-habitat associations, I

believe this system provides a strong model to test for MHC behaviour. I address three questions: 1) Do individuals move in order to match phenotype with environment? 2) Is MHC behaviour expressed equally during emigration and immigration? and 3) How may MHC contribute to homing? To address these questions I employed mark-transplant-recapture methods to manipulate phenotype-environment associations that forced movement decisions on individual pumpkinseeds (Edelaar et al. 2008). Under the matching habitat choice hypothesis, individuals select habitats that increase match between phenotype and habitat based on performance trade-offs. First, I tested whether the probability of leaving the release site (emigration) was increased when phenotypic mismatch to the release site was higher (P1). Then for individuals that left the release site, I tested whether the probability of switching habitats (immigration) was increased with greater mismatch to the release site (P2). My approach distinguishes MHC from other mechanisms that contribute to homing behaviour such as site fidelity and habitat preference and so avoids confounds that have obscured prior evidence of MHC (Bolnick et al. 2009; Edelaar et al. 2019; Camacho and Hendry 2020).

## **Methods**

### *Study System*

#### *Trophic Polymorphism in Pumpkinseed Sunfish*

Pumpkinseed sunfish (*Lepomis gibbosus*) are typically a shallow, inshore (littoral) lake habitat specialist (Scott and Crossman 1973) but also occupy deeper, offshore rocky shoals (pelagic) in some low diversity, post-glacial lakes (Robinson et al. 1993; Gillespie and Fox 2003; Weese et al. 2012). The separation of this species into multiple habitats has resulted in the

divergence of many morphological traits across habitat types which I refer to as ecotype in this paper (Robinson et al. 1993; Gillespie and Fox 2003). Ecotypes here are not genetically distinct populations but rather groups of individuals that are spatially segregated among different breeding habitats within a shared environment. Littoral and pelagic ecotypes differ not only in use of habitats that are distinguished by structural complexity, but also in their dominant prey types. The pelagic ecotype feeds extensively on large zooplankton prey while the littoral ecotype consumes a generalist diet of primarily benthic macroinvertebrates (Parsons and Robinson 2006; Berchtold et al. 2015). Pelagic ecotypes have smaller oral (Jarvis 2018; Axelrod et al. 2018) and pharyngeal (unpublished result) jaws, more closely spaced gill rakers (Robinson et al. 1993) and smaller heads (Jasterbski and Robinson 2004; Weese et al. 2012) relative to body size than littoral ecotypes. Smaller oral jaws are expected to increase suction feeding performance in fishes (Carroll et al. 2004; Collar and Wainwright 2009), which is beneficial when feeding on zooplankton prey, but their size also limits their ability to consume larger macroinvertebrate prey such as snails which are more common in the littoral habitat (Robinson et al. 1996). The larger oral jaws of the littoral ecotype provide a gape width large enough to consume macroinvertebrates while more robust pharyngeal jaws allow them to process these armored prey (Parsons and Robinson 2007). Indeed, laboratory foraging studies indicate that morphs face strong trade-offs in feeding performance between zooplankton and larger benthic invertebrate prey (Parsons and Robinson 2007), that may be the source of growth and condition performance trade-offs observed between ecotypes in the field (Robinson et al. 1996). Populations within each habitat contain a high level of morphological variability from the localized specialized form to an intermediate form common to both habitats. Population genetic studies suggest that the patterns

of diversification repeatedly observed in 12 post-glacial lakes more likely resulted from within lake divergence than from multiple colonization events (Weese et al. 2012). Phenotype is strongly influenced by plastic developmental responses especially in young-of-the-year fish, but evidence of genetic differences in body form has also been shown using reciprocal transplant common-garden experiments (Robinson and Wilson 1996; Parsons and Robinson 2006). Prior mark-recapture experiments using trophic ecotypes of pumpkinseed have identified high levels of site and habitat fidelity (McCairns and Fox 2004; Jarvis 2018), although the degree to which MHC contributes to these phenotype-habitat associations, if at all, is not known.

### *Ashby Lake Study System*

Ashby Lake (Lennox-Addington County, ON, 45°05'N, 77° 21'W) is a small (surface area of 2.59 km<sup>2</sup>, max depth of 36.6 m) oligotrophic, post-glacial temperate lake that contains distinct shallow and deep water habitats that have allowed the local pumpkinseed population to phenotypically diversify into habitat-specific ecotypes (Jasterbski and Robinson 2004; Jarvis 2018; Axelrod et al. 2018). I define two habitats here: the littoral habitat, which is characterized by shallow depth (<3m), soft organic muddy substrates with moderate macrophyte growth where macroinvertebrate prey are common on the benthos and submerged structure. The pelagic habitat is characterized by deeper (>3m depth) water punctuated by large rocky shoals that contain a high abundance of suspended zooplankton. Sunfish do not regularly occupy the open water that separates the inshore and offshore habitats, and so littoral and pelagic habitat represent spatially discrete habitat patches. Twenty sites were used: eleven from littoral and nine from pelagic and were selected based on prior knowledge of sunfish shoals and habitat characteristics (Fig. 1).

Littoral sunfish are known to exhibit high levels of site fidelity to embayment's along the

shoreline (McCairns and Fox 2004; Jarvis 2018) and so littoral sites were selected based on natural embayments. Pelagic sites are entirely submerged and so have no shoreline to identify boundaries, so sites were determined by the span of the rocky shoal (observed by snorkeling) out to a depth of 5m. The high propensity of site-specific philopatry in the system (Jarvis 2018), and in similar polymorphic lake populations of pumpkinseed in the region (McCairns and Fox 2004), makes me confident that the summer home ranges of pumpkinseeds here are typically limited to the sites I selected. The northwestern basin of Ashby Lake provided the greatest density of pelagic and littoral sites within the movement capabilities of a single sunfish, allowing for greater replication and a higher resolution depiction of movement in this part of the system.

## *Study Design*

### *Experimental Design*

I tested predictions of the MHC hypothesis by experimentally forming new phenotype-habitat associations and observing subsequent movement decisions to determine whether movement was influenced by phenotype. This was done by implementing a mark-transplant-recapture protocol (modified from McCairns and Fox, 2004) that revealed site and habitat fidelity in other polymorphic pumpkinseed populations in the region but with the additional quantification of phenotype. Mark-recapture methods provide instantaneous location data useful in estimating animal movement, especially in fishes where observation is difficult (McCairns and Fox 2004; Bolnick et al. 2009). By adding the experimental manipulation of reciprocal transplanting among sites within habitats and across habitats, I form new associations that force movement on each individual and allow direct tests of MHC behaviour independent of

phenotype-environment associations derived from natural selection and phenotypic plasticity. The habitat transplants here are not expected to generate individual plastic responses by adults (>75mm TL) in oral jaw width during the 3-month span of this study since these would require major changes to bone and soft tissue structure. Individual movement decisions can then be tested against predictions related to matching habitat choice, site fidelity and natal habitat preference or any combination of these mechanisms (Table 2).

#### *Capture, Tagging, Treatments and Recapture*

During the summer of 2019 from May 30 to July 18, I captured and tagged a total of 1985 pumpkinseed sunfish (994 littoral, 991 pelagic) for tagging and release. I used the original capture habitat of a fish to identify the fish as a littoral or pelagic ecotype for these analyses. I used a >75mm total length size criteria based on the high tag retention of 12mm PIT tags in a related species, bluegill sunfish (*Lepomis macrochirus*) greater than 75mm (Kaemingk et al. 2011). Flexible capture effort was allocated across all sites until approximately 1000 fish were tagged from each the littoral and pelagic habitat. Fish were captured primarily by angling which was more successful than trapping (angling n=1408, trapping n=577). Due to the uneven distribution of fish in the lake, each site had a capture quota which was based on prior estimates of relative fish abundances at sites (ranged from 30-150 individuals per site). Angling occurred between the hours of 07:00-19:00 and traps were checked every morning and evenings (deployment range 4-24 hours).

For tagging and phenotyping, fish were first anesthetized in a clove oil solution (6 ml clove oil:1000 ml water) until there was no active response after pinching the caudal peduncle.

PIT tags and needles were disinfected with ethanol and the abdomen of the fish was swabbed with Ovidine iodine solution (Western Chemical Inc, WA, USA) prior to insertion to minimize risk of infection. Fish were tagged with 12mm Passive-Integrated-Transponder (PIT) tags (GPA-12 tags, Biomark, Boise, Idaho, USA) inserted in the abdominal cavity using a 12-gauge needle and syringe. Oral jaw width was used to assess trophic phenotype (see below) and was measured as the lateral distance between mandibles using standard digital calipers (Thermo Fisher Scientific MA, USA) to the nearest hundredth of a mm. Total length was measured to the mm using a ruler and weight was measured by electronic scale (StarFrit, QC, CA) to the nearest gram. Fish were aged by counting annuli observed through a dissecting microscope from a sample of ten scales taken between the dorsal fin and the lateral line. Fish were also photographed in a clear, acrylic cuvette, using a Nikon COOLPIX L100 digital camera. To estimate PIT tag retention, I also clipped approximately one third to half of the 3<sup>rd</sup> dorsal spine of each fish in order to identify fish that expelled their internal PIT tags.

Overall, approximately 330 tagged individuals were assigned to each of three release treatment within each habitat. 1) release at home site (H) where a tagged individual was released at the site where it was first captured, 2) release at a same habitat as capture (SH) where a tagged individual was released at a different site in the same habitat type as the home site, or 3) release into alternate habitat type as that of initial capture (AH), where a tagged individual was released at a different site in the alternate habitat type as capture. The H treatment permits me to control for basal (natural) movement and movement induced by handling. The SH treatment allows me to remove site familiarity but maintain habitat effects and the AH treatments acts to induce phenotypic mismatch and remove site and habitat familiarity. I attempted to keep linear distance

between origin and release sites as similar as possible for the SH and AH release treatments to minimize the effects of distance on individual movement choices. Among all transplant treatments (SH and AH), distance from the initial capture site ranged from 60-350m which is well within the movement capabilities of an individual fish (Fish and Savitz 1983).

The recapture period began with the capture period but lasted until August 30 resulting in 268 total recaptures (recapture = 13.5%). Hence, the number of days available for a released individual to move ranged from 45-91 days. 48 fish were recaptured more than once, and in those cases, I only consider their final recapture location. This was done to estimate the final immigration choice of an individual to prevent accidental inference of immigration during transit. Recapture sampling effort was allocated in order to obtain high numbers of recaptures in both littoral and pelagic habitats. Recapture effort was evenly distributed among sites within habitats, but more effort was put into pelagic sites than littoral sites because pumpkinseed density is approximately seven times greater in the pelagic compared to the littoral habitat (Jarvis 2018), and so required more effort (72% more time spent in pelagic sites) for obtaining comparable recaptures. Recaptured individuals were identified using a PIT tag scanner (601 Fish Tag Reader, Biomark, Boise, Idaho, USA), weighed and measured for total length then released back at their recapture site. Of all recaptures obtained, 26 individuals scanned negative for a PIT tag, likely due to tag loss, yielding a tag retention estimate of 91% from June through the end of August.

### *Data Analysis*

#### *Homing Analyses*

Prior research has shown greater considerable homing in pumpkinseed sunfish (McCairns and Fox 2004) and so I first performed preliminary analyses to evaluate the strength of site and habitat homing across release treatments and ecotype. Because smaller individuals may experience greater costs of movement, I also compared the effects of body size and age on homing across ecotypes. Habitat and movement comparisons were completed using ANCOVAs and z-tests (after meeting assumptions of normality) to test for ecotype, release treatment, size and age effects. All analyses were completed in R Studio version 3.5.3 (R Core Team, 2013).

### *Phenotype-Habitat Associations*

MHC requires scoring a phenotypic trait that differs across habitats. I used oral jaw width as a measure of trophic phenotype because it is a well differentiated trait between ecotypes, is easy to measure in the field, and is correlated with a variety of other phenotypic traits such as body depth, and head size that distinguish pumpkinseed ecotypes (Jarvis unpublished). Because oral jaw width is allometrically related to body size, individual oral jaw widths adjusted for body size were estimated as residual values from an OLS regression of oral jaw width (mm) against total length (mm) using all 1985 tagged (original) individuals (Fig. 2).

MHC predicts that individuals select habitats that reflect phenotype-habitat associations and so pumpkinseed that select alternate habitats from their release habitat should have adjusted jaw sizes that differ from those that stay in the same release habitat (P2). In effect, MHC predicts that fish will try to re-establish patterns of phenotype-habitat associations after they are experimentally disrupted by release treatments. Because there are two habitat types, there are four possible movement responses from release habitat to their final recapture habitat; littoral –

littoral, littoral – pelagic, pelagic – littoral, pelagic – pelagic. I tested whether individuals that switched habitats differed in mean size-adjusted oral jaw width from those that stayed in the same habitat as release using a generalized linear regression to predict adjusted jaw size using the four movement response groups.

### *Estimating Phenotype-Habitat Mismatch*

To test the prediction that phenotype mismatch increases the probability of leaving the release site (P1) and switching habitats (P2), I required a statistic that reflects the degree of phenotypic mismatch of an individual to a reference habitat. The MHC hypothesis predicts that individuals most functionally mismatched to their local conditions are more likely to leave a site and to enter a new site where conditions better match their phenotype.

Individual mismatch was estimated by fitting a generalized linear model with a binomial error distribution (logistic model) using the original captured 1985 pumpkinseed. The model relates the effect of capture habitat (littoral or pelagic) with residual oral jaw width (adjusted for body size). Predicted values estimate the probability that an individual with a given oral jaw width was sampled from the littoral or pelagic habitat (Fig. 3). The residual probability values of an individual represents the difference between the predicted habitat probability and its actual original capture habitat, or the degree of phenotypic mismatch to its actual capture habitat. A greater difference between actual and predicted habitat indicates a greater phenotypic mismatch to a habitat (Fig. 3). Because there are two habitat types, individuals can be phenotypically mismatched to the pelagic or to the littoral habitat, reflecting direction in the negative or positive direction of body size adjusted oral jaw width respectively. I converted the residual probability

value of phenotypic mismatch to its absolute value so that littoral and pelagic individuals are both represented on a directionally uniform axis.

Because the AH treatments acts to alter original phenotype-habitat associations, I calculated all individual values of phenotypic mismatch to the release habitat. These absolute residual values of phenotypic mismatch applied to the release habitat reflect the intensity of phenotypic mismatch created by the particular experimental release treatment experienced by an individual. For this manipulation, the logistic model applied to the original capture habitat data above was instead applied to the release habitat and new residual probability values were calculated that represent the phenotypic mismatch of an individual to its release treatment habitat. (Fig. 4).

### *Emigration Analysis*

I first tested the prediction that the probability of leaving the release site (i.e. emigration) was increased by phenotypic mismatch to that habitat (P1). This result may occur in addition to (or independent of) other mechanisms of directed movement such as site fidelity and natal habitat preference. I test for effects of these mechanisms on the decision to stay or leave the release site (i.e. emigration) by fitting several logistic models (generalized linear models with a binomial family and logit link function) to binomial recapture data and comparing the predictive power of the various models using Akaike Information Criteria (AIC). The models reflect various combinations of independent variables detailed in Table 2. Individual responses were classified as either recaptured at the release site (staying = 0) or recaptured at a different site (leaving = 1). The MHC model incorporated the mismatch statistic derived above. The

independent site fidelity (SF) and natal habitat preference (NHP) models were coded based on predicted responses of emigration under each process (SF or NHP) across release treatments. For example, site fidelity predicts that individuals transplanted outside their original capture site should leave the release site (SH and AH treatment = 1), whereas individuals released at the original capture site will stay (H treatment = 0). Natal habitat preference predicts affinity to the original capture habitat type, so that individuals released at a site in the same habitat as capture will stay (H and SH treatment = 0), but leave the release site if it is in the alternate habitat type relative to capture type (AH treatment = 1). The models reflect alternative mechanisms that are not mutually exclusive. For example, MHC may act in combination with SF and/or NHP to influence movement. Hence, I also include a model combining all mechanisms. Finally, MHC behaviour may be shared by all individuals in a population or may differ among subgroups depending on the performance and fitness costs of phenotypic mismatch (Camacho and Hendry 2020). Hence, my model set also included a modified simple MHC model that allowed pumpkinseed ecotypes to have dissimilar MHC effects on movement decisions. To do this, I included an interaction term of initial capture habitat and mismatch to capture habitat to evaluate whether ecotypes differ in the effect of mismatch on emigration.

The combined model also provides the opportunity to evaluate the potential for indirect effects of MHC on emigration decisions through SF or NHP behaviour. There is a growing appreciation that site fidelity and habitat preferences may be influenced by phenotype (Bolnick et al. 2009; Jacobson et al. 2017; Camacho and Hendry 2020), in which case MHC may have subtle indirect effects on directed movement that are underappreciated. As far as I know, this possibility has never been formally evaluated. The potential for MHC to indirectly affect

movement decisions similar to SF and NHP outcomes was evaluated using a path analytic approach applied to the combined model. The strength of the indirect effect of MHC was estimated as the product of the Pearson correlation ( $r$ ) between MHC and another parameter (SF or NHP) and the standardized model coefficient of that other parameter in the model (Turnes and Ernst 2015). I interpret potential indirect effects of MHC on explaining variance in emigration (leave vs. stay) relative to the direct effects of the SF or NHP parameters to indicate an upper limit of the potential effect of MHC on homing mechanisms.

### *Immigration Analysis*

I then tested the prediction that the probability of switching habitat types is increased with increased phenotype mismatch to release sites (P2). My approach considers directed movement as a temporal sequence of movement decisions, and so I now focus only on the movement decisions of individuals that left the experimental release site. These individuals must have made the decision to ‘emigrate’ from the release site, in order to ‘immigrate’ into a new site. To test for directed movement on habitat choice (immigration), I fit similar models to those used to evaluate emigration decisions (logistic models with binomial family and logit link function), to predict habitat switching of the emigrants in response to the experimentally manipulated release habitat (Table 7). In this case, site fidelity (SF) and natal habitat preference (NHP) cannot be distinguished from each other because both predict that an individual will switch habitats when placed outside their original capture habitat type (AH treatment = 1) but not switch habitat otherwise (H and SH = 0). As a result, the model set had one less parameter to consider than for emigration models. In all other respects, the analysis proceeded as above for emigration.

### *Mismatch and Movement Responses*

The movement responses of individuals are expected to vary in accordance to the phenotypic mismatch of an individual at its release site (P2). There are three possible movement responses that an individual may exhibit from its release site: 1) stay at its release site, 2) move to other sites in the same habitat as its release site or 3) move to other sites in the alternate habitat type as its release site. MHC predicts that individuals more matched to their release site would exhibit either response 1 or 2, whereas mismatched individuals are more likely to switch habitats (response 3). After pooling all treatments, I used a multinomial generalized linear regression model (binomial family and logit link function) to predict movement responses as a function of phenotypic mismatch to the release site.

### *Change in Mismatch*

The second prediction of MHC, whereby the probability of switching habitats is increased with increased phenotype mismatch to release sites (P2), can also be further evaluated by observing the change in mismatch from release to recapture habitat. Mismatch does not change for individuals that choose not to emigrate or those that do not leave the release habitat, and so a change in mismatch can only be applied to individuals that switched habitats. I tested for a mean change in phenotype-habitat mismatch averaged among all emigrating individuals that switched habitats from their release site (eq. 2).

$$(1) \Delta mismatch = mismatch_{Recapture} - mismatch_{Release}$$

A negative  $\Delta$ mismatch value indicates that the mismatch at recapture for an individual was less than the habitat mismatch at release which is consistent with phenotype-matching behaviour (MHC). Positive  $\Delta$ mismatch values reflect a greater mismatch to the recapture compared to release habitat and is inconsistent with MHC.

## Results

Overall, 1985 pumpkinseed were capture, tagged and released between May 30 and July 18, 2019. Of these, 220 (11%) unique individuals were recaptured between June 10 and August 30, comprised of 136 individuals recaptured from littoral and 84 individuals from pelagic sites.

### *Homing Analyses*

There was strong evidence of homing behaviour by pumpkinseed that differed between ecotypes. Combining all release treatments, fish initially captured from littoral and pelagic habitats exhibited strong homing behaviour to their original capture habitat (91%) and to capture site (74%). Site homing was greater in the littoral ecotype (82%) than in the pelagic ecotype (61%) combining all release treatments ( $z = -3.49$ ,  $df = 218$ ,  $p < 0.001$ ). Site homing was more pronounced in smaller individuals ( $z = -2.51$ ,  $df = 218$ ,  $p = 0.01$ ) but not younger individuals ( $z = -0.17$ ,  $df = 203$ ,  $p = 0.86$ ). 20% of all individuals experimentally released to their initial capture site (Home) were recaptured elsewhere, reflecting either basal movement or responses to sampling manipulation. Pelagic fish tended to move more in the home treatment than littoral individuals (14% more site leaving in pelagic), but this difference was not significant ( $z = -1.57$ ,  $df = 93$ ,  $p = 0.11$ ). Ecotypes did not differ in habitat switching in the home treatment (littoral: 5%, pelagic: 6%,  $z = 0.15$ ,  $df = 93$ ,  $p = 0.88$ ). However, for releases to the Same Habitat (SH)

treatment, the pelagic ecotype moved more frequently to different pelagic sites compared to the littoral ecotype movement among littoral sites (movement frequency was 27% greater in the pelagic ecotype:  $z = 2.32$ ,  $df = 69$ ,  $p = 0.02$ ). In the Alternate Habitat (AH) treatment, both ecotypes exhibited strong homing to their initial capture habitat (83% total returned; and was similar between both ecotypes (89% in littoral, 78% in pelagic;  $z = 1.08$ ,  $df = 52$ ,  $p = 0.28$ ) indicating a willingness to switch habitats (from release). The frequency of habitat switching away from the initial capture habitat did not differ between ecotypes across all treatments (littoral: 7%, pelagic: 13%,  $z = 1.60$ ,  $df = 218$ ,  $p = 0.11$ ).

### *Phenotype-Habitat Associations*

Phenotypic variation in this system was spatially organized as found in prior studies and may influence pumpkinseed movement. Size-adjusted oral jaw width was 8.31% larger in littoral compared to pelagic pumpkinseed using all originally captured fish ( $t = -18.57$ ,  $df = 1983$ ,  $p = <0.0001$ , Fig. 5). This difference was consistent in the recapture dataset ( $t = 7.87$ ,  $df = 164$ ,  $p <0.0001$ , see Appendix A for dataset comparisons). Size-adjusted oral jaw width was also a significant predictor of initial capture habitat justifying estimates of a phenotype-habitat mismatch statistic ( $R^2 = 0.12$ ,  $z = -15.83$ ,  $df = 1983$ ,  $p < 0.0001$ , Fig. 3). Total length was 10% higher ( $t=11.4$ ,  $df = 1983$ ,  $p=<0.0001$ , Fig. 6) and body mass and 37% greater ( $t = 9.97$ ,  $df = 1983$   $p=<0.0001$ , Fig. 6) in the pelagic compared to the littoral ecotype.

Preliminary phenotype analysis detected that the mean size-adjusted oral jaw width differed between ecotypes that stayed in, versus switched their release habitat, consistent with P2. Generalized linear regression analysis indicated that release-recapture group was a

significant predictor of oral jaw phenotype ( $F_{3,216} = 17.47$ ,  $R^2 = 0.18$ ,  $p < 0.0001$ ). Fish released to littoral sites and subsequently moved to pelagic sites had significantly smaller jaws compared to those that stayed in, or selected new littoral sites ( $t = -4.42$ ,  $df = 42$ ,  $p < 0.0001$ , Fig. 7). Fish released to pelagic sites that moved to littoral sites had significantly larger jaws than those that stayed in, or selected pelagic habitats ( $t = -4.88$ ,  $df = 59$ ,  $p < 0.0001$ , Fig. 7).

### *Emigration Decisions*

Evidence that phenotypic mismatch directly influenced site emigration decisions was equivocal (P1). Overall, 126 (57%) individuals left their release site and 94 (43%) stayed (Table 3). Model parameter estimates and significance tests can be found in Table 4. There was weak evidence that mismatch alone increased the probability of leaving behaviour ( $z = 1.89$ ,  $df = 218$ ,  $p = 0.059$ , Fig. 8). Model comparisons identified the homing model (Site Fidelity + Natal Habitat Preference) as the most parsimonious fit to the emigration data (Table 5). Nevertheless, the 2<sup>nd</sup> and 3<sup>rd</sup> ranked models were similar in performance to this homing model based on AIC (Table 5), where the 3<sup>rd</sup> ranked model included the MHC parameter, suggesting that MHC could also influence emigration decisions. It was also possible that the influence of mismatch may have differed between ecotypes ( $z = -2.01$ ,  $df = 216$ ,  $p = 0.04$ ), where the littoral ecotype was more likely to emigrate from release sites in which they were phenotypically mismatched than pelagic ecotypes ( $z = -2.01$ ,  $df = 216$ ,  $p = 0.04$ ). When modeled separately, phenotypic mismatch increased the probability of leaving release sites in littoral fish ( $z = 2.57$ ,  $df = 134$ ,  $p = 0.01$ ), but not in pelagic ecotypes ( $z = -0.50$ ,  $df = 82$ ,  $p = 0.62$ ). There also was weak evidence that larger individuals had a higher likelihood of leaving release sites ( $z = -2.0$ ,  $df = 218$ ,  $p = 0.05$ ).

The similarly high performance of site fidelity models without and with the MHC parameter suggested that MHC might indirectly affect the probability of emigration through influences on other movement mechanisms. MHC was positively correlated with both the theoretical site fidelity parameter ( $r = 0.23$ ,  $p < 0.001$ ) and the theoretical natal habitat preference parameter ( $r = 0.46$ ,  $p < 0.001$ ). Using standardized coefficients estimated for site fidelity in the combined model (MHC + SF + NHP), I estimate that phenotype mismatch may account for up to 21% of the effect of SF on leaving behaviour and up to 20% of the effect of NHP on leaving behaviour (Fig 9)..

### *Immigration Decisions*

I found evidence that MHC influenced habitat switching decisions (P2). For those fish emigrating from their release habitat, phenotypic mismatch increased the probability of switching habitats by 21% ( $z = 5.51$ ,  $df = 124$ ,  $p < 0.0001$ , Fig. 10). In addition, there was strong evidence that phenotypic mismatch increased the likelihood of switching habitats after accounting for other directed movement mechanisms ( $z = 2.95$ ,  $df = 123$ ,  $p = 0.003$ , Fig. 10). Overall, 56 (57%) individuals switched habitats types from release and 70 (43%) stayed in the same habitat type (Table 6). Specific model parameters, estimates and significance are presented in Table 7. AIC model comparisons identified the combined model (MHC + NHP/SF) as the highest-ranking model and most parsimonious fit to the data (Table 8). This model was superior all other models including the theoretical SF/NHP model that also predicted habitat selection (Table 8). Ecotype did not affect habitat switching ( $z = 1.50$ ,  $df = 122$ ,  $p = 0.13$ ), nor interact with phenotypic mismatch to affect habitat switching ( $z = -1.26$ ,  $df = 122$ ,  $p = 0.21$ , Table 7).

There was no evidence that body size ( $z = -0.04$ ,  $df = 124$ ,  $p = 0.97$ ) influenced the likelihood of switching habitats.

The best fitting model included MHC in addition to the general homing parameter (SF/NHP), allowing me to evaluate any indirect effects of MHC operating through homing behaviour (SF/NHP) on immigration (Fig. 11). MHC was positively correlated with the general homing parameter ( $r = 0.54$ ,  $p < 0.001$ ) indicating potential additional indirect effects of mismatch. I estimate that phenotype mismatch may account for up to 47% of the effect of homing on habitat switching behaviour here.

#### *Mismatch and Movement Responses*

Phenotype mismatch to the release site also was a significant predictor of movement response among the three possible movement responses (stayed in home site, switched sites in the same habitat or switched habitats,  $R^2 = 0.20$ ,  $df = 217$ ,  $p < 0.0001$ , Fig. 12), consistent with P2. There was strong evidence that individuals that switched habitats were more mismatched to their release site compared to those that stayed at the release site ( $z = 4.26$ ,  $df = 217$ ,  $p < 0.0001$ ), and also compared to those that selected sites in the same habitat as the release site ( $z = 5.81$ ,  $df = 217$ ,  $p < 0.0001$ ). In contrast, there was weak evidence that individuals that stayed at their release site differed in mismatch to the release site compared to those who left and selected sites in the same habitat as the release site ( $z = -1.81$ ,  $df = 217$ ,  $p = 0.07$ ).

#### *Change in Mismatch*

The net effect of habitat switching decisions tended to increase the match between oral jaw width (size-adjusted) and lake habitat. There was an average decrease in mismatch of 20% for the individuals that switched habitat types from their release treatment (Fig. 13).

## **Discussion**

I found evidence that matching habitat choice influences movement decisions by pumpkinseed that is not equally expressed in emigration as in immigration. Specifically, phenotypic mismatch increased the probability of switching habitats by 21% (P2) but did not increase the probability of leaving the release site (P1). Indeed, dividing individual movement into sequential stages of dispersal revealed that MHC had a more direct effect on site immigration decisions than on site emigration decisions, after accounting for effects of site fidelity and habitat preference behaviour. My results suggest that individuals that selected alternate habitat types were doing so to increase their phenotypic match to conditions in the new habitat. My approach also revealed that phenotype matching may be one of various mechanisms that also contributes to homing behaviour. This is important because site homing was the most common form of movement observed here in both littoral and to a lesser extent, pelagic individuals. Multiple direct and indirect effects of MHC may then operate to direct movement behaviour among sites and habitats.

My study strongly supports the idea that many factors including site fidelity, natal habitat preference and potentially phenotype matching can influence leaving decisions by an individual to leave a local site. I identified minor effects of MHC that may operate indirectly through site homing where it can explain up to 21% of site homing behaviour but model selection identified site fidelity as the primary mechanism tested for homing (present in all top three models).

Staying in a familiar site may provide valuable information regarding feeding, movement and predator avoidance which may increase individual fitness more so than trophic phenotype under certain conditions (Stamps 2006; Merkle et al. 2014). MHC was not a significant factor in predicting leaving decisions on its own. Decisions to leave sites are expected to reflect a combination of condition- and phenotype-dependent factors that shape decisions which may include age, body condition or size (Matthysen 2012). I found no influence of age on emigration decisions but there were minor effects of size even though I focused on near-adult and adult individuals. Larger individuals were more likely to leave release sites but capture site homing was significantly greater in smaller individuals. This may be due to the higher competitive ability and reduced predation risk of larger individuals more able to cope with the incurrent costs of dispersal. Conversely, the increased site homing among smaller individuals may also reflect competitive inferiority, such that the advantages of inhabiting a natal patch is greater than dispersing to neighboring patches or habitats.

Phenotypic mismatch to the release site influenced habitat switching as predicted but seems to operate in addition to mechanisms of homing. Indeed, with increasing phenotypic mismatch to the release site, the probability of switching habitat types from the release site was increased by 21%. Additionally, individuals typically moved to increase phenotypic match to a new habitat. Here, the average change of phenotype mismatch from release habitat to recapture habitat was decreased by 20% (Fig. 13). This pattern was replicated when comparing jaw sizes across groups that exhibited different movement responses, where individuals that switched habitats had phenotypes that resembled the recipient population instead of the population at release (Fig. 7). A similar pattern was observed in lake-stream forms of three-spine stickleback

which may suggest that MHC may be a trait that evolves relatively quickly as fish populations diverge between alternate habitats (Bolnick et al. 2009). Jacob et al. (2015) also suggest that MHC operating during immigration contributes to phenotype-habitat associations. This in turn may promote population differentiation and spatial heterogeneity of phenotypes, even when emigration is random (Jacob et al. 2015). This occurs because individuals that do leave, settle in a habitat where phenotypic performance is increased resulting in the formation of phenotype-habitat associations (Jacob et al. 2015). While potentially true when all individuals exhibit distinct phenotypes and perfect matching habitat choice (always settling in habitats reflective of phenotype-habitat associations), the effects of imperfect MHC, where individuals vary in phenotype, their costs, and in their movement behaviours are not clear.

I found no evidence of mean differences in MHC tendencies between pumpkinseed ecotypes, but variation among individuals in MHC behaviour within ecotypes seems likely. Individuals were found to vary in the strength of matching habitat choice and so may vary with respect to the costs incurred by dispersal and phenotypic mismatch with a local habitat (Camacho and Hendry 2020). Specifically, only the subset of individuals that left release sites were more likely to match phenotype to a new habitat. Furthermore, larger individuals left sites more frequently than smaller individuals perhaps because larger body sizes mitigate dispersal costs through more efficient swimming, reduced predation risk or social status at a new site. This suggests that individual variation in MHC behaviour likely exists here because individuals vary in dispersal costs. Alternatively, given that phenotype is related to foraging performance on habitat specific prey (Robinson et al. 1996; Parsons and Robinson 2007), it is expected that more extreme phenotypes may accrue a greater performance cost of phenotypic mismatch than an

intermediate pumpkinseed phenotype. For example, the mismatch incurred by an intermediate phenotype may be mild relative to those incurred by a specialist. The advantages of phenotype-matching by an intermediate ‘generalist’ phenotype may not outweigh the costs associated with leaving a site, which would tend to promote site residency behaviour.

During the span of my study, the phenotype-habitat associations that are formed as a result of movement are likely as a result of MHC instead of plasticity or selection. Phenotype-habitat associations in a natural population may result from any combination of adaptive evolution, phenotypic plasticity or phenotypically directed movement behaviour (Edelaar et al. 2008). Identifying the role of directed movement in phenotype-habitat associations then, requires separation of those associations formed by local adaptation and plasticity (Edelaar et al. 2008; Jacob et al. 2017; Camacho and Hendry 2020). By transplanting individuals across sites and habitats, however, I remove site and habitat familiarity and experimentally break down pre-existing phenotype-habitat associations and observe if those associations are re-formed through movement. For this pumpkinseed polymorphism, prior research suggests that phenotypic divergence in ecotypes reflects both adaptive responses to habitat types (Jastrebski and Robinson 2004; Parsons and Robinson 2006; Weese et al. 2012) and plastic morphological responses to habitats (Robinson and Wilson 1996; Parsons and Robinson 2007). The short-term nature of movement decisions observed here are not expected to generate plastic responses by adults (>75mm TL) in oral jaw width since these would require changes to hard bone structure in addition to soft tissue. Furthermore, mortality is unlikely to differ across habitats based on individual phenotype because predation of sunfish by large piscivores declines rapidly over lengths of 50-100mm TL (Mittelbach 1984). Also, we do not expect that the effects of jaw size

on feeding to cause significant mortality because it does not exclude feeding on alternative prey (Parsons and Robinson 2007). In fact, the wide overlap of oral jaw width across habitats (Fig. 2) and high frequency of intermediate forms suggest that if mortality selection occurs, it is unlikely to be strong and enough to influence these results. Hence, I am confident that the phenotype-habitat associations formed by the movement of fish here primarily reflect the effects of phenotypically directed movement, and so conclude that these contribute to phenotype-habitat associations in this system in addition to plastic and evolutionary responses.

Homing was the most prevalent form of directed movement observed here, consistent with other divergent lake populations of fishes. Of all pumpkinseed movement observed, returning to the initial habitat type was the most prevalent outcome (91% of all individuals returned to their initial source habitat type across all experimental release treatments). Comparisons of this level of homing to other similar divergent fish systems allows me to interpret whether this is an unusual value, such as the genetically divergent lake and stream stickleback and other phenotypically divergent pumpkinseed populations. Bolnick et al. (2009) transplanted tagged stickleback from both populations to an intermediate location at the stream mouth and observed subsequent individual movement decisions. They report similar levels of habitat homing (88.1%) to the habitat fidelity observed here (91.0%). McCairns and Fox (2004) observed consistent habitat fidelity (90.5%) by pumpkinseed ecotypes in another polymorphic lake population similar to that studied here. Hence, the high rate of homing observed does not seem unusual.

My ability to control for homing, another important factor influencing movement decisions, allows me to also conclude that phenotype has unique effects on movement apart from

those generated by homing to site or habitat. For example, an individual with a phenotype matched to habitat A that is transplanted to habitat B may return to habitat A due to phenotype matching and/or due to habitat or site homing. Traditionally, when an individual returns to a location, homing is the preferred inference, usually without understanding why the individual has homed. Both site fidelity and natal preference are two scales of homing that can occur to site or habitat (Hert 1992; Stamps and Swaisgood 2007; Piper 2011). Homing is relatively common in centrarchid fishes (Parker and Hasler 1959) and my results are consistent with previous transplant studies that conclude pumpkinseed have high fidelity to initial capture sites (McCairns and Fox 2004; Jarvis 2018). By comparing explicit models that account for other movement behaviours, my approach builds on these studies by explicitly distinguishing the effect of phenotype from effects of other mechanisms that cause site or habitat homing. In addition, my analysis suggests that up to 20% of the effect on emigration decisions and up to 47% of the effect on immigration decisions typically attributed to homing may be indirectly influenced by MHC effects. Future studies are required to further evaluate how phenotype matching may influence homing behaviours in fish and in other animals. Nevertheless, despite site fidelity and natal habitat preference being an important factor in movement decisions by these pumpkinseed, I identify unique effects of phenotype matching on pumpkinseed movement.

The evolutionary consequences of the directed movement observed are difficult to predict without genetic analyses of adaptive traits that distinguish ecotypes or information on post-movement reproduction. Both homing to a habitat and MHC can favour phenotypic diversification if they reduce migration load (the import of maladaptive alleles into a population). Homing to a natal habitat or site decreases the number of non-native immigrants, whereas

phenotype matching may introduce a phenotypic bias on immigrants that increases population fitness (Bolnick et al. 2009). I found strong evidence of both site homing and matching habitat choice that may contribute to the further diversification of pumpkinseed ecotypes. Selective habitat choice can be a major factor driving sympatric divergence by limiting maladaptive gene flow between diverging populations when it strongly enforces spatial assortative mating (Via 1999). In pea aphids (*Acyrtosiphon pisum*), habitat choice among preferential host plants led to spatial assortative mating which likely was a significant factor in generating reproductive isolation between host plant specialists. For this to drive further evolutionary diversification of sympatric pumpkinseed ecotypes, it must be the case that, 1) the recapture location is the locale chosen by an individual in which to mate, and 2) some aspect of traits that provide performance advantages in each habitat reflect underlying genetic differences.

There is strong evidence of spatial assortative mating between pumpkinseed ecotypes in this population (Colborne et al. 2016) in addition to weak mate choice (Jarvis et al. 2017), both of which should be amplified by the homing and MHC observed here (Bolnick et al. 2009). However, I do not have data to evaluate whether the individuals that switched habitats in response to phenotype matching are now more likely to spawn in the final recapture habitat. Evidence that individuals that switched habitats will return to that habitat consistently over consecutive years and are spawning in that habitat are now required to evaluate this uncertainty. Furthermore, we do not know anything about the ontogeny of MHC behaviour or in most other systems. It is not clear that pumpkinseed <75mm total length express similar levels of MHC behaviour, which would be important if most movement that contributes to gene flow occurs in juveniles.

There is some evidence that trophic traits that affect performance are heritable. Performance trade-offs exist relating to feeding in the lab (Parsons and Robinson 2007) and in natural populations (Robinson et al. 1996), although it is not clear which trophic traits most strongly affect feeding performance. There is also evidence of genetic differences in body form among pumpkinseed ecotypes attained through common garden studies (Robinson and Wilson 1996; Parsons and Robinson 2006), although these did not focus on variation oral jaw size specifically. This suggests that this pumpkinseed system may meet these two general requirements that should promote further diversification. However, population genetic studies reveal very little neutral genetic divergence between ecotypes (Weese et al. 2012), indicating sufficient gene flow to homogenize neutral alleles here despite the presence of assortative mating, some mate choice and the directed movement now observed here. Hence, there is still uncertainty about how mechanisms of movement interact to influence adaptive divergence. As demonstrated in a recent study, Jacob et al. (2017) found that the rates of adaptation to different temperature regimes in ciliates were influenced in unexpected ways by genetic identity of migrants interacting with the rate of migration among subpopulations. Perhaps this complexity reflects the consequences of variation in MHC behaviour among members of a population (Camacho and Hendry 2020) or pleiotropic genetic effects. Nevertheless, my study has revealed a new mechanism that may influence gene flow in these recently diverged pumpkinseed populations.

Future studies that identify other potential systems in which to explore how MHC behaviour develops, evolves and influences diversification are required, and this work can be promoted by identifying conditions that make the evolution of MHC behaviour more likely. The

evolution of MHC is thought to be enhanced by spatial heterogeneity in environmental conditions, reliable and available environmental information, low dispersal costs and strong phenotype performance trade-offs across different environmental conditions (Edelaar et al. 2008; Jacob et al. 2017; Camacho and Hendry 2020). Large post-glacial temperate lakes often provide distinct littoral and pelagic habitats which are relatively stable which have repeatedly allowed for phenotypic differentiation of sunfish (Robinson et al. 2000; Weese et al. 2012) and other fish species (Robinson and Wilson 1994), suggesting they represent alternative adaptive peaks that can support unique fish populations. Pumpkinseed here and elsewhere (McCairns and Fox 2004) had a high propensity to leave transplant sites which suggest that movement costs are minimal, although natural rates of movement are relatively low (Jarvis 2018). Edelaar et al. (2008) suggest that MHC may only arise when the costs of phenotypic mismatch are greater than those of dispersal, and therefore may be most prevalent in systems with low dispersal costs and/or high costs of phenotype mismatch. Phenotypically mediated trade-offs in feeding performance occur between habitats in these pumpkinseed to which pelagic ecotypes seem most susceptible (Parsons and Robinson 2007), but this did not translate into variation in MHC behaviour among ecotypes. Given the characteristics of this system and its similarities to other systems where MHC behaviour occurs, environmental heterogeneity, reliable environmental information, low costs of movement and strong phenotypic trade-offs seem to be likely conditions that promote the evolution of MHC.

## **Conclusion**

I provide strong evidence that matching habitat choice contributes to phenotype-habitat associations in a polymorphic population of pumpkinseed sunfish. Such tests of MHC are rare in

natural populations and under field conditions. MHC behaviour occurred independently from additional mechanisms such as site fidelity and natal habitat preference that also generated homing tendencies in these pumpkinseeds. The roles and importance of these mechanisms in movement varied over sequential stages of dispersal, suggesting that deconstructing movement into sequential decisions may provide opportunities to reveal how these different mechanisms affect, and interact with one another. For example, MHC had a greater effect on site immigration decisions than on emigration decisions here. By developing a model set that included combined and independent mechanisms that influence movement and comparison by AIC, I identify unique effects of phenotype after controlling for the effects of other mechanisms such as site fidelity and natal habitat preference. An additional benefit of this approach was the estimation of potential indirect effects of phenotype matching on homing-related mechanisms. These indirect effects were potentially present in both emigration and immigration decisions. The existence of MHC behaviour in this recently diverged population suggests that MHC behaviour may play a significant role in the phenotypic diversification of littoral and pelagic ecotypes of pumpkinseed. Understanding what processes influence individual movement is a difficult task, especially in the field, but is crucial for understanding the ecological factors that regulate phenotypic diversity and adaptive population divergence.

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processes interact to shape patterns of within-lake phenotypic divergences in polyphenic pumpkinseed sunfish, *Lepomis gibbosus*. *Ecol Evol* 2:574–592.  
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## TABLES

**Table 1. Breakdown of predictions from compared directed movement processes across sequential dispersal stages.**

Research Hypotheses	Sequential Dispersal Stages	
	Emigration prediction (P1) (stay or leave release site?)	Immigration Prediction (P2) (select same or alternate habitat type from release site?)

Matching habitat choice	Individuals leave habitats due to high phenotypic mismatch to the release site	Individuals switch habitats to decrease phenotypic mismatch to the release site
Site fidelity	Individuals leave release sites to return to initial capture site	Individuals select habitats to return to their initial capture site or habitat
Natal habitat preference	Individuals leave release sites to return to initial capture habitat	

**Table 2. Model variables used in analysing movement decisions of pumpkinseed.** These models reflect potential explanations of movement behaviour of pumpkinseed released into one of three treatments: H – release at home site, SH – release at a different site in the same habitat as the home site, AH – release in the alternate habitat of the home site. Site fidelity and natal habitat preference were modelled by assigning dummy coded variables based on theoretical predictions of individual responses to each release treatment. Ecotype models were developed to identify possible differences in matching habitat choice between littoral and pelagic pumpkinseed.

<b>Stage</b>	<b>Model</b>	<b>Variables</b>
Emigration Leaving release site (1 = Leave, 0 = Stay)	Matching Habitat Choice	Mismatch (continuous)
	Site Fidelity	Theoretical SF responses (0 – H; 1 – SH & AH)
	Natal Habitat Preference	Theoretical NHP responses (0 – H & SH; 1 – AH)
	Ecotype	Initial Capture Habitat + Mismatch + Capture Habitat : Mismatch
Immigration Switch habitats relative to release habitat (1 = Yes, 0 = No)	Matching Habitat Choice	Mismatch
	Site Fidelity / Natal Habitat Preference	Theoretical SF responses (0 – H & SH; 1 – AH)
	Ecotype	Capture Habitat + Mismatch + Capture Habitat : Mismatch

**Table 3. Sample sizes of emigration decisions by recaptured pumpkinseed sunfish.**

Emigration Leave release site?	Total Sample	Littoral fish	Pelagic fish
Yes	126	75	51
No	94	61	33
Total	220	136	84

**Table 4. Emigration models, parameters and Wald's tests with probability values for transplanted pumpkinseed.** Matching habitat choice (MHC), site fidelity (SF) and natal habitat preference (NHP) unstandardized parameter estimates are shown for predicting stay/leave decisions. MHC may operate in addition to (or without) mechanisms of homing (SF and NHP) so models were developed in any combination of these three mechanisms. The ecotype model was developed to identify possible differences in MHC between littoral and pelagic pumpkinseed.

Model	Parameters	$\beta$	SE $\beta$	Wald's $X^2$	df	p
MHC	Intercept	-0.2401	0.3107	0.6	1	0.44
	Mismatch	1.2311	0.6508	3.6	1	0.059
SF	Intercept	-1.3863	0.2565	29.2	1	6.49e-08
	Theoretical SF	3.1688	0.3615	76.8	1	<2e-16
NHP	Intercept	-0.1448	0.1556	0.87	1	0.35
	Theoretical NHP	2.4272	0.4945	24.1	1	9.2e-07
SF + MHC	Intercept	-1.2378	0.4150	8.9	1	0.0029
	Theoretical SF	3.2124	0.3771	72.6	1	<2e-16
	Mismatch	-0.3942	0.8760	0.2	1	0.65
NHP + MHC	Intercept	0.1739	0.3379	0.26	1	0.61
	Theoretical NHP	2.6427	0.5396	24.0	1	9.7e-07
	Mismatch	-0.8452	0.7978	1.1	1	0.29
SF + NHP	Intercept	0.2000	0.0384	27.2	1	4.3e-07
	Theoretical SF	0.6169	0.0587	110.5	1	<2e-16
	Theoretical NHP	0.0905	0.0675	1.8	1	0.18
Combined (SF + NHP + MHC)	Intercept	-0.9644	0.4382	4.8	1	0.028
	Mismatch	-1.1448	0.9956	1.3	1	0.25
	Theoretical SF	2.9026	0.4035	51.7	1	6.3e-13
	Theoretical NHP	1.0729	0.6167	3.0	1	0.082
Ecotype	Intercept	-0.6925	0.3862	3.2	1	0.073
	Mismatch	2.2457	0.8744	6.6	1	0.01
	Capture Habitat	1.3867	0.6884	4.1	1	0.044
	Mismatch x Capture Habitat	-2.7748	1.3786	4.1	1	0.044

**Table 5. Model summaries and AIC comparisons of emigration decisions of transplanted pumpkinseed sunfish.** Comparisons of matching habitat choice (MHC), site fidelity (SF) and natal habitat preference (NHP) models used to predict binomial decisions to stay/leave the release site. The homing model (including theoretical SF and NHP) provided the most parsimonious fit to emigration choices, although the performance of the homing model was only slightly greater than the SF alone model and the combined model including MHC.

Model	df	Log-Likelihood	AIC	$\Delta_i$	$w_i$	Rank
Homing (SF + NHP)	3	-98.00	201.99	0	0.32	1
Site Fidelity (SF)	2	-99.06	202.12	0.12	0.31	2
Combined (SF + NHP + MHC)	4	-97	202.64	0.65	0.24	3
SF + MHC	3	-98.96	203.91	1.92	0.13	
Natal Habitat Preference (NHP)	2	-131.29	266.57	64.58	3.11e-15	
NHP + MHP	3	-130.72	267.44	65.44	2.02e-15	
Ecotype + MHC	4	-146.16	300.32	98.32	1.47e-22	
Matching Habitat Choice (MHC)	2	-148.32	300.65	98.65	1.24e-22	
Null	1	-150.16	302.31	100.31	5.39e-23	

**Table 6. Sample sizes of Immigration decisions by recaptured pumpkinseed sunfish.**

Immigration Switch Habitats?	Total Sample	Littoral fish	Pelagic fish
Yes	56	30	26
No	70	45	25
Total	126	75	51

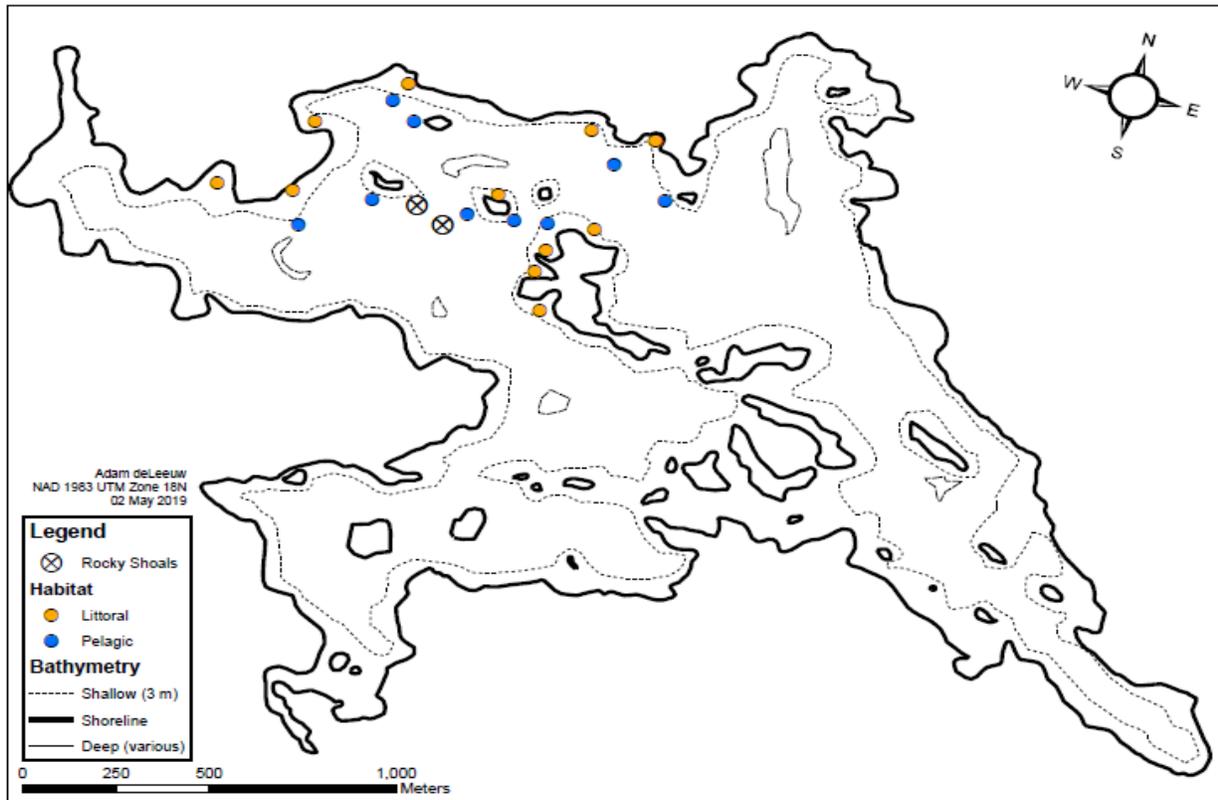
**Table 7. Immigration models, parameters and Wald's tests with probability values for transplanted pumpkinseed sunfish.** Matching habitat choice (MHC), site fidelity and natal habitat preference (SF/NHP) unstandardized parameter estimates are shown for predicting habitat switching decisions. MHC may operate in addition to mechanisms of homing (SF and NHP), so models were developed with any combination of parameters. The ecotype model was used to identify possible differences in MHC between littoral and pelagic pumpkinseed.

Model	Parameters	$\beta$	SE $\beta$	Wald's $\chi^2$	df	p
MHC	Intercept	-3.6276	0.6587	30.3	1	3.6e-08
	Mismatch	7.2568	1.3161	30.4	1	3.5e-08
SF/NHP	Intercept	-6.0039	0.8345	51.8	1	6.49e-08
	Theoretical SF	4.2121	0.6150	46.9	1	7.5e-12
Combined (MHC + SF/NHP)	Intercept	-7.5251	1.1808	40.6	1	1.9e-10
	Mismatch	5.1305	1.7371	8.7	1	3.1e-03
	Theoretical SF	-3.6101	0.6429	31.5	1	2.0e-08
Ecotype	Intercept	-4.5566	0.9979	20.8	1	5e-06
	Mismatch	8.7912	1.9629	20.1	1	7.5e-06
	Capture Habitat	2.0362	1.3543	2.3	1	0.13
	Mismatch x Capture Habitat	-3.3785	2.6919	1.6	1	0.21

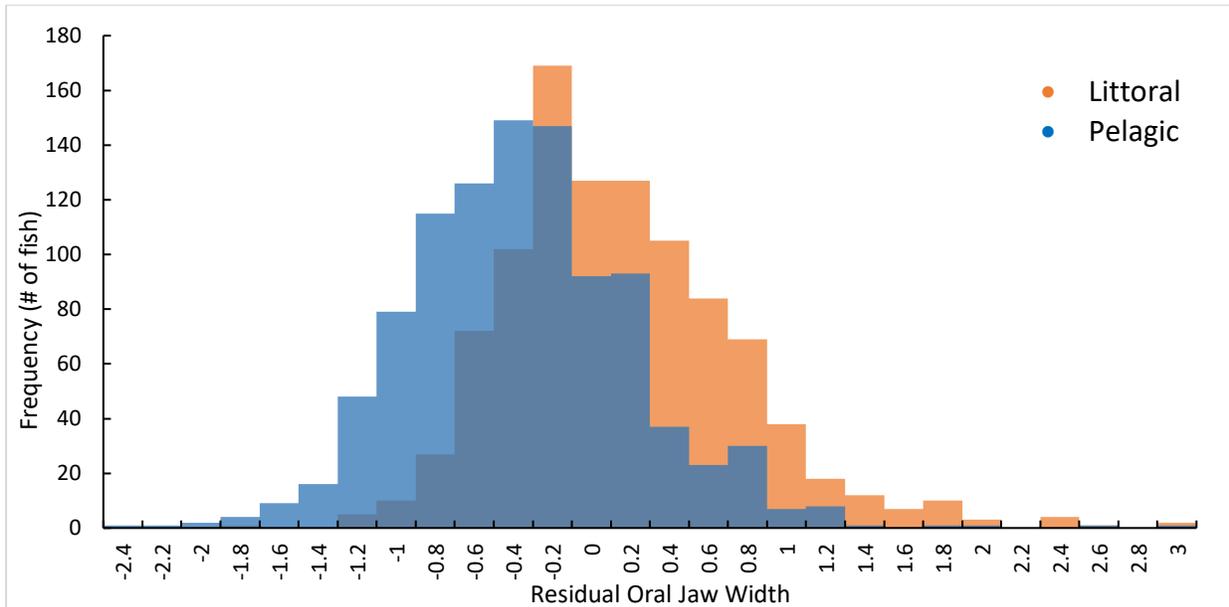
**Table 8. Model summaries and AIC comparisons of immigration decisions of transplanted pumpkinseed.** Comparisons of matching habitat choice (MHC), and site fidelity (SF) / natal habitat preference (NHP) used to predict binomial habitat switching decisions relative to the release habitat. The combined model (homing and matching habitat choice) had the most parsimonious fit to the data, with other models suffering significant reductions in fit.

Model	df	Log-Likelihood	AIC	$\Delta_i$	$w_i$	Rank
SF/NHP + MHC	4	-40.47	86.93	0	0.98	1
Site Fidelity (SF/NHP)	2	-45.43	94.87	7.93	0.02	2
Matching Habitat Choice (MHC)	2	-62.78	129.56	42.62	5.45e-10	3
Ecotype + MHC	4	-61.50	130.99	44.06	2.66e-10	
Null	1	-86.56	175.11	88.18	1.63e-76	

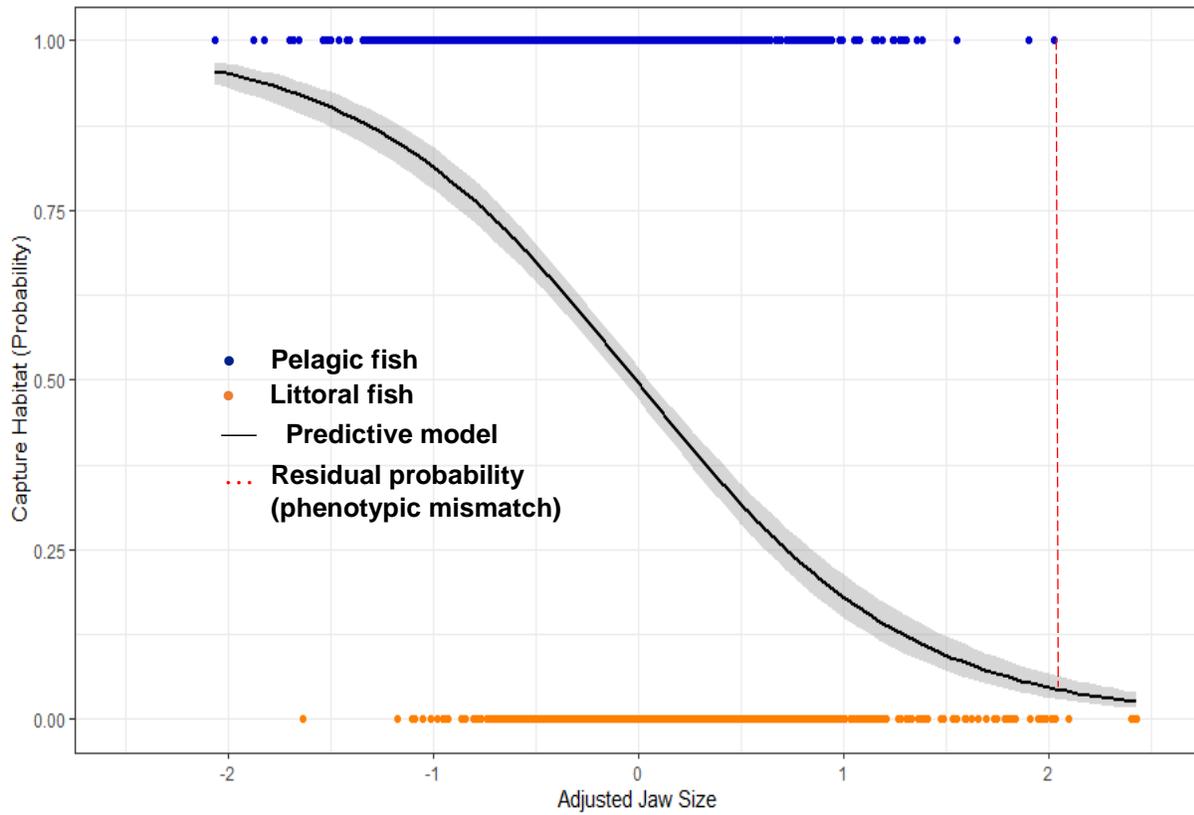
## FIGURES



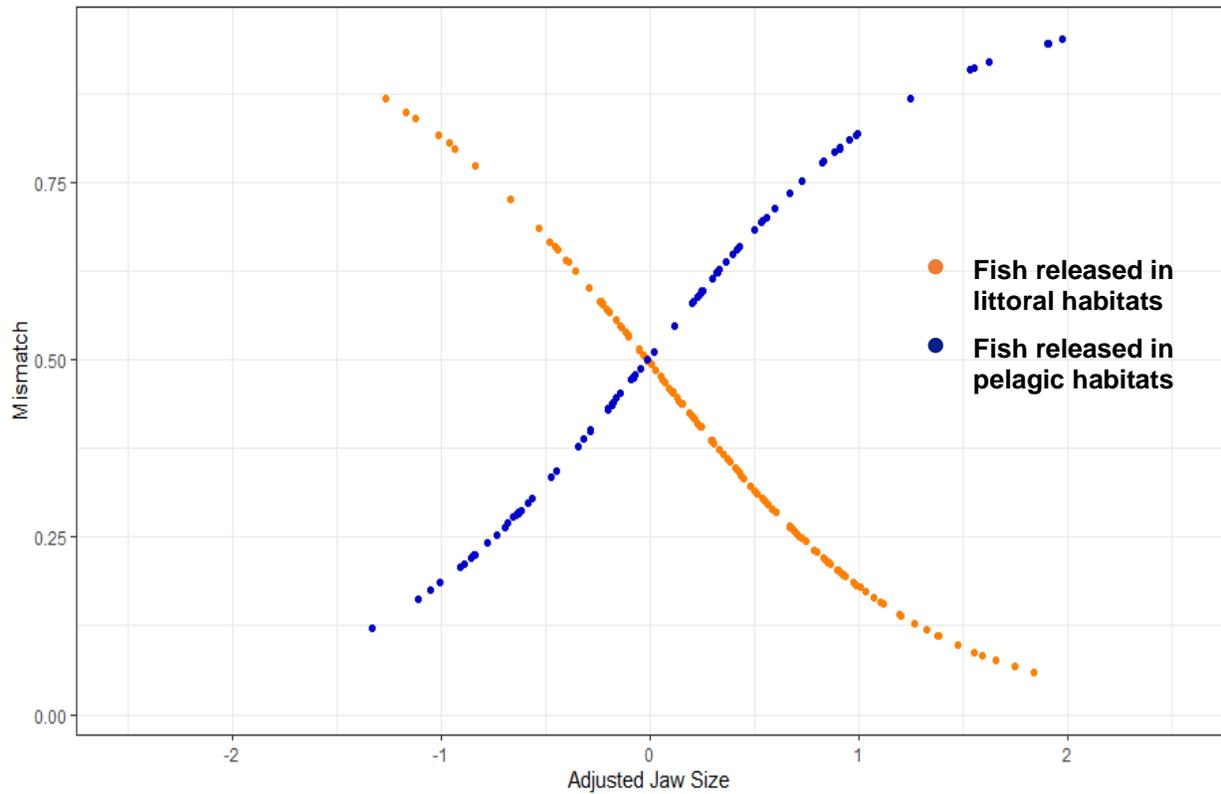
**Fig.1. Map of sites used for capture, transplanting and recapturing of pumpkinseed sunfish in Ashby Lake, ON.** Littoral sites are in small bays characterized by soft sediments, macrophyte growth and structural complexity. Pelagic sites are rocky shoals surrounded by open water.



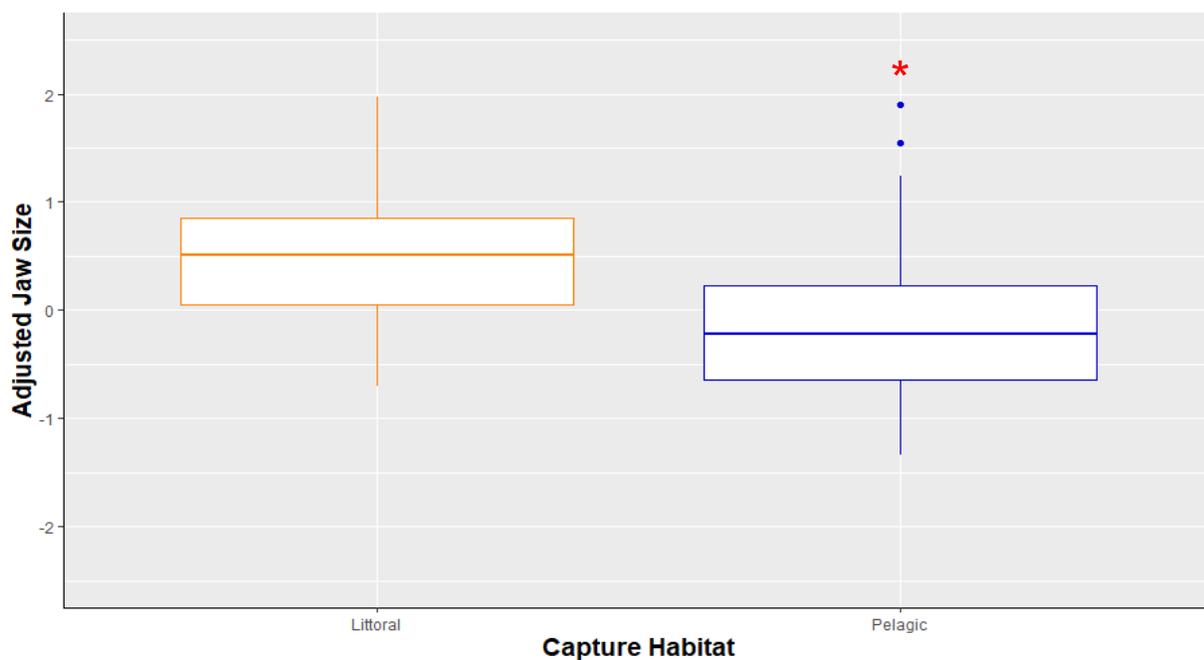
**Fig. 2. Distribution of body size adjusted oral jaw width between littoral (orange) and pelagic (blue) habitats of pumpkinseed sunfish in Ashby Lake.** Residual oral jaw width was obtained through an ordinary least squares (OLS) regression of oral jaw width against total length for all initially captured individuals combined (n=1985). The mean adjusted jaw size is larger in littoral fish than in pelagic fish ( $t = -18.57$ ,  $df = 1983$ ,  $p < 0.0001$ ) and expected to influence feeding performance in each habitat. Larger jaws improve feeding on larger benthic macroinvertebrates that predominate in the littoral habitat whereas smaller jaw improve feeding on smaller zooplankton in the pelagic habitat.



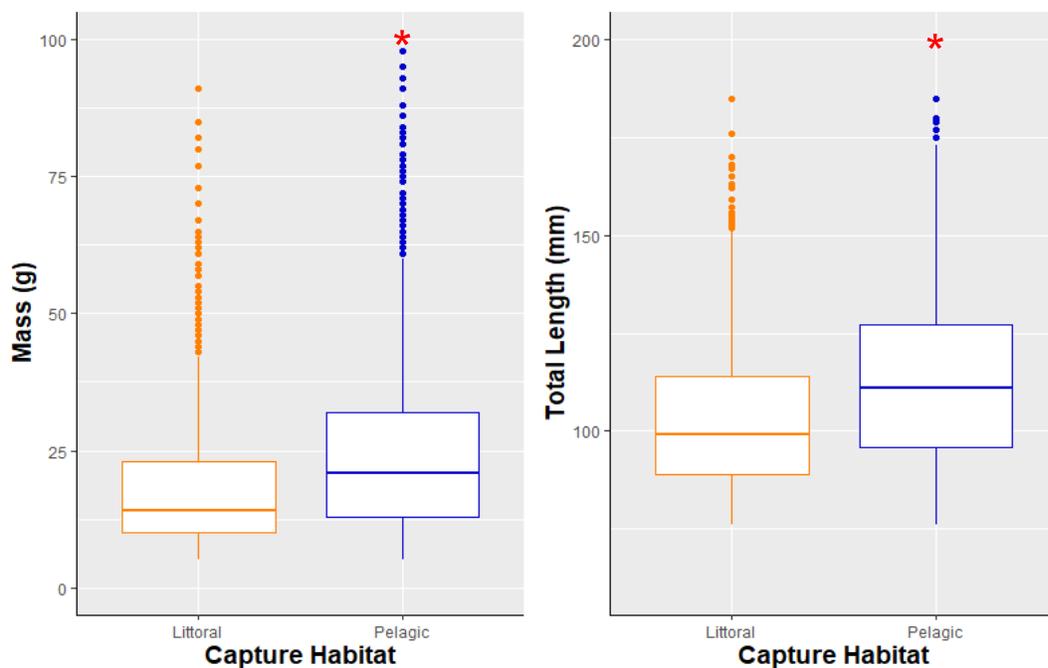
**Fig. 3. Probability curve between capture habitat (0 (orange) = littoral, 1 (blue) = pelagic) in relation to residual oral jaw width after adjusting for body size.** Each point at 0 and 1 on the y-axis represents the body size-adjusted oral jaw width of an individual fish (see Fig. 2). The curve was fitted using a binomial generalized linear model (logit link function) and reflects the probability that a given jaw size was sampled from the pelagic habitat. For example, individuals with unusually large oral jaws for their size had a lower probability of having been sampled originally from the pelagic habitat. When an individual with large jaws was sampled from the pelagic habitat, its large residual probability represents its phenotypic mismatch with respect to the pelagic habitat (red dashed line). The shaded area reflects the 95% confidence interval.



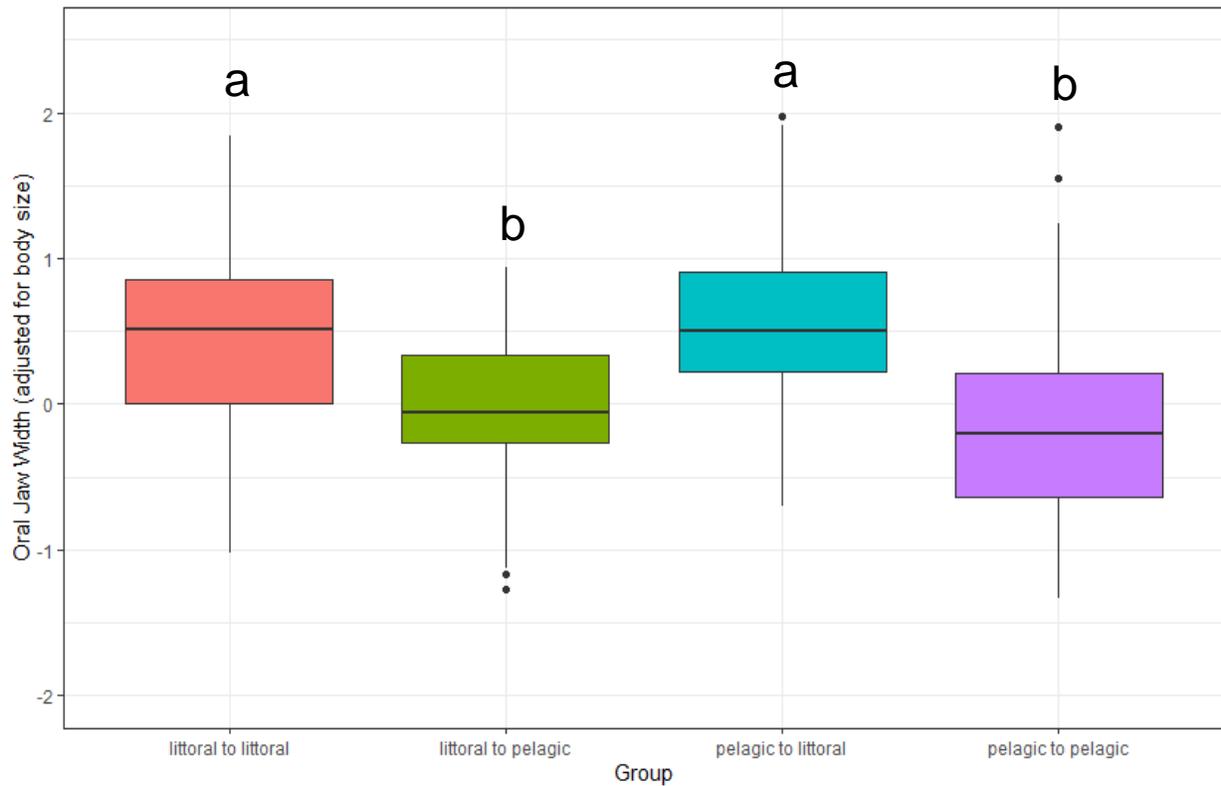
**Fig. 4. Estimated phenotypic mismatch values of pumpkinseed experimentally released into either the littoral (orange) or pelagic (blue) habitat.** Curves reflect the probability differences between actual and predicted recapture habitat (Fig. 3). Larger jaws are better matched to the littoral habitat while small jaws are better matched to the pelagic habitat. Mismatch was calculated as the absolute value of the residuals from the ordinary least squares (OLS) regression of initial capture habitat predicted by size adjusted oral jaw width (Fig. 3) now applied to experimental release habitat.



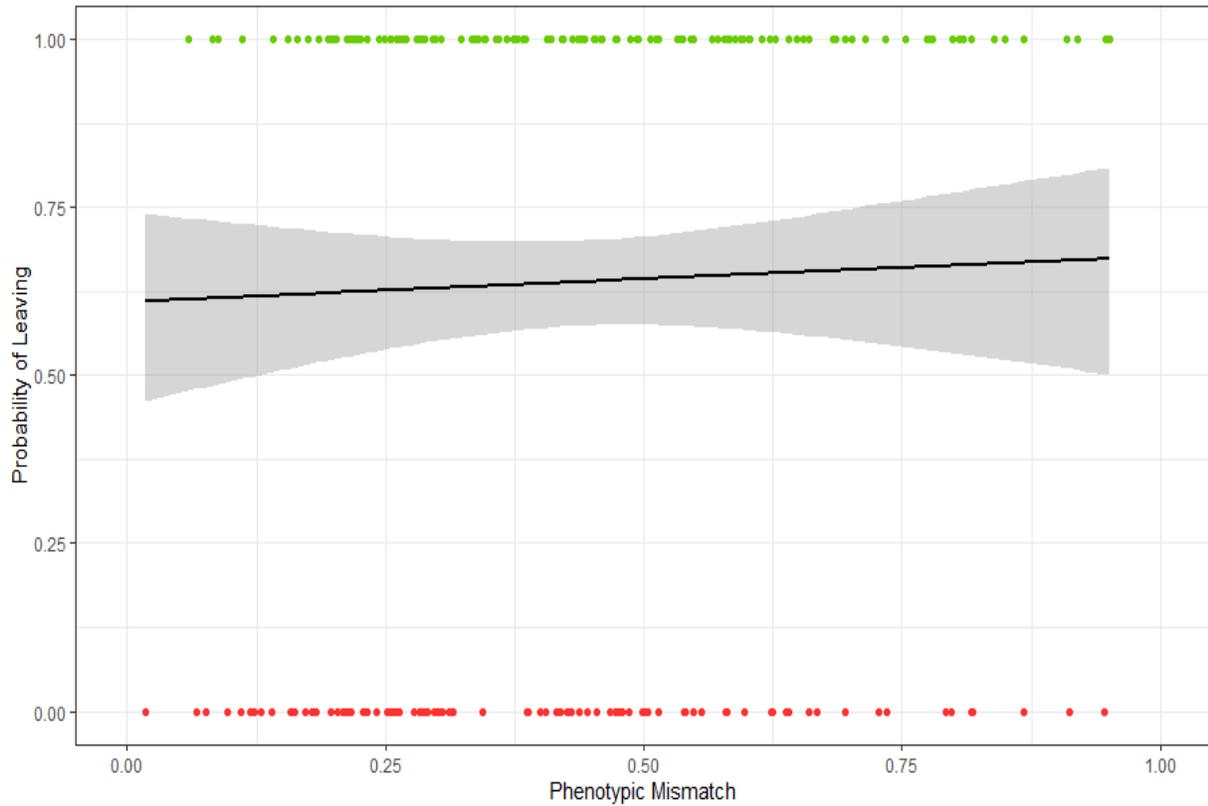
**Fig. 5. Oral jaw width (adjusted for body length) between recaptured littoral and pelagic pumpkinseed sunfish (n=220).** Capture habitat refers to the initial habitat type in which the fish was first captured. Body size adjusted oral jaw width among recaptures was significantly larger in littoral compared to pelagic ecotypes ( $t = -8.04$ ,  $df = 218$ ,  $p < 0.0001$ ).



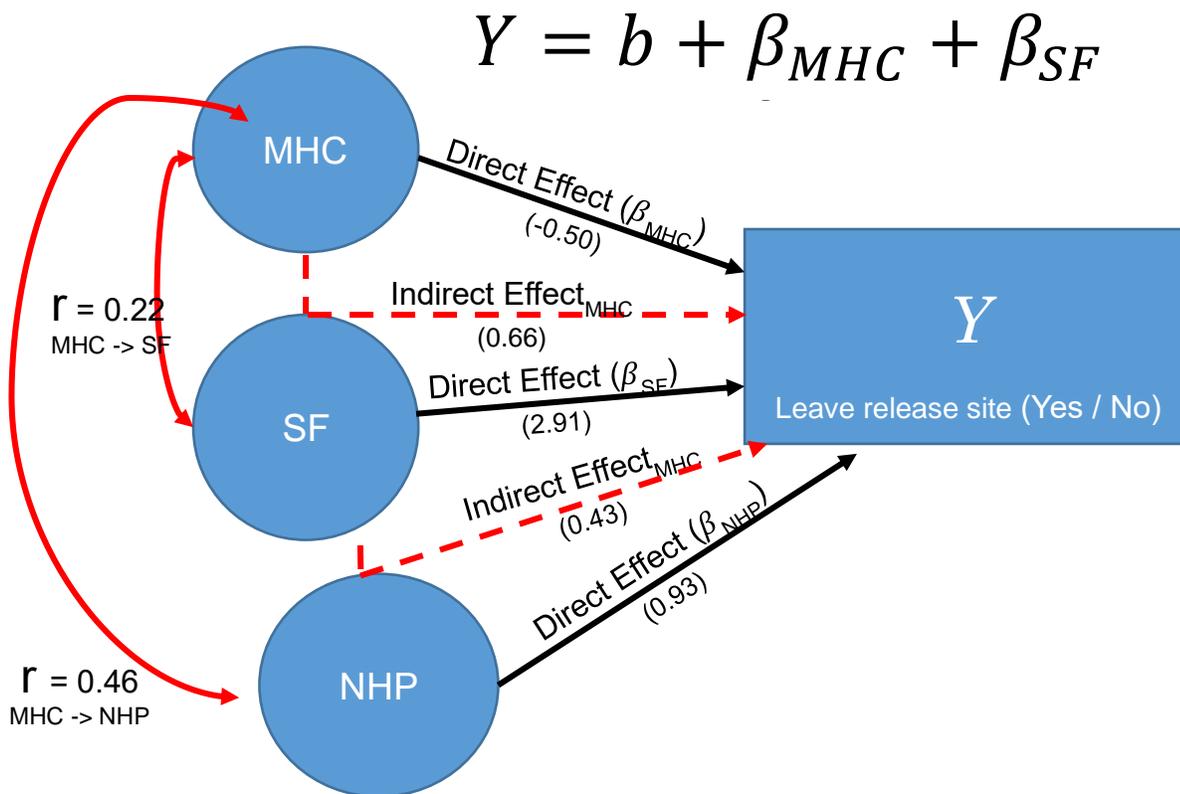
**Fig. 6. Body mass and total length of tagged littoral and pelagic pumpkinseed sunfish.** Capture habitat refers to the habitat type in which the fish was initially captured. Pelagic sunfish on average, have a higher body mass and total length than littoral sunfish.



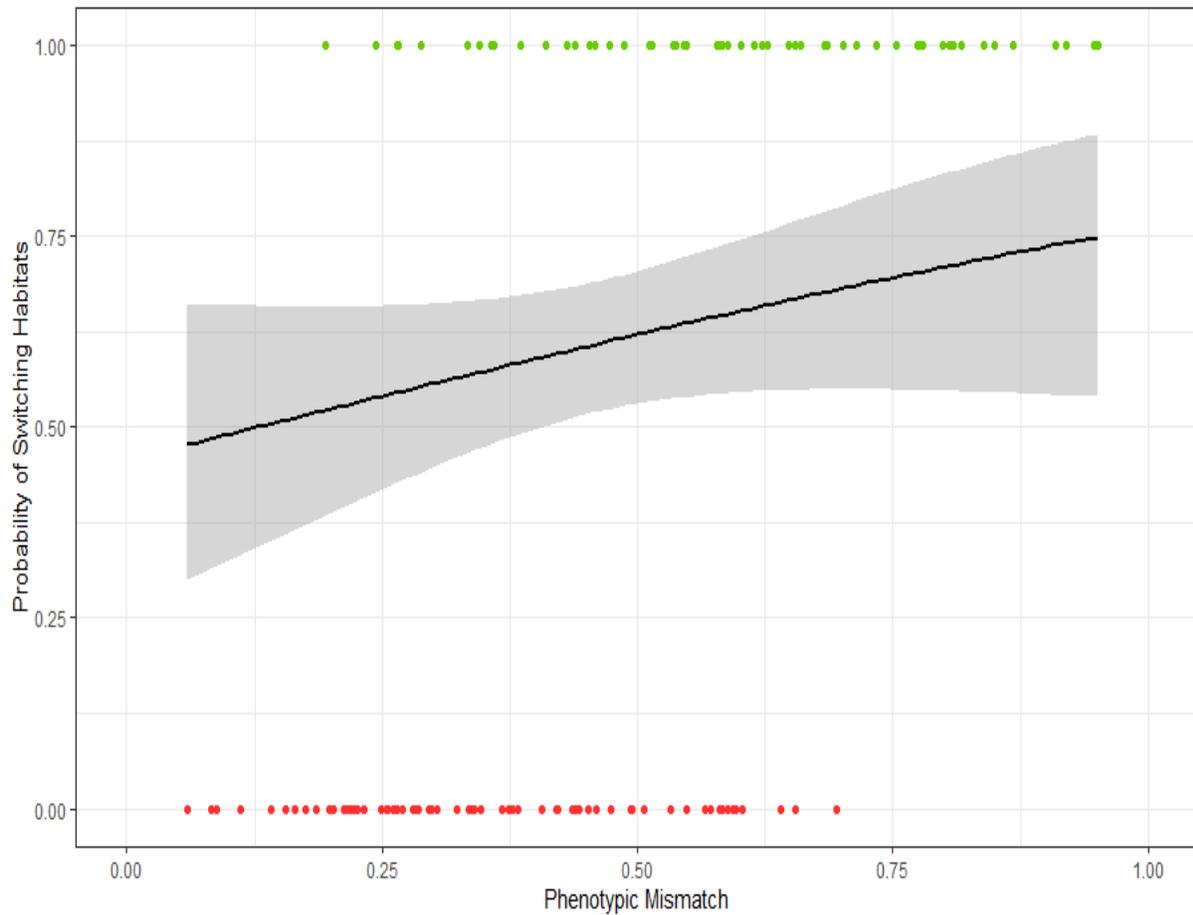
**Fig. 7. Relationship between oral jaw width (adjusted for body size) and movement responses on transplanted pumpkinseed.** Recaptured fish are grouped into four categories based on their release and recapture habitats as indicated. Pumpkinseed that selected the alternate habitat relative to their release habitat had significantly different oral jaw sizes compared to fish who remained in the release habitat (combining all individuals that did not emigrate or who emigrated but remained in the release habitat). The phenotypes of switching individuals (littoral-pelagic and pelagic-littoral) resembled those of the recipient population in both littoral and pelagic ecotypes. Different letters indicate significant differences between pairs that shared a common release habitat.



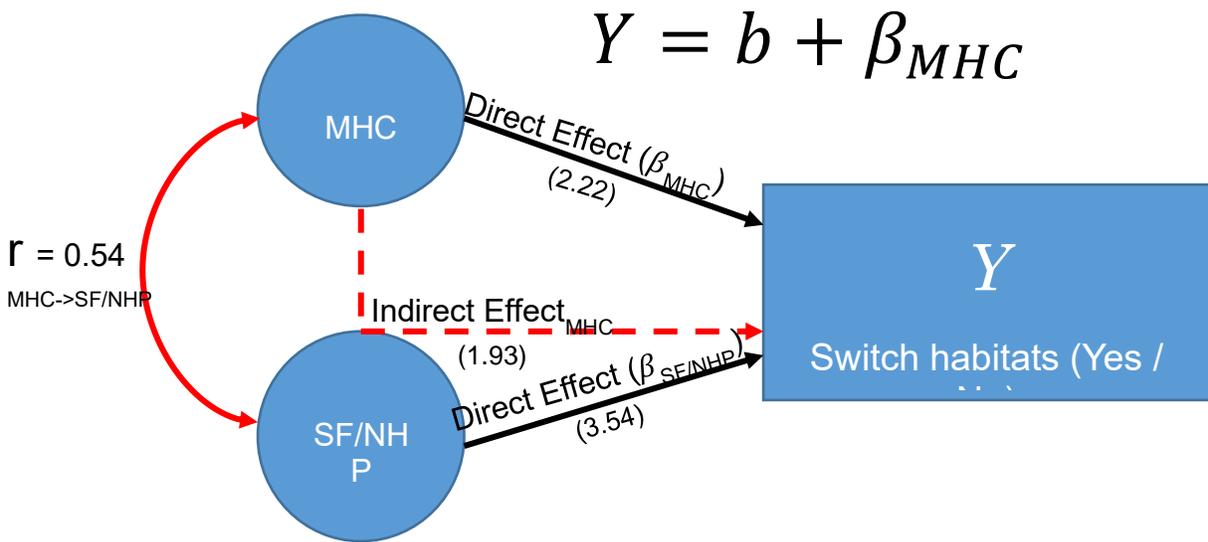
**Fig. 8. Influence of phenotypic mismatch on the probability of emigration from the release site of transplanted pumpkinseed.** There was only weak evidence that the phenotypic mismatch to its release habitat increased the probability of leaving the release site ( $z = 1.89$ ,  $df = 218$ ,  $p = 0.059$ ). Phenotypic mismatch was estimated as the residuals of an OLS regression of oral jaw size (adjusted for body size) against release habitat (Fig. 4). Shading represents the 95% confidence interval of the model.



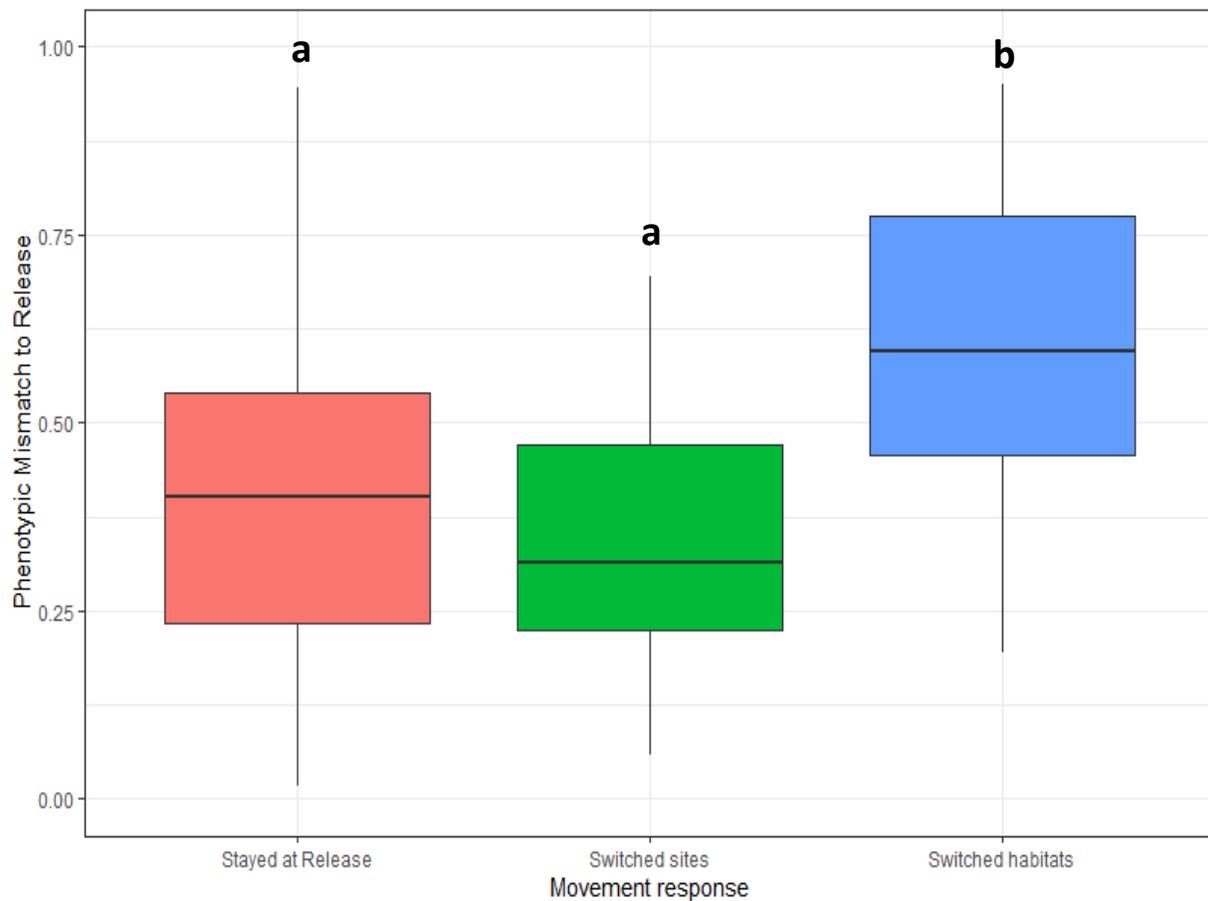
**Fig. 9. Summary of path analysis used to express direct effects (solid black arrows) and infer potential indirect effects (dashed red arrows) of matching habitat choice (MHC) on the probability of emigration for transplanted pumpkinseed.** Parameter direct effects are estimated by the standardized coefficients from a binomial generalized linear regression including matching habitat choice (MHC), natal habitat preference (NHP) and site fidelity (SF) parameters (Table 5). When controlling for both homing parameters (SF and NHP), the direct effect of MHC on emigration is negative. However, MHC is positively correlated with both SF and NHP (double headed red arrows), indicating that MHC may potentially have a net indirect positive influence on emigration decisions through its correlation with the SF and NHP effect.



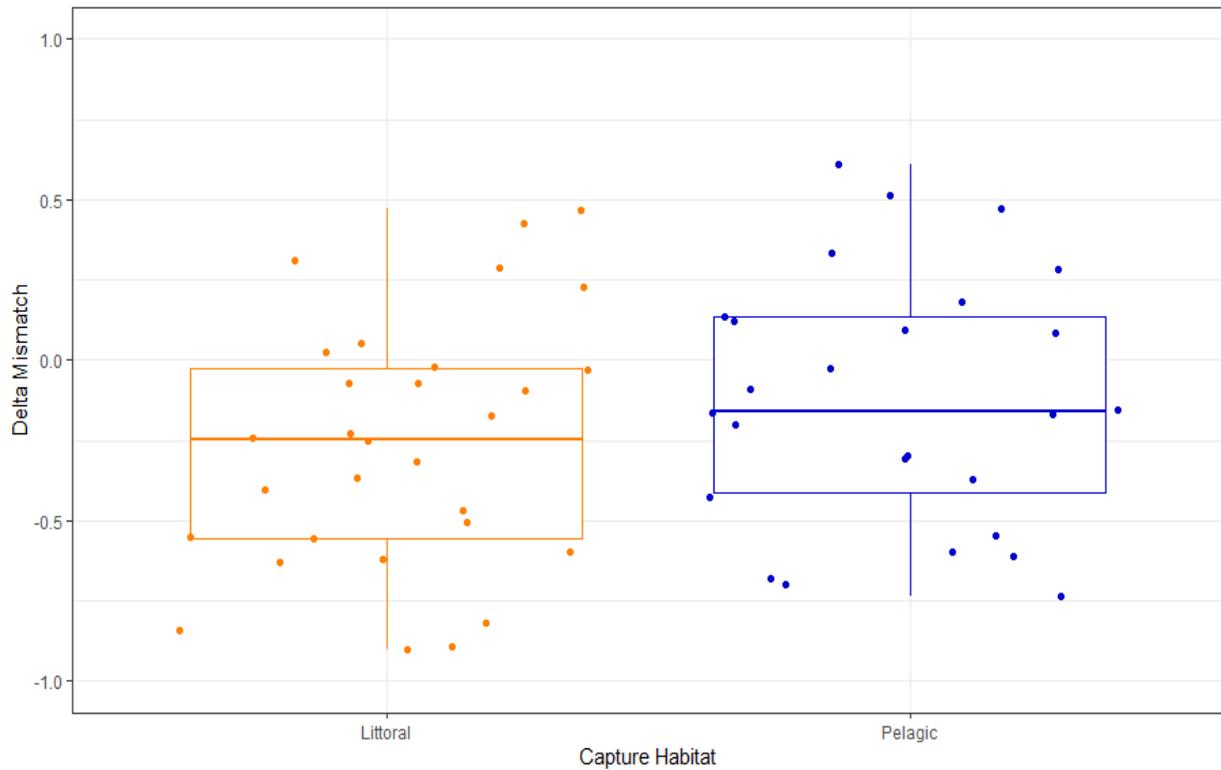
**Fig. 10. Influence of phenotype mismatch on the probability of habitat switching from the release habitat of transplanted pumpkinseed sunfish.** Phenotypic mismatch to release site increased the probability of switching habitats by 21%. In addition, there was strong evidence that the phenotypic mismatch to its release habitat increased the probability of switching habitats from their release habitat after accounting for other directed movement mechanisms ( $z = 2.95$ ,  $df = 123$ ,  $p = 0.003$ ). Phenotypic mismatch was estimated as the residuals of an OLS regression of oral jaw size (adjusted for body length) against release habitat (Fig.4). Shading represents the 95% confidence interval of the model.



**Fig. 11. Path analysis of indirect effects of matching habitat choice on probability of habitat switching of transplanted pumpkinseed sunfish.** Parameter direct effects are estimated by the standardized coefficients from a binomial generalized linear regression including matching habitat choice (MHC) and theoretical homing (site fidelity [SF] and natal habitat preference [NHP]) parameters. However, MHC is positively correlated with the homing parameters ( $r = 0.54$ ) indicating the potential that MHC influences immigration decisions through the SF or NHP effect.



**Fig. 12. Phenotypic mismatch of transplanted pumpkinseed that exhibited different movement responses.** Different letters indicate significant differences in mean mismatch among response groups. Movement responses reflect the response relative to release treatment. Individuals that switched habitats were more mismatched to their release site compared to those that stayed at the release site, and also compared to those that selected sites in the same habitat as the release site. In contrast, there was only weak evidence that individuals that stayed at their release site differed in mismatch to the release site compared to those who left and selected a new site in the same habitat as the release site. Phenotypic mismatch was estimated for the release habitat (Fig. 4).



**Fig. 13. Change in phenotypic mismatch of transplanted littoral and pelagic pumpkinseed sunfish that switched habitats relative to the release habitat.** Each point represents the change in mismatch by an individual fish that switched habitats from release site.  $\Delta$ mismatch was calculated by subtracting mismatch to release site by mismatch to recapture site. Mismatch is estimated by the residual value of an OLS regression of phenotype against release/recapture habitat (see methods). Ecotypes did not differ in  $\Delta$ mismatch ( $p = 0.20$ ). Across both ecotypes, pumpkinseed switched habitats to increase phenotypic match by an average of 20%.

## Appendix A – Dataset Comparison

**Table i. Comparison between capture and recapture datasets and littoral (L) and pelagic (P) ecotypes for data on total length (TL), body mass (Mass) and condition indices (SMI).**

Variable	$\mu_{\text{capture}}$	$\mu_{\text{recapture}}$
oral jaw width (adjusted for body size)	L: 0.25 P: - 0.25 <i>P &lt; 0.0001</i>	L: 0.52 P: - 0.17 <i>P &lt; 0.0001</i>
Total Length	L: 103.80 P: 114.19 <i>P &lt; 0.0001</i>	L: 108.33 P: 120.04 <i>P &lt; 0.001</i>
Mass	L: 18.87 P: 25.90 <i>P &lt; 0.0001</i>	L: 21.19 P: 30.01 <i>P &lt; 0.001</i>
Body condition (Scaled Mass Index)	L: 19.61 P: 19.36 <i>P &lt; 0.01</i>	L: 19.96 P: 19.52 <i>P = 0.08</i>

## **Appendix B – Competition**

MHC behaviour and other mechanisms of directed movement may be influenced by spatial variation in competition, in which case individuals may make movement decisions based on local population densities and resource abundance in addition to or instead of phenotype-environment interaction effects on performance. I did not find strong evidence that competition potentially contributed to emigration decisions as I discuss next.

Direct estimates of competition require data on resource availability, quality and local population density which were outside the scope of this study. Instead, I evaluated the opportunity for spatial variation in competition among sites and between the two habitats by spatially comparing site mean body condition and catch-per-unit-effort (CPUE), and by their effects on site leaving decisions of recaptures. I assume that sites with lower mean individual body condition (estimated from original captures at each site) have less resources available per individual, thereby reflecting higher competition. If competition promotes site leaving decisions, then I expect a negative relationship between release site mean body condition and leaving decisions because lower site mean body condition would encourage leaving the site in search of a site with more resources. I calculated body condition using the Scaled Mass Index (SMI) for all individuals on initial capture. SMI is preferred over other condition indices such as Fulton's index because it allows the relationship of mass and length to deviate from perfect isometric growth, which typically underestimates the relationship between body size and energy reserves (Peig and Green 2009, 2010). The Scaled Mass Index was calculated here according to Peig and Green (2009, eq. 1).

$$(2) \hat{M}_i = M_i \left[ \frac{L_0}{L_i} \right]^{b_{SMA}}$$

Where  $\hat{M}_i$  is the predicted body mass for an individual  $i$  that has mass  $M_i$  and length  $L_i$  standardized to  $L_0$  (the mean body length of the population) with the scaling exponent  $b_{SMA}$  calculated from a standard major axis (SMA) regression of  $M$  on  $L$ .

Spatial variation in competition may also be related to differences in local population density, assuming equivalent resource abundance and quality across spatial units. In the absence of data on actual local population densities, I use mean site CPUE calculated as the number of individuals caught divided by the relative effort (fishing time multiplied by number of anglers) for each site that is averaged over the recapture period (June-August). Again, if competition promotes site leaving decisions, then I expect a positive relationship between site CPUE and leaving decisions because higher CPUE implies higher sunfish density which would encourage leaving the site for one with a lower density of fish.

I then fit two separate generalized logistic models (binomial family and logit link function) to test whether the probability of leaving the release site was related to release site mean body condition or to site mean CPUE. I then refit these logistic models now including a site fidelity parameter, in order to evaluate the effects of competition while accounting for the strong effects of site fidelity discovered during my analysis of MHC. Competition might influence site immigration decisions, but there was no reason to expect that variation in competition would influence habitat switching decisions, and so I only analyzed the effects of competition on site leaving (emigration decisions).

The effects of competition on site leaving behaviour were equivocal. When modelling leaving decisions against release site mean body condition, lower release site mean SMI significantly increased the probability of leaving ( $df = 218, z = -2.615, p = 0.009$ ). However, this effect was non-significant once the site fidelity parameter was included as a covariate in the model ( $df = 217, z = 1.355, p = 0.18$ ), suggesting that competition may have little direct effect on site leaving decisions. When modeling leaving decisions against site CPUE, release site CPUE did not predict leaving behaviour ( $df = 219, z = 0.625, p = 0.532$ ). Taken together these results suggest minimal competitive effects on emigration decisions in these pumpkinseed.

**Table ii. Competition models, parameters and Wald’s tests with associated significance for transplanted pumpkinseed sunfish.** Body condition (SMI), site fidelity (SF) and catch-per-unit-effort (CPUE) unstandardized parameter estimates are shown. Body was calculated as the mean site scaled mass index (SMI) of captured individuals according to Peig and Green (2009). Catch-per-unit-effort was calculated as the mean number of individuals captured per hour of angling effort averaged across the recapture period (June-August, 2019). Asterisks indicate significance.

Model	Parameters	$\beta$	SE $\beta$	$df$	$p$
Body condition	Intercept	-26.1006	9.8764	1	3.6e-08*
	SMI	-1.3206	0.5051	1	8.9e-03*
Body condition + SF	Intercept	15.9627	12.7974	1	0.21
	SMI	-0.8866	0.6546	1	0.18
	SF	3.1222	0.3631	1	2e-16*
CPUE	Intercept	0.0702	0.3810	1	0.85
	CPUE	0.0159	0.0255	1	0.53
CPUE + SF	Intercept	-1.6638	0.5468	1	2.4e-03*
	CPUE	0.0197	0.0339	1	0.56
	SF	3.1720	0.3623	1	2e-16*

## **Appendix C – Terms relating to matching habitat choice**

Phenotypic sorting

Phenotype-dependent dispersal

Phenotype-sensitive dispersal

Phenotype-specific habitat selection

Genotype-specific microhabitat partitioning

Genotype-specific habitat selection

Adaptive / refined habitat selection

Phenotype-matching habitat selection

Fitness-dependent dispersal

Directed movement

Fitness-driven dispersal

Conditional movement

Fitness-associated dispersal

Colonization effect