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Boldness and dispersal tendency of native and invasive pumpkinseed (*Lepomis gibbosus*): is spatial sorting creating superior invaders?

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This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (http://www.icais.org/html/previous19.html). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

The ability of non-native species to establish populations, expand their range, and ultimately affect native species is influenced by the behaviour and dispersal potential of individuals. Spatial sorting theory predicts that individuals with greater dispersal tendencies will accumulate at the invasion front and promote further territorial expansion. The theory predicts that the reproduction of such individuals at the front as a result of their proximity leads to selection for better dispersers. To test for evidence of this phenomenon in successful invasive populations, we compared boldness and dispersal tendency in one native North American and two invasive European populations of pumpkinseed (*Lepomis gibbosus*) reared in a common environment, with a second wild native population from a local water body added to the comparison. Tests were conducted in a 4-metre long artificial flume with a video capture system recording movement. Boldness was measured using the time it took an individual to emerge from a sheltered area, and dispersal tendency was assessed using a combination of movement metrics as the fish explored the flume. Contrary to expectations, native North American populations were bolder than invasive European populations, and there was no significant difference in dispersal tendency between native and invasive pumpkinseed. Females were bolder and showed greater dispersal tendency than males. Post-hoc analysis indicates that populations originating from lotic water bodies had significantly lower dispersal tendency than those from lentic water bodies regardless of native/invasive status, suggesting that habitat of origin may affect dispersal tendency.

Key words: freshwater fish, exploratory behaviour, invasive species, range expansion

Introduction

Range expansion is an important process in species invasions, as it extends the area of impact beyond that of the initial establishment. If we assume that a population contains individuals that differ in their mobility and rate of dispersal, then range expansion will be the result of heterogeneous movement patterns. Indeed, empirical studies of invading populations have identified leptokurtic movement distributions, where most individuals move little, while a smaller proportion of the population moves extensively (Skalski and Gilliam 2000; Rodriguez 2002). Thus, physiological or behavioral traits within this small, highly active proportion of the population likely play an important role in the rate of range expansion in invasive populations.

Spatial sorting theory predicts that during a species invasion, individuals exhibiting high dispersal ability will accumulate at an expansion front, and that

increased rates of dispersal will evolve in the population regardless of whether natural selection favours high dispersal tendency or not (Shine et al. 2011). Spatial sorting is based upon non-random mating of rapid dispersers at the edge of an expanding range (the Olympic Village effect; Phillips et al. 2008), producing progeny with higher rates of dispersal than the population at large as well as their parents. Support for the predictions of spatial sorting in range-expanding populations has been found in a variety of species (reviewed in Shine et al. 2011; Chuang and Peterson 2016); most notably in the cane toad (*Rhinella marina* Linnaeus, 1758) in Australia, where the species has experienced accelerated invasion rates associated with increases in leg length (Phillips et al. 2006), as well as greater movement and higher endurance at the expansion front (Llewelyn et al. 2010). Using an individual based-model, Dytham et al. (2014) showed that individual variation in dispersal rates increases the rate of territorial expansion in an invasion scenario, implicating a trade-off favouring dispersal-enhancing traits even at the expense of adult survival.

Certain behavioural traits, like exploration and boldness, may have a role in dispersal tendency and ultimately, invasion success (Pintor et al. 2009; Cote et al. 2010a, 2010b). Past studies examining boldness in fish show that invasive species are more likely to disperse sooner and to greater distances than their native congeners (Rehage and Sih 2004). Individuals that are bolder are more likely to colonize new habitats since they tend to make up a higher proportion of the leading edge of an invading population (Rehage and Sih 2004; Myles-Gonzalez et al. 2015). These patterns suggest that behavioral traits associated with increased dispersal tendency in fish may also be selected for during range expansion.

A number of studies have explored differences in behavioural traits in invasive populations relative to their position within an expanding range (e.g., Phillips et al. 2006; Groen et al. 2012; Lopez et al. 2012 and references therein). In contrast, there are relatively few comparisons of behavioural traits associated with range expansion between native and invasive populations, and much of this research is on terrestrial fauna (e.g., Brodin et al. 2013). Part of this research gap may be attributable to the challenges of quantifying heritability of behaviour, and the necessity of isolating behaviour from effects of the environment using artificial selection and genetic analysis (Conrad et al. 2011). These challenges are particularly difficult with longer-lived species, and given the ethical restrictions associated with transporting and raising invasive species.

In this study, we use the pumpkinseed (*Lepomis gibbosus* Linnaeus, 1758) to explore the role that behavioural traits play in invasion success, specifically by examining differences in boldness and dispersal tendency between native and invasive populations. The pumpkinseed is a small, warmwater sunfish native to the eastern and central portions of North America (Scott and Crossman 1973). The species has been successfully introduced in many countries, and is considered invasive in parts of southern Europe (Copp and Fox 2007). The pumpkinseed was imported into Europe in the late 19th century (Lever 1977; de Groot 1985). It spread through France and was first detected in the Iberian Peninsula around 1910 (García-Berthou and Moreno-Amich 2000), where it is considered to be a highly successful invader (Godinho et al. 1997, García-Berthou et al. 2005). The negative effect of pumpkinseed on native species in Spain appears to be mainly through trophic interactions: pumpkinseed are aggressive and cause other species to retreat even in the presence of small pumpkinseed (Almeida et al. 2014).

If spatial sorting results in selection for individuals with higher dispersal rates over the course of an invasion (Shine et al. 2011), it follows that individuals from an introduced lineage that has experienced extensive range expansion would show higher dispersal rates than individuals from native populations living in the original source area. Based on this premise and the linkage between boldness, exploratory behavior, and dispersal, we hypothesized that pumpkinseed from invasive populations would be bolder and show greater dispersal tendencies than those originating from native populations.

Methods

Study system

The pumpkinseed used in our study originate from two invasive European populations from Catalonia, Spain (Ter River: 42º01′N; 3º12′E; Susqueda Reservoir: 41º58′N; 2º30′E), and two native North American populations from Ontario, Canada (Otonabee River: 44º23′N; 78º16′W, Rice Lake: 44º10′N; 78º10′W). Pumpkinseed from Canada and New York State, USA were introduced into northern Europe several times in the late 19th century (Mather 1889; Künstler 1908; Arnold 1990). Iberian pumpkinseed were introduced through France (García-Berthou et al. 2005), and microsatellite DNA analysis confirms that wild Iberian pumpkinseed, including the populations used in our study, have an Atlantic North American origin (Detta 2011).

Pumpkinseed were first discovered in the Iberian Peninsula around 1910 in Lake Banyoles in the northeast (García-Berthou and Moreno-Amich 2000; Elvira and Almodóvar 2001). As Lake Banyoles is connected to the Ter River via a short tributary, pumpkinseed may have been present in the river site for as long as 100 years. Pumpkinseed are abundant in the mid-lower Ter River, and are one of several exotic species, including largemouth bass *Micropterus salmoides* (Lacepède, 1802)*,* found in that part of the river (Boix et al. 2010). Susqueda Reservoir, located in the middle portion of the Ter River, approximately 30 km upstream from our Ter River site, was created to provide drinking water to Barcelona, and it experiences very high water level fluctuations as a result of high drawdown rates, its steep-sided topography, and the region's dry climate (Vila-Gispert et al. 2007). The pumpkinseed is one of three dominant fish species in the reservoir; the other two being common carp *Cyprinus carpio* (Linnaeus, 1758) and exotic largemouth bass (Carol et al. 2006).

Native pumpkinseed populations used in our study originate from the Otonabee River and Rice Lake; both part of the Trent-Severn Waterway which connects Georgian Bay in Lake Huron to the Bay of Quinte in Lake Ontario. The Otonabee River is the largest tributary of Rice Lake, a relatively shallow, highly vegetated, mesotrophic lake. Water level in the lake and river is regulated through a series of dams for navigation of recreational boats though the system. Pumpkinseed are common in both water bodies in addition to a diverse fish community including bluegill *Lepomis macrochirus* (Rafinesque, 1819), rock bass *Ambloplites rupestris* (Rafinesque, 1817), yellow perch *Perca flavescens* (Mitchill, 1814), native largemouth bass, and exotic common carp (Gutowsky and Fox 2012).

Adult pumpkinseed from the four study populations were used to establish colonies in 2008 at a pond facility located at Sir Sandford Fleming College, Lindsay, Ontario (44º20′N; 78º43′W). The adults ($N \geq 20$ per population) were electrofished from the two Spanish populations in the spring of 2007, flown to Canada in the late fall, and held over the winter period in a laboratory holding facility. Adults from the two native populations (again*,* ≥ 20 per population) were collected with a bag seine in early June 2008, and were transported by truck to the laboratory holding facility. Adults from all four populations were transported by truck to the pond facility, and stocked in mid-June 2008.

Colonies were housed in artificial outdoor ponds (9 m length × 3 m width, contoured to a maximum depth of 3 m); one pond per population. For this study, we collected individuals 60–100 mm total length from the colonies with the exception of the Otonabee River population. This colony suffered heavy mortality in the winter of 2014 due to failure of the aeration unit that keeps part of the pond ice-free, and individuals of the desired size were unavailable. As a result, we substituted wild pumpkinseed sampled from the Otonabee River, itself. Individuals from all sites were collected from July 8 to September 12, 2014. Using pumpkinseed from these colonies, we were able to compare populations of successful invaders and populations from the native range that were reared in a common environment (with the one exception as noted), thus reducing the potential effect of environmental variability on the outcome of our study.

Collected pumpkinseed were transported immediately to Trent University in aerated coolers, where they were individually held in 19 L aerated aquaria at approximately 21 °C under a 14:10 hour light:dark cycle. Each aquarium was wrapped with opaque material to eliminate visual contact between fish, and was outfitted with a PVC pipe shelter and artificial vegetation. Individuals were held for no more than 7 d before undergoing experiments, and were fed *ad libitum* for 15 minutes daily with frozen adult brine shrimp.

Assessment of boldness and dispersal tendency

Boldness and dispersal tendency were assessed using a protocol modified from that of Myles-Gonzalez et al. (2015). The trials were run on 19–25 individuals per population (85 pumpkinseed in total). Experiments were conducted in a Plexiglas flume (0.5 m width × 0.7 m depth × 6 m length with a 4 m experimental arena), covered on all sides with opaque material. An off-white gravel substrate was used in the arena, with rocks (diameter > 10 cm) randomly placed throughout to provide structure. Filtered Otonabee River water was chilled to approximately 21 ± 1 °C, before entering the flow-through system of the flume. Water flow was maintained at a negligible velocity. Prior to each trial, an individual pumpkinseed was placed in a 0.6 m long enclosed segment at the downstream end of the flume and allowed to acclimate for 30 min. This acclimation zone was filled with plastic aquarium plants for shelter and was separated from the testing arena by a corrugated plastic door, which was operated remotely. After the acclimation period, the plastic door was raised, allowing the test fish access to the experimental arena for 30 min.

Boldness was assessed as the time taken by an individual fish to emerge from the acclimation zone (Brown et al. 2005). Fish that did not emerge at any point during the trial were excluded from the main

analyses (3/21 from Rice, 2/20 from Otonabee, 7/19 from Susqueda, 8/25 from Ter). Fish were considered to have been startled by the gate if they exited the acclimation zone immediately upon opening of the gate (< 2 s) or if they were observed on camera darting towards the back of the acclimation zone as the gate was being raised. Eight individuals were startled during their trials, and they were completely excluded from analysis of behavioural traits along with the fish that did not emerge (Myles-Gonzalez et al. 2015).

Dispersal tendency was assessed using five measures of velocity and distance that describe fish exploratory movement in the experimental arena. These metrics were total distance travelled, total mean velocity, mean velocity while moving, total time spent moving, and farthest distance reached; these metrics have been used in several previous studies to assess dispersal in animals (Rehage and Sih 2004; Cote et al. 2010b; Myles-Gonzalez et al. 2015). Movement of the fish during each trial was recorded with two HD digital cameras, positioned 3 m above the flume and spaced so as to capture the entire length of the arena in the field of view. Videos were converted to one frame per second image sequences using MPEG Streamclip, and analyzed using ImageJ software with a manual tracking plug-in to quantify fish movement (http://rsb.info.nih.gov/). Each fish was subjected to a single trial, after which it was euthanized (MS-222), total length (TL), and wet weight recorded, and its sex determined by examination of internal organs.

Data analysis

An information theoretic approach (Burnham and Anderson 2002) was used to assess whether population origin accounted for differences among individuals in boldness and dispersal tendency. We also considered the effects of two other variables: sex and body size (total length). All two-way interaction terms were included in these analyses. Models were evaluated with Akaike's information criterion corrected for small sample bias (AICc); the best model for each dependent variable was considered to be that with the lowest AICc. Models with an AICc difference (Δi) < 2 from the best model were considered to have strong support, whereas those with $2 < \Delta i < 4$ were considered to have moderate support (Burnham and Anderson 2002).

To assess boldness, the dependent variable tested (time to emergence from the shelter) was $\log_e$-transformed prior to analysis to correct for non-normality and heteroscedasticity. As previously noted, trials in which an individual was startled or did not emerge from the shelter were excluded from the afore-

mentioned model selection procedures. To test for differences in emergence rate between native and non-native pumpkinseed, we used a chi-square analysis with populations and sexes pooled.

To assess dispersal tendency, the five movement variables were initially collapsed into a smaller number of measures using principal component analysis (PCA), with the PCA conducted on the correlation matrix of the standardized raw measures. PCA scores of individual trials for axes with eigenvalues near or above 1 were then used to evaluate alternative models (see López et al. 2005).

Examination of the results suggested an apparent effect of waterbody type (lentic vs lotic) on boldness and dispersal tendency. For this reason, we conducted post-hoc analyses, including this factor in model selection tests in place of population (both could not be run concurrently on account of singularity).

Results

The proportion of fish that emerged from the acclimation zone was significantly higher in the native (Canadian) populations than the invasive (Spanish) populations (86 vs 48%, respectively; $\chi^2 = 10.8$, $P = 0.001$). For those that emerged, the strongest model for predicting time to emergence included population and sex as main effects (Table 1A). Females emerged sooner from the acclimation zone than did males in all four populations (Figure 1). Individuals from the native populations generally emerged sooner than those from the invasive populations; the one exception being that females from Susqueda emerged sooner than females from Rice Lake.

The PCA for dispersal tendency produced one PC axis with an eigenvalue ≥ 1, which accounted for 78% of the explained variation (Table 2). This PC1 axis showed a strong significant positive correlation with total distance travelled, mean velocity, amount of time in motion and mean velocity while moving, and also correlated positively with longitudinal distance travelled. As with boldness, the strongest model for predicting measures of dispersal, as indicated by the PC1 score, included population and sex as main effects (Table 1B). Unlike boldness however, dispersal tendencies were not consistently different between native and invasive populations (Figure 2).

Differences between populations in both boldness and movement showed consistent habitat-related results. In the native populations, pumpkinseed from Rice Lake were bolder (Figure 1) and had a higher dispersal tendency (Figure 2) than pumpkinseed from the Otonabee River. Similarly, in the invasive populations, pumpkinseed from Susqueda Reservoir were

Table 1. Model selection results from an analysis of the effects of population (pop), sex and body length (TL) on (A) time to emergence and (B) indicators of dispersal tendency as assessed with PCA (PC1 axis score) in pumpkinseed from two native and two invasive populations in behavioural trials. Candidate models are ranked by the Akaike Information Criterion corrected for small sample size (AICc), with the differences between models shown as Δi. All combinations of the three independent variables were assessed; the models displayed are those classified as strong ($\Delta i \leq 2$) and moderate ($4 \leq \Delta i \leq 2$). Also shown for each model are the number of parameters including the error term and intercept (K), Akaike weight (w_i), relative likelihood (RL), and significance probability (Prob).

Model terms	K	AICc	RL	Δ_i	w_i	Prob
A. Time to emergence (log$_e$-transformed)						
pop, sex	4	191.11	1.0	0	0.47	0.016
pop, sex, TL	5	193.21	0.35	2.10	0.16	0.029
pop	3	194.52	0.18	3.41	0.08	0.080
pop, sex, TL, TL*sex, TL*pop	7	195.07	0.14	3.96	0.06	0.017
sex	3	195.10	0.14	3.99	0.06	0.17
B. Dispersal tendency (PC1)						
pop, sex	4	270.43	1.0	0	0.47	0.0014
pop	3	270.77	0.84	0.34	0.39	0.0016
pop, sex, TL	5	272.69	0.32	2.26	0.15	0.0032

Figure 1. Time taken by male and female pumpkinseed to emerge from an acclimation zone at the downstream end of a 4 m flume, comparing individuals collected from native populations (Rice Lake and Otonabee River, Canada; black bars), and invasive populations (Susqueda Reservoir and Ter River, Spain; grey bars). Values shown are back-transformed means with 95% confidence intervals.
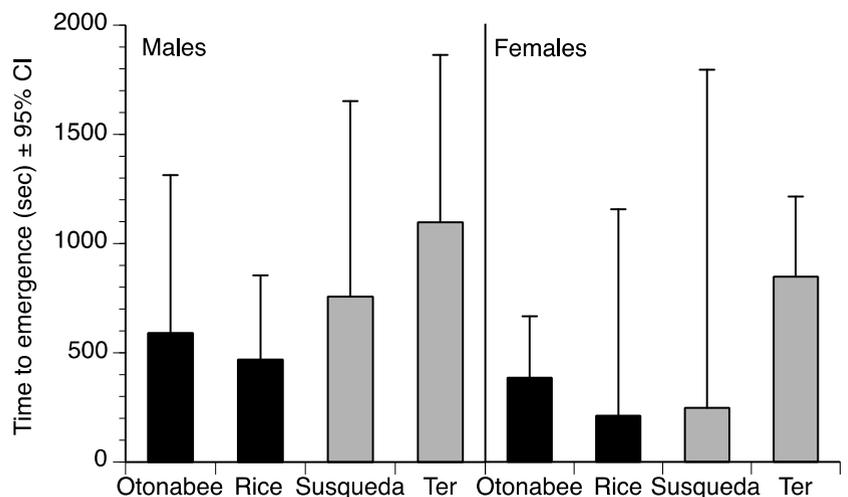
bolder and had a higher dispersal tendency than those from the Ter River. The differences were evident in both sexes, and suggested that waterbody type (lentic vs lotic) might have an effect on these characteristics. Post-hoc analysis showed that water body type was one of the strongest factors in predicting boldness (Table 3) and dispersal tendency (Table 3, Figure 3) in our trials. These results suggest that pumpkinseed from lacustrine water bodies are bolder and have a higher dispersal tendency than those from fluvial systems, regardless of geographic origin.

Discussion

We found that native North American pumpkinseed were bolder than invasive European pumpkinseed, averaging less time to emerge from a sheltered space, and that dispersal tendency of the European pumpkinseed was not greater than that of the North American populations. Neither of these results supported our hypotheses that invasive populations would be bolder and show greater dispersal tendency.

The hypotheses that the invasive pumpkinseed would be bolder and have greater dispersal tendency than the native populations was based on spatial sorting theory and the assumption that animals with high dispersal tendency tend to be bold individuals. Though some studies have linked boldness to greater dispersal tendency (e.g., Chapman et al. 2011; Brodin et al. 2013; Myles-Gonzalez et al. 2015), correlations between these characteristics are not always evident. For example, Cote et al. (2010b) found that dispersal distance in mosquitofish *Gambusia affinis* (Baird and Girard, 1853) was unrelated to the boldness of an individual. Correlations between boldness and dispersal tendency were weak in African jewelfish

Table 2. Results of PCA on five variables related to dispersal potential as assessed in pumpkinseed from two native and two invasive populations during behavioural trials. Variables were standardized prior to analysis. Shown are the eigenvalues for each axis and the loadings for the correlation matrix. Numbers in boldface indicate significant correlation with PC axis.

Axis	Eigenvalue	Percent variation explained			
1	3.88	77.7			
2	0.76	15.2			
3	0.30	6.0			
4	0.06	1.1			
5	0.0004	0.1			
Loading matrix					
Original variable	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Total distance	**0.97**	-0.16	-0.06	-0.11	-0.01
Longitudinal distance	**0.61**	**0.78**	0.17	-0.03	0.0004
Mean velocity	**0.97**	-0.18	-0.07	-0.11	0.01
Mean velocity when moving	**0.87**	-0.29	**0.39**	0.11	0.0002
Time moving	**0.92**	0.12	**-0.33**	0.14	-0.0001

Figure 2. Comparison of mean PCA scores of male and female pumpkinseed collected from native (black bars) and invasive (grey bars) populations. The PC1 axis shown was generated from measurements of total and longitudinal distance travelled, average velocity, velocity while moving, and time spent moving during 30 minute trials in a 4 m long flume. These variables were used as indicators of dispersal potential. Correlations between the PC1 axis and the original variables is provided in Table 2. Error bars represent SE.
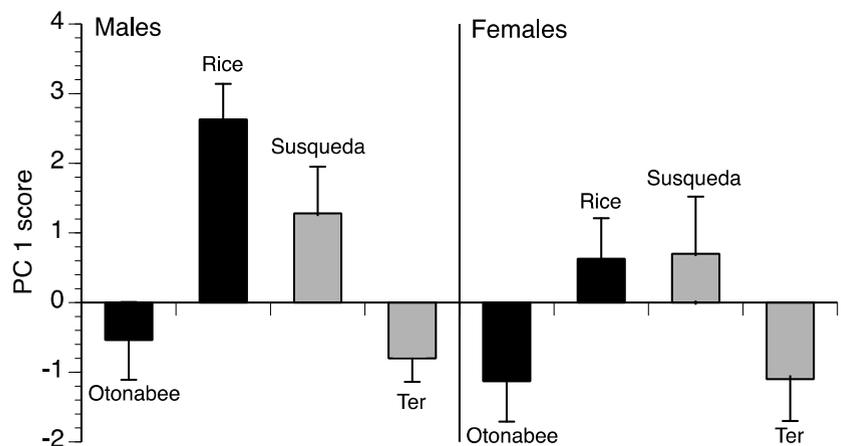
Hemichromis letourneuxi (Sauvage, 1880) captured from both invasion fronts and established areas in the Florida Everglades, and there was no significant difference between these groups in either factor (Lopez et al. 2012). Thorlacius et al. (2015) found no significant correlations between boldness and either dispersal latency or dispersal distance in round goby *Neogobius melanostomus* (Pallas, 1814) in newly-established or older populations in Canada. Despite weak within-population correlations, Thorlacius et al. (2015), and Groen et al. (2012) demonstrated that round goby from the invasion front are bolder and more aggressive than those from an older, more established areas on the same water body.
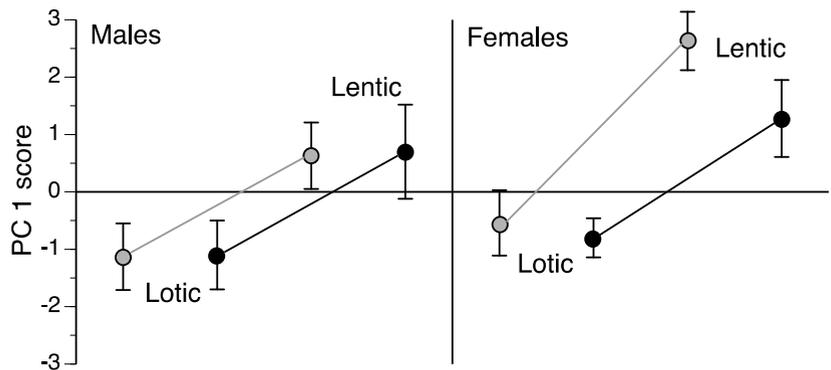
One possible reason why native pumpkinseed might be bolder than those from invasive populations could relate to the presence of a strong congeneric competitor in the native range. Much of the native pumpkinseed range overlaps with that of the bluegill (Scott and Crossman 1973), and the two congeners compete strongly for prey resources (Keast 1978;

Osenberg et al. 1988). The bluegill is rare or totally absent from Europe, but co-occurs in many North American waterbodies where the pumpkinseed is found (Fox 1994; Fox and Copp 2014), including the Rice Lake and Otonabee River sites used in our study. Experiments with the two species have demonstrated that the pumpkinseed responds morphologically to the presence of bluegill (Yavno et al. 2014), and that pumpkinseed from waterbodies where bluegill co-occur grow faster than those from waterbodies where bluegill are absent, an example of countergradient variation in response to interspecific competition between the two species (Arendt and Wilson 1999). While there are no studies relating interspecific competition to boldness in pumpkinseed, a study of interspecific interactions between threespine and ninespine stickleback (*Gasterosteus aculeatus* and *Pungitius pungitius* Linnaeus, 1758) demonstrated that when heterospecific pairs competed for prey, bolder individuals consumed a greater share of the resources (Webster et al. 2009). If the same response is

Table 3. Model selection results from an analysis of the effects of waterbody type (wb), sex and body length (TL) on (A) time to emergence and (B) indicators of dispersal tendency as assessed with PCA (PC1 axis score) in pumpkinseed from two native and two invasive populations in behavioural trials. Candidate models are ranked by AICc); models displayed are those classified as strong ($\Delta i \leq 2$) and moderate ($4 \leq \Delta i \leq 2$). Parameters and abbreviations are provided in Table 1.

Model terms	K	AICc	RL	Δ_i	w_i	Prob
A. Time to emergence ($\log_e$-transformed)						
wb, sex	4	192.25	1.0	0	0.25	0.035
wb, sex, TL, sex*TL	6	193.20	0.62	0.96	0.15	0.037
wb, sex, wb*sex	5	193.35	0.58	1.10	0.14	0.049
wb	3	194.24	0.37	2.00	0.09	0.10
wb, sex, TL	5	194.39	0.34	2.15	0.09	0.077
sex	3	195.10	0.24	2.85	0.06	0.173
wb, sex, TL, sex*TL, TL*wb	7	195.17	0.23	2.93	0.06	0.060
wb, sex, TL, wb*sex	6	195.64	0.18	3.40	0.05	0.095
B. Dispersal tendency (PC1)						
wb, sex	4	265.87	1.0	0	0.26	< 0.001
wb	3	266.19	0.85	0.32	0.22	< 0.001
wb, sex, wb*sex	5	267.70	0.40	1.83	0.10	0.0004
wb, sex, TL	5	268.18	0.31	2.31	0.08	0.0005
wb, TL	4	268.34	0.29	2.47	0.08	0.0004

Figure 3. PC1 axis scores from Figure 2, re-oriented to illustrate the relationship of dispersal tendency with waterbody type. Native and invasive pumpkinseed population means (±1 SE) shown with black and grey circles, respectively. Lentic water bodies are Rice Lake (Canada) and Susqueda Reservoir (Spain); lotic water bodies are Otonabee River (Canada) and Ter River (Spain).

true of pumpkinseed that co-occur with bluegill, it would explain why the native pumpkinseed used in our experiment were bolder than the invasive pumpkinseed from Spain.

Another possible reason why native pumpkinseed might be bolder than those from invasive populations could be that there is a greater predation risk in the native environment. Pumpkinseed in the Otonabee River and Rice Lake are exposed to three obligate piscivores: largemouth bass (*Micropterus salmoides*), muskellunge (*Esox masquinongy*) and walleye (*Stizostedion vitreum*). While there are introduced largemouth bass in the Spanish waterbodies, along with congeneric equivalents to muskellunge and walleye, it has been argued that European pumpkinseed are at lower risk of predation because the dominance of easier to consume cyprinid species in European waterbodies makes introduced pumpkinseed a less desirable target for these piscivores (Fox and Copp 2014 and references therein). In experiments

with poeciliids from eight native Panama populations varying in level of predation pressure, Brown et al. (2005) found that individuals from high-predation areas were bolder than those from low-predation areas. The researchers explained their findings as high-predation fish responding to the need to carry out critical activities under constant threat, and argued that there should be strong selection pressure favouring boldness under these circumstances.

Regardless of the reasons for the difference in boldness, the results do not imply that only bold phenotypes are found in native populations, or that only shy individuals are found in invasive populations. Shy-bold is part of a well-studied personality syndrome, and has been extensively explored in fishes (reviewed in Conrad et al. 2011). The high within-population variability in time to emergence (Figure 1) has been found in other studies of boldness and exploratory behaviour (e.g., Gifford et al. 2014; Myles-Gonzalez et al. 2015), and suggests

that there were a mixture of shy, intermediate, and bold phenotypes in each of the four populations.

The absence of greater dispersal tendency in the invasive Spanish populations is not, in itself, evidence that spatial sorting does not occur in invading pumpkinseed populations. However it does suggest that if spatial sorting did occur during this active invasion, selection for dispersal ability was not maintained in this species. Thorlacius et al. (2015) found evidence for personality-dependent dispersal in newly-established invasive round goby populations, but not in populations established for > 20 years. They suggested that the adaptive value of a rapid dispersal phenotype declines over time as selection strengthens for other traits favoured by increasing population densities and thus, higher levels of interspecific competition. Similarly, the behavioral trait of straight-line movement patterns in an invasive cane toad population in Australia weakened over a 10 year period, suggesting a loss of this dispersal enhancing trait during the course of this invasion (Brown et al. 2014). The adaptive value of rapid dispersal phenotypes may decline with time post-invasion as selection strengthens for other life-history traits that are favoured under increasing population densities (i.e. trade-offs: Chuang and Peterson 2016; Perkins et al. 2016). Given that invasive pumpkinseed have been present in Spain for over 100 years, it is possible that greater boldness or dispersal tendencies that were present when the species first invaded the region have been lost since the species became established. If time since establishment has an effect on selection pressure for dispersal phenotypes, this could potentially explain the difference in dispersal ability between the two Spanish populations in our study (Figure 2). Susqueda Reservoir is 30 km upstream of the part of the Ter River we used in our study. Given the likely downstream origin of the initial invasion of pumpkinseed into this system (from Lake Banyoles; see García-Berthou and Moreno-Amich 2000; Elvira and Almodóvar 2001), one might expect greater selection for high dispersal phenotypes in Susqueda Reservoir. Alternatively, the reasons for greater dispersal potential in Susqueda than in the Ter River could be due to differences in pumpkinseed density or habitat type. Unfortunately, no population assessment data are available to enable us to compare pumpkinseed density in these two systems.

Our finding that boldness and dispersal tendency are sex-linked is not surprising, as several other studies on fishes have shown male-female differences in at least one of these parameters. For example, Congdon (1994) showed that female mosquitofish dispersed farther than males in low velocity trials;

however another experiment with mosquitofish showed that males dispersed farther than females (Cote et al. 2010b). Greater dispersal distance in round goby males was also found by Marentette et al. (2011) in both lab trials and mark-recapture assessments; however Brownscombe and Fox (2012) found a female biased sex-ratio at the leading edge of an actively expanding round goby population. These inconsistent sex-linked patterns in dispersal tendency suggest that sex-biased dispersal in fish may be strongly context dependent.

In our study, consistent differences in dispersal tendency between study populations from lentic and lotic water bodies suggest that habitat of origin may influence selection for high dispersal phenotypes. Fish living in fluvial environments could have a lower dispersal rate due to more strenuous hydro-dynamic conditions (Ryder and Pesendorfer 1989). The gibbose body form of pumpkinseed is better suited to complex manoeuvring in lake environments, rather than in a river current (Webb 1998), and lake and river pumpkinseed show differences in morphology specific to locomotion (Brinsmead and Fox 2002). Smaller fin size may reduce drag in a current, and thus may be favoured in river fish where movement requires more exertion (Brinsmead and Fox 2002). However, individuals with smaller fins may require more energy than those with larger fins for upstream migration and thus, even pumpkinseed that are adapted to foraging in a fluvial environment may not be as disposed to dispersal as those living in lacustrine water bodies. The difference between lentic and lotic habitat is one of several possible reasons for the pattern in dispersal potential found in our study. At minimum, additional behavioural research comparing a larger number of lentic and lotic populations at different invasion stages would be required to determine if dispersal potential is influenced by the flow characteristics of the water body of origin.

Pumpkinseed have demonstrated variable success in establishing populations throughout Europe, and are only considered invasive in certain regions, mainly in the southern range (Copp and Fox 2007). This variability in invasion success may be caused by different selective pressures in these novel environments, or it may be a result of different adaptive responses and flexibility of behaviour of the individuals within the established populations (Sol et al. 2002; Dingemanse et al. 2007). Spatial sorting theory predicts that behavioural traits that promote rapid dispersal may be selected for during invasion, suggesting that high dispersal traits are likely to be more common in invasive populations. In contrast to these predictions, our study demonstrated that the progeny of native Canadian pumpkinseed were

bolder than those of invasive Spanish populations reared under a common environment, and that dispersal tendency showed no consistent native-invasive differences. The long period of time since the establishment of our two invasive populations in the wild may have allowed density-dependent or other trade-off mechanisms to reduce the occurrence of high-dispersal phenotypes overtime within these invasive populations. Our study identified greater dispersal potential in individuals originating from lentic water bodies than those from originating from rivers, regardless of region of origin. As such, future studies comparing dispersal traits among invasive and non-invasive fish populations should consider the effect of habitat of origin on comparisons, even when individuals are reared under a common environment.

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