Biological Invasions

Invasion of pumpkinseed Lepomis gibbosus is facilitated by phenotypic plasticity across its invasion gradient

--Manuscript Draft--

Manuscript Number:	BINV-D-20-00416R2
Full Title:	Invasion of pumpkinseed Lepomis gibbosus is facilitated by phenotypic plasticity across its invasion gradient
Article Type:	Research paper
Keywords:	Life history traits, establishment, invasion stage, stream, Lepomis gibbosus
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Funding Information:	Türkiye Bilimsel ve Teknolojik Araştirma Kurumu (115Y660) — Prof. Ali Serhan Tarkan
Abstract:	As a non-native species invades a new region, individuals at the range front are often characterised by differences in their traits and behaviours versus individuals in the established 'core' area. Here, differences in life history traits of invading pumpkinseed Lepomis gibbosus were assessed across a fine spatial scale in the Sarıçay Stream, Turkey, with seven locations sampled between their core area and the invasion front. Following genetic analyses that suggested the pumpkinseed all originated from the same founding population, life history trait analyses revealed pronounced differences between the sites at the core and front that were independent of environmental factors. Pumpkinseed in sites towards the invasion front were of relatively low abundance and displayed relatively high reproductive investment, with heavier gonads, higher fecundity and smaller eggs. They also had faster growth rates than pumpkinseed in sites towards the core of the invasion. The traits displayed by pumpkinseed at sites in the mid-range were intermediate between the extremes displayed by fish at core and frontal sites. These results suggested high plasticity in life history traits of these pumpkinseed across this fine-scale invasion gradient and was considered to be most likely due to responses to low intra-specific competition at the invasion front that had shifted selective pressures towards higher investment in somatic growth and reproduction.
Response to Reviewers:	COMMENTS TO THE AUTHOR: Reviewer #1: The manuscript is substantially improved after revisions. The writing is

clearer such that the manuscript is easy to follow. Suggestions were incorporated and the study framed well within current invasion biology research. The majority of my comments are related to wording in the Results section: I still had to spend some time reviewing the Tables to understand your interpretation. I have offered some alternate wording in the hope that it will help with final revisions, and look forward to seeing your paper published.

p. 8, Line 177 Suggest wording "in combination with fish rejected due to regenerated scales, …"

Response 1.1: Done (line 176)

p. 12 Line 297 Suggest wording "Both mean TL and TW were highest in the most…"

Response 1.2: Fixed (line 296)

p. 12 Lines 298-299 Suggest wording "Minimum TL and TW occurred at two invasion front locations (VI and AV; Table 2)."

Response 1.3: Done (line 297-298)

p. 13 Line 306 Use lowercase location x sex as this is the convention used elsewhere in the manuscript

Response 1.4: Fixed (line 305)

p. 13 Lines 323-325 Wording is confusing. Looking at Table 4, my interpretation is "Age and length at maturity decreased with distance from the core (Table 4)."

Response 1.5: Corrected (line 324-325)

p.14 Lines 331-334 Suggest wording "Mean egg diameters (in mm) were significantly larger in core locations (UP = 1.10 …) than in intermediate (AK = …) and front sites (BR =…) (Table 5).

Response 1.6: Fixed (line 331-334)

p. 15 Line 356 Change rations to ratios

Response 1.7: Done (line 356)

p. 28 Lines 11-12 There's a typo in the mean TLs for the front fish - the values are too small.

Response 1.8: Corrected – thank you!

Reviewer #2: With great interest I received for a second round the revision the manuscript "Invasion of pumpkinseed Lepomis gibbosus is facilitated by phenotypic plasticity across its invasion gradient" by Tarkan et al. The manuscript has been greatly improved its merit and deserves acceptance but with some very minor revisions. The authors responded to all comments and left no pending doubts. Also, I will suggest another proof reading by an english speaker. English has been improved well but still a second reading/correction is required. Below you can find some minor comments:

MINOR COMMENTS

Materials and Methods
Lines 254, 264: The authors use "PERANOVA" and "PERMANOVA" to refer to the same analysis. I would advice to change to PERMANOVA and applied it elsewhere in the text

Response 2.1: We used ‘permutational univariate analysis of variance’ so its correct abbreviation should be ‘PERANOVA’, whereas PERMANOVA is the name of the statistical adding tool for PRIMER. Hence, we retained the current usage.

RESULTS
Line 297 change WAS to WERE

Response 2.2: Changed (line 296)

LINE 298 change MINIMUMS to MINIMUM and MEAN to MEANS

Response 2.3: Fixed (line 297-298)

LINE 322 change WAS to WERE

Response 2.4: Done (line 322)

DISCUSSION
Line 416 change to COULD HAVE BEEN RELATED

Response 2.5: Done (line 417)

REFERENCES
Some of the references do not follow the general format
Britton et al 2010
Murphy and Willis 1996
Nolan and Britton 2019

Response 2.6: All fixed

FIGURE CAPTION
In Fig, 2, 3 and 4 change to POPULATION IS GIVEN...

Response 2.7: All fixed

TABLE 4 needs a better alignment

Response 2.8: Whole alignment has been revised

FIGURES 2 to 5
I would advice the authors to plot the figures in ggplot for better visualisation but only if they know how to use the ggplot2 package

Response 2.9: Thank you for the advice. We prepared all these figures in R but not with ggplot. As per requested, we have plotted these figures with ggplot however it did not make much difference in terms of a better visualisation therefore we retained the current figures.

Invasion of pumpkinseed *Lepomis gibbosus* is facilitated by phenotypic plasticity across its invasion gradient

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Abstract

As a non-native species invades a new region, individuals at the range front are often characterised by differences in their traits and behaviours versus individuals in the established ‘core’ area. Here, differences in life history traits of invading pumpkinseed *Lepomis gibbosus* were assessed across a fine spatial scale in the Sarıçay Stream, Turkey, with seven locations sampled between their core area and the invasion front. Following genetic analyses that suggested the pumpkinseed all originated from the same founding population, life history trait analyses revealed pronounced differences between the sites at the core and front that were independent of environmental factors. Pumpkinseed in sites towards the invasion front were of relatively low abundance and displayed relatively high reproductive investment, with heavier gonads, higher fecundity and smaller eggs. They also had faster growth rates than pumpkinseed in sites towards the core of the invasion. The traits displayed by pumpkinseed at sites in the mid-range were intermediate between the extremes displayed by fish at core and frontal sites. These results suggested high plasticity in life history traits of these pumpkinseed across this fine-scale invasion gradient and was considered to be most likely due to responses to low intra-specific competition at the invasion front that had shifted selective pressures towards higher investment in somatic growth and reproduction.

Key-words: Life history traits, establishment, invasion stage, stream, *Lepomis gibbosus*

Introduction

For a non-native species to become invasive requires its introduction into a region outside of its natural range, followed by the establishment of sustainable populations that then disperse naturally (Kolar and Lodge 2002). Thus, understanding the probability of invasion success requires data on their population dynamics at each invasion stage (Williamson 1999), especially as different biological characteristics are often required for completing the different stages (Ribeiro et al. 2008). As an invading species spreads, densities at the invasion front tend to be relatively low compared with those in established core areas (Masson et al. 2016; Paton et al. 2019). Differences between these areas are then often also apparent in traits, such as body size and fecundity, with these differences having been recorded in a broad range of taxa (e.g., amphibians, decapods and fishes; Hudina et al. 2012; Perkins et al. 2013; Masson et al. 2016).

These differences in trait expression between the core area and invasion front are driven by phenotypic plasticity, natural selection and spatial sorting (Messager and Olden 2019). Phenotypic plasticity is strongly evident in many successful invaders as it enables adaptation to new environments more rapidly than natural selection alone (Chevin et al. 2010). Life history theory is well suited to predict differences in the traits of invaders across their range (e.g., changes in growth, reproductive allocation, size at maturity) (Bøhn et al. 2004; Fox et al. 2007), with strategies varying from ‘opportunistic’ at the front (earlier maturity, smaller size at maturity, and higher reproductive allocation) to ‘equilibrium’ in core areas (later maturity, larger size at maturity, and relatively low reproductive allocation) (Winemiller and Rose 1992). Selection pressures at the front drive the expression of traits that facilitate the faster dispersal of individuals, accelerating the invasion (Chuang and

Peterson 2016; Weiss-Lehman et al. 2017; Dominguez Almela et al. 2020), with low rates of intra-specific competitive interactions also shifting selective pressures towards faster rates of somatic growth (Brownscombe and Fox 2012) and reproduction (Grabowska et al. 2021), with the opposite usually evident in the core area (Ribeiro et al. 2008). There are then areas within the invasion range that lie between these extremes, where the species is at intermediate stages of invasion. Here, it is likely that fish abundances will be increasing exponentially, resulting in life history trait expression differing from both the core and front (Moyle and Light 1996).

The North American freshwater sunfish *Lepomis gibbosus* (‘pumpkinseed’) provides a strong model species for identifying whether substantial shifts in life history traits can facilitate the establishment of non-native species in novel environments. Following its introduction into Europe in the late 19th century (Maes 1898), pumpkinseed has established invasive populations in at least 28 Eurasian countries (Copp and Fox 2007) and is considered to be one of the most introduced aquatic species worldwide (García-Berthou et al. 2005). Its high establishment success has been associated with its tolerance to harsh environmental conditions, including high temperatures and hypoxia (Farwell et al. 2007). It generally displays an ‘equilibrium’ life-history strategy under the classification system of Winemiller and Rose (1992), although it does demonstrate some ‘opportunistic’ traits, such as nest guarding (Crivelli and Mestre 1988). Its inherently high phenotypic plasticity ensures they are a robust model species for testing fine scale spatio-temporal effects on plasticity and in relation to invasion success (Cucherousset et al. 2009).

There have been numerous studies on the biology and ecology of pumpkinseed in their native and non-native ranges (e.g., Copp and Fox 2007), including intercontinental comparisons of life history traits (e.g., Fox and Copp 2014), and finer scale comparisons between recent and long-established non-native pumpkinseed (e.g., Konečná et al. 2015). These studies have sought to understand the factors facilitating the establishment of populations, including how the variability in life history traits expression contributed to their high invasiveness and impacts on native species (Garcia-Berthou and Moreno-Amich 2000). However, there is still a substantial knowledge gap on how the expression of these traits varies across invasion gradients within river basins, which is compounded by a lack of information on how differences in trait expression across larger spatial areas are affected by differences in their source populations and the specific environmental factors in the invaded area.

Here, the aim was to determine differences in pumpkinseed life history traits across an invasion gradient in a relatively small river basin in Turkey into which the species had been initially introduced in 1990. Following determination of the genetic variation in the invasive pumpkinseed across the basin, spatial differences in the expression of their life history traits were measured, including their growth rates, condition factors and reproductive traits. These data were used to test the prediction that individuals would have contrasting life history trait expression between their core and front areas (such as higher reproductive investment at the front) that are consistent with opportunistic responses to novel environments.

Material and Methods

Study area

The study was conducted in the Sarıçay Stream, which flows through the Muğla-Milas region in south-west Anatolia and into the Gulf of Güllük (Fig. 1). The stream is approximately 70 km long with a mean width of ≈10 m and has a range of habitat and substrate types (predominantly sand, gravel, mud+stone, and rock). The fish assemblage comprises of some endemic species (Smyrna chub *Petroleuciscus ninae*, Aegean chub *Squalius fellowesii*, loach *Oxynoemacheilus* sp.), as well as non-native species (gibel carp *Carassius gibelio*, common carp *Cyprinus carpio*, eastern mosquitofish *Gambusia holbrooki*, topmouth gudgeon *Pseudorasbora parva*) (Top et al. 2016). Pumpkinseed were introduced into the Geyik reservoir (Fig. 1) through an accidental contaminant of common carp *Cyprinus carpio* stockings for fisheries purposes. Pumpkinseed have since become the dominant fish species (numerically and by biomass) in the reservoir (Top et al. 2012, 2016).

Sampling design and laboratory processing

Pumpkinseed individuals were sampled from seven sites along their invasion gradient in the Sarıçay stream. The furthest upstream sampling site ('UP') was located 5 km above the site of initial pumpkinseed introduction, the Geyik reservoir, from which samples were also collected ('GE') (Fig. 1). The next two sampling sites, Akgedik reservoir ('AK') and Sandpit ('SA'), were also lentic environments located 10.1 and 20.6 rkm downstream of GE (Fig. 1). The last three sampling sites were lotic habitats located 23.6 rkm (Bridge 'BR'), 28.2 rkm (Village, 'VI') and 34.6 rkm (Avşar 'AV') downstream from GE (Fig. 1).

Sampling was conducted in April 2017 and 2018 to coincide with the pre-reproduction period of mature fish in the stream (Top 2012). Fish were captured using a portable electrofishing device in the lotic habitats without stop nets, and electrofishing from a boat in lentic habitats. In all sites, fishing was continuous within 10-minute blocks to enable a catch per unit effort (CPUE) metric to be used as a measure of relative fish abundance, expressed as the number of pumpkinseed captured per 10 minutes fishing (Murphy and

Willis 1996; Copp et al. 2005. After capture, fish were euthanized (anaesthetic overdose; 2-phenoxyethanol) and transported to the laboratory on an ice water slurry. Muscle tissue samples were dissected and preserved in 95% ethanol for molecular analysis and kept at -20°C until DNA extraction. Just prior to the fish sampling, five environmental variables (water temperature, conductivity, dissolved oxygen, salinity and pH) were measured at each sampling location using a YSI 556 MPS probe (Table 1).

In the laboratory, fish were measured (total length (TL); nearest mm) and weighed (total weight (TW) and eviscerated weight (EW); to 0.1g). The latter was used to avoid bias from the weight of gonads in providing a true representation of the Gonadosomatic Index (GSI). Scale samples were taken for ageing from the area between lateral line and dorsal fin. Gonads were removed for sex determination and weighed wet (GW; to 0.001 g). Females with ovaries containing yolked eggs were classified as mature, and those with non-yolked or indistinguishable eggs were classified as immature. To enable fecundity estimates, sub-samples of weighed ovaries were taken from the anterior, middle, and posterior portions of each lobe and then fixed in 3.6 % buffered formaldehyde. Fecundity and egg diameters were determined from the sub-samples using a stereomicroscope. The diameters of the first 50 oocytes were measured using a stereomicroscope.

Ageing of all pumpkinseed specimens was completed by counting true annuli (after Vilizzi et al. 2013). Scale impressions on acetate strips (10 scales for each specimen) were read on a micro-projector (magnification: ×48). Two independent interpreters estimated the ages without prior biological knowledge of the fish; when age estimates differed, a final evaluation was made. If the age disagreement remained, the sample was rejected. In combination with fish rejected due to regenerated scales, 5.2 % of all sampled fish were excluded from subsequent analyses. Following age estimation, one scale per fish was measured for its total scale radius and the distances from the scale focus to each annulus for back-calculation of length at age (Bagenal and Tesch 1978).

Total DNA Extraction, PCR Amplification and Sequencing

DNA isolations were conducted in compliance with the manufacturers protocol using Qiagen DNeasy Blood & Tissue Kit, with minor adjustments. A Colibri spectrophotometer measured the quality and quantity of extracted DNA and samples were diluted to a final concentration of 50 ng/µl. PCR reactions were performed using fish-specific universal primers; FishF1: TCAACCAACCACAAAGACATTGGCAC and FishR1: TAGACTTCTGGGTGGCCAAAGAATCA (Ward et al. 2005), targeting a 650 base pair long fragment of mitochondrial cytochrome c oxidase I (COI) gene. PCR amplifications were performed using 8 ml of 5x FIREPol Master Mix Ready to Load (12.5 mM MgCl2) (Solis BioDyne, Estonia), 1 ml of each primordial (F, R), 2 ml of DNA template and 28 ml of ultrapure water with a total 40 ml reaction volume. Thermal cycler conditions were set at 2 minutes at 95°C for an initial denaturation, 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 60 s, with a final extension of 72 °C for 10 min. PCR yields were visualized on 2 % agarose gels stained with ethidium bromide and purified using the Wizard SV Genomic DNA Purification Kit (Promega, USA). PCR yields were subjected to bidirectional sequencing using the SeqStudio platform. Sequencing was carried out using the same primers designed for the PCR. Four sequences were removed from the dataset as they included too many unidentified nucleotides. The remaining 206 sequences were trimmed to 558 base pairs for homogenizing all the sequences, with codon usage analyses revealing there were no stop codons.

Data analyses

The obtained genetic sequences were aligned using MEGA X and subsequently edited manually, and then compared to existing reference data from GENBANK. Nucleotide composition, codon usage and genetic distances were calculated using Jukes Cantor model, as it was the most suitable substitution model of our dataset according to BIC scores (Bayesian Information Criterion) and AICc value (Akaike Information Criterion, corrected).

Regarding life history traits, somatic growth rates were assessed using lengths-at-age (LaA), which were back-calculated using the Fraser-Lee equation (Francis 1990): $L_t = c + (\mathrm{TL_c} - c)(S_\mathrm{t} / R)$, where L_t is TL when annulus t was formed, $\mathrm{TL_c}$ is TL at capture, S_t is the distance from scale focus to the annulus t, R is scale radius, and c is the intercept on the length axis from the linear relationship of TL versus scale radius ($\mathrm{TL} = 9.704 \times R + 22.703$, $r^2 = 0.741$, $P < 0.001$). The body-scale intercept ($c = 22.7$ mm) was used a fixed value for each sampled site to reduce bias due to differences in the size distribution. To compare the relative growth performance of pumpkinseed across the sampling locations, a relative growth index (GI) was calculated (Hickley and Dexter 1979). After using the Walford (1946) method to obtain a straight line by plotting TL at mean age n of all specimens against TL at age $n + 1$, the GI was calculated as the mean value of the growth in each age class of pumpkinseed for a certain location relative to TLs at age, using the following formulae:

$$l_n = L_\infty (1 - k^n)$$

where $L_\infty = l_t \div (1 - k)$ and l_t is the intercept, l_n is the TL at age n and k is the slope of the Walford plot (after Hickley and Dexter 1979), and:

$$GI = \Sigma TL_{oi} \div TL_{ri} \times 100$$

where TL_{oi} and TL_{ri} are the observed and reference mean total lengths respectively of the pumpkinseed at age i. Only the first three age classes (i.e., comparable ages) of the fish from each site were used in the calculations, because high levels of variability in the estimated length at older ages in the reviewed dataset were detected, suggesting some potential issues of ageing accuracy and precision (Beamish and McFarlane 1983).

Variation in body condition was measured using the relative body condition (RC) of Le Cren (1951) according to W/W', where W is the actual weight of an individual and W' is the expected weight from the WL relationship. RC values >1 or <1 indicate that individuals are in better/worse condition than the other fish at the same TL range. The index requires populations to be sampled at the same time of year (Knaepkens et al. 2002) and correspondingly, samples were collected from all sampling locations on the same day. Mean age at maturity was calculated from the percentage of mature males and females at each age-class using the formula proposed by DeMaster (1978):

$$\alpha = \sum_{x=0}^{w} (x)\left[f(x) - f(x-1)\right]$$

where α is the mean age of maturity (AaM), x is the age in years, $f(x)$ is the proportion of fish mature at age x, and w is the maximum age in the sample. A modified version of this formula (10 mm TL intervals in place of age-classes; Trippel and Harvey 1987) was used to calculate mean TL at maturity (LaM). Absolute fecundity (AF) was estimated gravimetrically as: $AF = W_G \times D$, where W_G is the weight of ovary, and D is the density of number of mature oocytes per g of ovarian tissue, while relative fecundity (RF = number of oocytes g^{-1} of female) was calculated as RF = AF/TW (Bagenal 1978). The gonado-somatic index (GSI) was calculated as: GSI = (GW/(EW–GW) x 100 (Wootton 1990).

Statistical analyses

Deviations from 1:1 in the sex ratio between males and females were tested using chi-square (χ^2) tests for equality. Permutational univariate analysis of variance (PERANOVA) was used to test for significance differences in back-calculated LaA, mean CPUE, TL, TW, GSI, AF, RF, and RC of pumpkinseed between the sampled pumpkinseed and years. CPUE (with location) was based on a one fixed-factor design, whereas a two-way factorial design for TL and TW (with location and sex), GSI, AF, RF, and RC (with location and TL), and a three-factor design for LaA (with age, location and sex) were used, all fixed and crossed. In each case, data were normalized according to Clarke and Gorley (2006). Following normalisation of the data, an Euclidean distance measure produced a distance matrix that was subjected to 9999 permutations of the raw data. Statistically significant effects for the main and interaction effects were tested and it was followed by *a posteriori* pairwise comparisons ($\alpha = 0.05$). Statistical analyses were carried out in PERMANOVA v1.0.1 for PRIMER v6 (Anderson et al. 2008).

Results

Genetic distances between the sampling sites

Analysis of the nucleotide composition revealed that the mean frequencies for the complete data set were 22.1% for A, 30.8% for C, 17.4% for G and 29.7% for T. Nucleotide pair frequency analysis of the complete data set resulted in 537 conserved sites out of 558 (96.24%) base pairs, and 21 variable sites out of 558 (3.76%) base pairs, where parsimony information sites were only 5 out of 558 (0.90%). The Jukes Cantor model was used to infer genetic distances in terms of the number of base substitutions per site, with mean pairwise genetic distance estimated as 0.0006 (S.E. 0.0002) across all of pumpkinseed sequences. The highest genetic distance between two specimens was 0.014 (1.4%) and all the genetic distances were found to be within intraspecific genetic thresholds for freshwater fish species (up to 3%), indicating no indication of multiple introductions from different gene pools.

Sample sizes, CPUE and body size

Environmental variables were generally similar between sampling locations, with the exception of salinity and conductivity that increased slightly with distance downstream (i.e., towards the invasion front) and dissolved oxygen in two sampling stations with stagnant waters (Table 1). In total, 1499 pumpkinseed were collected from all locations, varying from 82 at invasion front (VI) to 406 fish at core of the invasion (GE). Since no significant between-years differences were found in mean TL and W of pumpkinseed from each location ($P^{\#} > 0.05$), they were combined. Fish lengths and weights varied between 23 and 128 mm and 0.16 and 46.01 g respectively (Table 2).

Significant differences in relative abundance (as CPUE) were apparent between the sampling locations ($F^{\#}_{2,84} = 22.36$, $P^{\#} = 0.001$), with values being significantly higher at the core site (GE) and decreasing with distance downstream towards the invasion front (Fig. 2). In two sampling sites (AK, BR), there were also significant differences in CPUE between sampling years ($t_s^{\#} > 4.33$, $P_s^{\#} < 0.009$) (Fig. 2). There were significant differences in mean TL and TW across the locations and between sex, but not for the location × sex interaction (Table 3). Both mean TL and TW were highest in the most upstream location (UP), followed by the intermediate and front of the invasion (SA and BR, respectively). Minimum TL and TW occurred at two invasion front locations (VI and AV; Table 2).

Condition and growth

As differences in pair-wise comparisons for each location between years were not significantly different ($t_s^\# < 0.981$, $P_s^\# > 0.05$) then the growth and condition data were combined across the two years of sampling for each site. There was significant variation in the relative condition of pumpkinseed between locations, but not between the sexes or the interaction of location × sex (Table 3). Fish relative condition was higher in the more frontal stream sites (BR, VI and AV) than in the lotic locations in the intermediate and core areas of the invasion. This was also the case for the most upstream site (UP) (Fig. 1).

Significant differences in LaA were found for age, location, and the interaction of location × age, but not for sex and the interaction of age × sex (Table 3). The greatest LaA values were recorded at site BR towards the invasion front and higher growth increments at all comparable ages were evident at the invasion front versus the core and intermediate locations, and between the fish at the intermediate versus core areas for all ages (except year 1). Additionally, the relative growth index revealed significant differences across the locations (Table 3), being highest in the downstream frontal stream locations and upstream location (UP) than in the core and intermediate lentic locations (Fig. 3). No significant differences in the relative GI were found between the two years of sampling ($t_s^\# < 0.314$, $P_s^\# > 0.05$).

Reproductive traits

The reproductive traits did not significantly vary between years ($t_s^\# < 0.981$, $P_s^\# > 0.05$). Sex ratios were skewed towards males in all locations, but were only significantly different from 1:1 in two lentic locations (AK: $\chi^2 = 4.84$, $P = 0.02$; SA: $\chi^2 = 6.09$, $P = 0.01$), and in a lotic location towards the invasion front (BR: $\chi^2 = 5.12$, $P = 0.02$) (Table 4). Age and length at maturity decreased with distance from the core and was lowest at the invasion front (Table

4). Conversely, GSI was significantly higher in the frontal locations than locations at the invasion core (Table 5; Fig. 4). Both absolute and relative fecundity were significantly correlated with length at all stages of invasion, although the regression coefficient *b* did not differ between sites (Fig. 5), with significant differences in absolute and relative fecundity, being higher at the front locations (Table 5). The effect of the interaction of location × TL on fecundity was not significant ($P_s^{\#} > 0.05$). Mean egg diameters (in mm) were significantly larger in core locations (UP = 1.10 ± 0.03, GE = 1.13 ± 0.05) than in intermediate (AK = 1.05 ± 0.04, SA= 1.06 ± 0.03) and front sites (BR = 1.03 ± 0.03, VI: 1.01 ± 0.01 and AV = 0.89 ± 0.01) (Table 5).

Discussion

The genetic data indicated that all of the invasive pumpkinseed in the Sarıçay stream basin originated from a single founding population. Despite this, there was considerable variation in the expression of pumpkinseed life history traits along their invasion gradient in the basin, with faster growth, smaller body sizes, higher condition, earlier maturity and greater reproductive investment (higher but smaller egg production) evident in fish at the invasion front versus those in the core and intermediate areas. This expression of highly opportunistic life-history traits by pumpkinseed at the invasion front is similar to the traits detected in low-density fish populations experiencing high resource availability (Graboswka et al. 2021). These traits have also been displayed in other introduced fishes during their range expansion phase, including in species as diverse as vendace *Coregonus albula* and round goby *Neogobius melanostomus* (Bøhn et al. 2004; Gutowsky and Fox 2012). In these studies, drivers of this trait expression at the front included relatively low intra-specific competition that shifts selective pressures towards faster growth (Brownscombe and Fox 2012) and higher rates of reproduction (Grabowska et al. 2021), with epigenetic mechanisms (phenomena that alter gene expression without changing DNA sequences) also contributing to phenotypic variation (Bossdorf et al. 2010; Liebl et al. 2013).

In general, the pumpkinseed at the core sites had sex ratios that did not differ significantly from 1:1. The sites where the sex ratios did deviate from equality were male dominated and comprised of two intermediate sites and a site at the front. These male dominated sites also had pumpkinseed with wider age distributions and/or relatively high lengths at age compared with other sites. The equal sex ratio in the core of their range is consistent with some other invasive pumpkinseed populations, including in Central Europe (Konečná et al. 2015), although the male dominance in the intermediate locations is a novel finding for the species. In round goby, males tend to dominate long-established populations, whereas females usually dominate recently established populations (Gutowsky and Fox 2012; Brandner et al. 2013). This has been explained by females being dominant at the invasion front due to high male mortality rates resulting from their higher energy investment in territorial defence and parental care (Brownscombe and Fox 2012). Although pumpkinseed and round goby share a similar reproductive strategy through parental care and male nest guarding, female dominance was not apparent in our pumpkinseed sites at the range front.

The spatial relationships detected in the pumpkinseed life history traits across their invasion gradient in the Sarıçay stream were largely consistent with the proposed negative relationship between juvenile growth and age at maturity of pumpkinseed (Copp and Fox 2007). This relationship suggests that the extent of pumpkinseed invasiveness is predictable from their age of maturity and somatic growth rates, with populations that mature earlier and have faster growth rates having elevated ‘invasiveness’ (Copp and Fox 2007). This well-defined life history theory, as applied to invasion biology, involves individuals in recently colonised areas being faster growing and investing more heavily in reproduction when compared with those in more established populations (Phillips 2009). Our results were consistent with this theory. Given the limited differences in water temperatures between the sites then these spatial differences in somatic growth rates were considered as temperature-independent. This is important, as water temperature has been suggested as an explanatory variable of faster growth in invasive pumpkinseed populations at larger spatial scales (Cucherousset et al. 2009). Indeed, differences in temperature, often expressed by latitude as a proxy variable, are also considered as an important driver of the growth rates of other invasive fish species across large spatial areas, including largemouth bass *Micropterus salmoides* (Britton et al. 2010) and pikeperch *Sander lucioperca* (Nolan and Britton 2019).

It was predicted that the pumpkinseed at sites at the invasion front of the Sarıçay stream would exhibit higher reproductive investment, and this was well supported by our results. This higher reproductive investment might simply be the result of varying intra-specific competition across the invasion gradient that increased the energy allocation to reproduction in the areas of lower competition pressure at the invasion front (e.g., Bøhn et al. 2004; Gutowsky and Fox 2012). Irrespective, elevated reproductive output in newly invaded areas, mainly through producing more offspring, is also associated with ‘colonizer syndrome’, which describes a strategy for further colonization of novel environments (Baker 1965). In the Sarıçay stream, it appeared that this was occurring at a relatively small spatial scale, which would be facilitating increased pumpkinseed reproduction success at the invasion front (Ling et al. 2008; Amundsen et al. 2012). However, increased fecundity at the front was in a trade-off with oocyte size, as egg size was reduced in sites where female fecundity was higher. This was considered to be due to the allocation of resources into early reproduction, where priority was given to increasing egg number rather than egg quality (as egg size). This trade-off between egg number and size has been demonstrated in other invasive fish across relatively small spatial areas, such as vendace (Bøhn et al. 2004). It could relate to a strategy in which pioneering individuals ensure they are able to maximise their reproduction success in areas of low abundance and/or that have an unpredictable environment (Roff 1992).

The study design involved the application of relatively high sampling effort to provide pumpkinseed samples from seven locations across the Sarıçay stream basin that represented a gradient of sites between the core and frontal areas. Despite this sampling effort, the physical modifications of the basin meant that the only locations that could be sampled in the intermediate area of the invasion gradient were lentic environments. Consequently, when comparing pumpkinseed traits between the intermediate areas and the invasion front, some caution is needed in that comparisons are being made between lentic and lotic populations. Thus, some of the differences detected in how the traits were being displayed between these sites could have been related more to the physical habitat differences rather than differences driven by the invasion gradient. For example, pumpkinseed populations in southern England tend to be more abundant in lentic than lotic environments, although this is exacerbated by their reproductive failure in lotic environments due to lower water temperatures and/or habitat constraints (Copp and Fox 2007). Differences in pumpkinseed abundances potentially then have important density-dependent effects on their life history traits, with more abundant populations likely to be slower growing and less fecund (Zięba et al. 2020). In addition, while our findings were consistent with previous research on pumpkinseed and other invasive species (Bøhn et al.

2004, Gutowsky and Fox 2012), it is acknowledged that this study involved only one invasive species in one river basin that primarily assessed their life history traits. Correspondingly, it is recommended that future intra-basin studies on invasive pumpkinseed develop greater complexity in their designs by examining life history traits in response to diet (Wolfram-Wais et al. 1999), survivorship of adults relative to juveniles (e.g., Bertschy and Fox 1999), disease emergence (Ercan et al. 2015), and effect of predators (Fox and Copp 2014). More importantly, the underlying mechanisms of these changes should be explored (e.g., relative contributions of plastic versus epigenetic components).

In summary, the results of this study revealed high phenotypic plasticity in the life history traits of invasive pumpkinseed that were associated with their stage of invasion and were not related to underlying genetic differences. Phenotypic shifts were observed mainly in higher reproductive investment and fecundity, and greater condition and growth, at the invasion front versus the core and intermediate areas. These strong and clear changes suggested that the life history traits consistently evolve during range expansion and are apparent even at relatively fine spatial scales. Understanding how these patterns in life history traits vary over time and space, and in relation to population abundance, is thus important for understanding their roles in determining the invasion probability of non-native fishes following introductions into new river basins.

Acknowledgements

This study was supported by The Scientific & Technological Research Council of Turkey (TÜBİTAK) (Project No: 115Y660).

Data Availability Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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Figure captions

Fig. 1. Location of the sampling sites in the new (Bridge – BR, Village – VI, Avşar – AV), old (Upstream – UP, Geyik – GE) and intermediate (Akgedik – AK, Sandpit – SA) invasion fronts of the populations of pumpkinseed.

Fig. 2. Catch per unit Effort (CPUE) of pumpkinseed along the invasion gradient. Distance of each population from the core (0) population is given in parenthesis (km). Upper panel: 2017, lower panel: 2018. See Table 1 for site abbreviations.

Fig. 3. Relative Growth Index (GI) of pumpkinseed along the invasion gradient. Distance of each population from the core (0) population is given in parenthesis (km). Upper panel: 2017, lower panel: 2018. See Table 1 for site abbreviations.

Fig. 4. Gonadosomatic Index (GSI) of pumpkinseed along the invasion gradient. Distance of each population from the core (0) population is given in parenthesis (km). Upper panel: 2017, lower panel: 2018. See Table 1 for site abbreviations.

Fig. 5. Fecundity-total length relationships of pumpkinseed populations from three representative invasion stages (Core – GE, Intermediate – SA, Front – AV).

Tables

Table 1. Latitude (Lat.), longitude (Lon.), altitude (Alt., m), mean temperature (T, ºC), pH, dissolved oxygen (DO, mg^{-1} and %), conductivity (EC, µS cm^{-1}) and salinity (S, ‰) of sampling points measured in April 2017 and 2018 along the invasion gradient in the Sarıçay stream.

Location	**Lat.**	**Lon.**	**Alt.**	**T**	**pH**	**DO**	**DO (%)**	**EC**	**S**
Upstream (UP)	37°25'55''	27°52'29''	487	12.5	7.6	9.4	101.8	189	0.11
Geyik (GE)	37°23'49''	27°52'57''	470	13.8	8.2	11.3	129.5	159	0.08
Akgedik (AK)	37°19'41''	27°49'07''	61	13.5	8.1	9.3	102.2	340	0.18
Sandpit (SA)	37°21'03''	27°44'58''	23	12.9	7.5	2.3	24.2	613	0.34
Bridge (BR)	37°20'37''	27°43'43''	24	13.4	8.1	9.5	102.4	435	0.23
Villiage (VI)	37°18'56''	27°41'49''	15	14.0	7.7	2.8	31.6	649	0.34
Avşar (AV)	37°17'03''	27°40'53''	8	14.2	8.0	10.2	112.2	3430	1.99

Table 2. Number of specimens (n), minimum (Min), maximum (Max), mean and standard deviation (SD) of total length (TL) and weight (W) of male (M) and female (F) *Lepomis gibbosus* populations from different stages of invasion in the Sarıçay stream basin. See Table 1 for abbreviations of the sampling points.

		TL				W			
Location	n	Min	Max	Mean	SD	Min	Max	Mean	SD
UP (F)	41	40	90	72.80	1.59	0.99	12.69	7.75	3.93
UP (M)	54	43	98	83.80	0.31	1.13	17.60	12.80	5.32
GE (F)	178	31	88	63.61	1.10	0.32	11.09	4.11	1.98
GE (M)	228	27	98	66.84	1.16	0.33	29.36	5.31	3.89
AK (F)	46	24	46	61.00	0.52	1.27	4.75	3.15	0.90
AK (M)	100	53	78	66.94	1.54	1.81	7.90	4.26	1.54
SA (F)	64	34	110	75.67	1.78	0.47	19.62	7.61	4.72
SA (M)	110	43	128	80.46	2.21	0.96	46.01	10.72	9.02
BR (F)	78	23	112	66.31	1.87	0.16	30.19	6.50	6.26
BR (M)	114	28	124	73.33	2.39	0.26	39.17	10.06	10.66
VI (F)	38	42	81	57.16	0.97	1.07	9.74	3.51	2.10
VI (M)	44	43	81	60.41	1.07	1.29	8.39	4.09	2.24
AV (F)	48	42	81	57.38	0.94	1.07	9.74	3.41	2.10
AV (M)	56	43	85	59.79	1.06	1.29	8.39	3.98	2.25

Table 3. PERANOVA results on the differences in growth parameters (TL - total length, W – weight, RC – Relative Condition, GI – Growth Index, and TL at age) by Location, Sex and Age. Statistically significant effects (α = 0.05) in bold.

	df	MS	*F*	*P*
TL				
Location	6	8.881	19.605	**0.0002**
Sex	1	4.659	16.897	**0.0243**
Location×Sex	6	0.606	0.649	0.5191
W				
Location	6	14.393	26.224	**0.0001**
Sex	1	5.242	14.491	**0.0155**
Location×Sex	6	1.566	1.775	0.1714
RC				
Location	6	12.676	15.401	**0.0001**
Sex	1	0.423	0.514	0.4701
Location×Sex	6	0.847	1.028	0.3592
GI				
Location	6	9.156	21.140	**0.0009**
TL at age				
Age	8	36.588	639.36	**0.0001**
Location	6	4.172	72.911	**0.0001**
Sex	1	0.219	3.828	0.0525
Age×Location	14	1.095	19.142	**0.0001**
Age×Sex	8	0.001	1.135	0.3399
Location×Sex	6	0.001	0.001	0.9575
Age×Location×Sex	14	0.001	0.771	0.6250

Table 4. Mean age at maturity (AaM, in years), mean total length (TL) at maturity (TLaM, in mm) and sex ratio of *Lepomis gibbosus* populations in different stages of invasion in the Sarıçay stream basin. M = males, F = females. See Table 1 for abbreviations of the sampling points.

Reproductive parameters	**UP**	**GE**	**AK**	**SA**	**BR**	**VI**	**AV**
AaM (M)	2.4	2.5	2.2	2.1	1.6	1.5	1.1
AaM (F)	2.5	2.7	2.4	2.2	1.7	1.7	1.2
TLaM (M)	50	51	48	47	47	44	45
TLaM (F)	53	55	52	50	50	47	47
Sex ratio (M:F)	1.29:1.00	1.28:1.00	2.17:1.00*	1.72:1.00*	1.59:1.00*	1.16:1.00	1.17:1.00

*Difference from 1:1 significant at $P < 0.05$ (chi-square equality test).

Table 5. PERANOVA results on the differences in reproductive parameters (AF – Absolute fecundity, RF – Relative fecundity, GSI – Gonadosomatic Index, ED – Egg diameter) by Location and Total length (TL). Statistically significant effects ($\alpha = 0.05$) in bold.

		df	MS	*F*	*P*
AF					
	Location	6	1.491	33.130	**0.0001**
	TL	46	1.495	69.559	**0.0001**
	Location×TL	22	0.001	2.629	0.0863
RF					
	Location	6	3.046	73.140	**0.0001**
	TL	46	1.266	24.637	**0.0001**
	Location×TL	22	0.001	1.730	0.1884
GSI					
	Location	6	8.445	9.950	**0.0009**
	TL	71	0.836	0.989	0.4764
	Location×TL	49	0.627	0.741	0.7752
ED					
	Location	6	18.457	420.83	**0.0001**
	TL	46	0.841	19.167	**0.0001**
	Location×TL	22	0.139	3.165	**0.0015**

Fig. 1

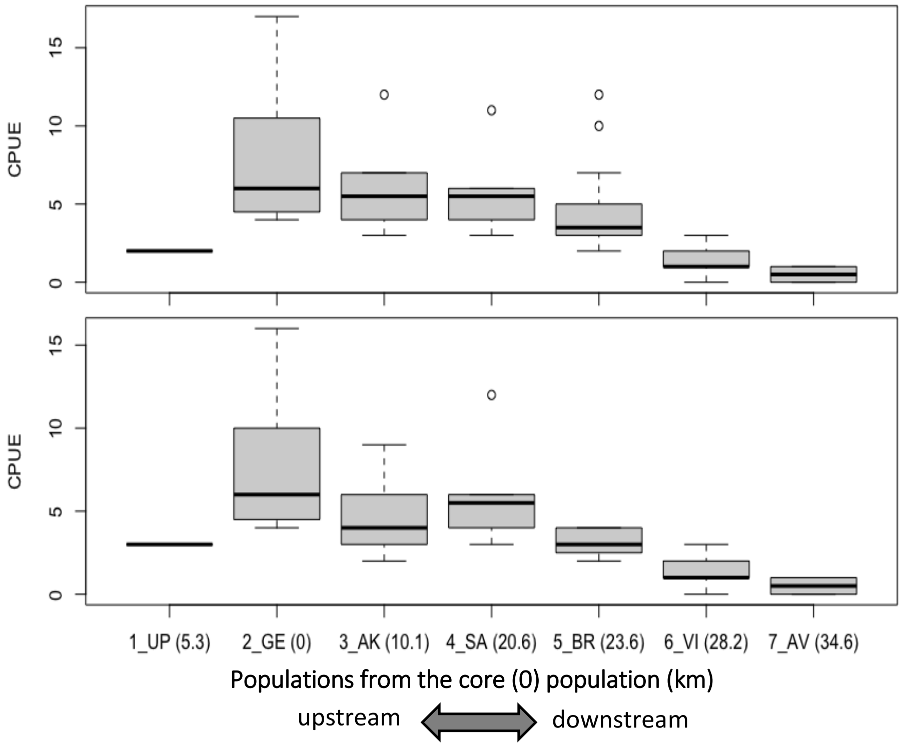
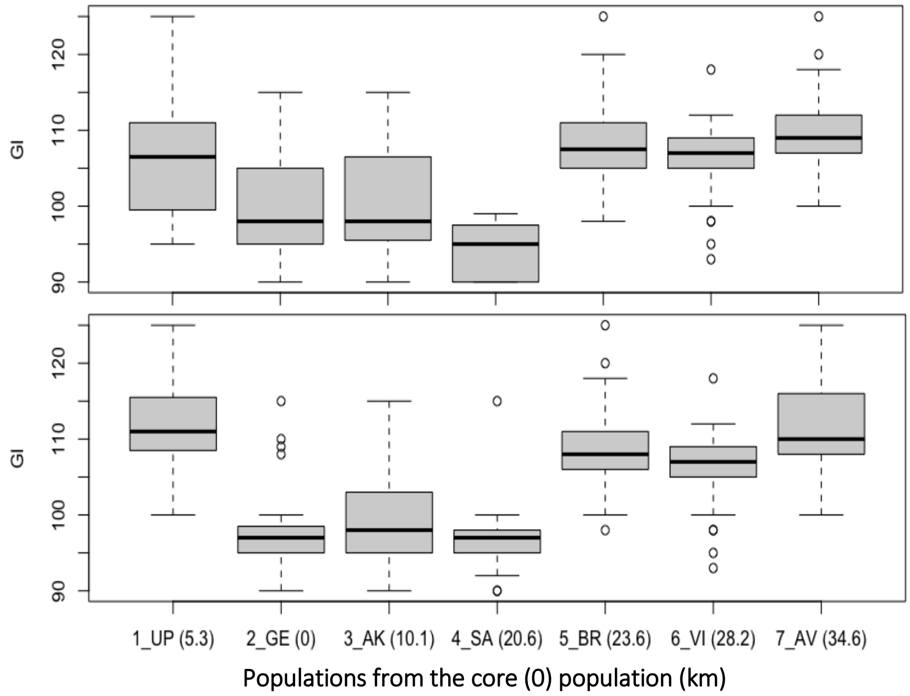

CPUE
0
5
10
15
CPUE
0
5
10
15
1_UP (5.3)
2_GE (0)
3_AK (10.1)
4_SA (20.6)
5_BR (23.6)
6_VI (28.2)
7_AV (34.6)
Populations from the core (0) population (km)
upstream
downstream

Fig. 2


GI
90
100
110
120
GI
90
100
110
120
1_UP (5.3)
2_GE (0)
3_AK (10.1)
4_SA (20.6)
5_BR (23.6)
6_VI (28.2)
7_AV (34.6)
Populations from the core (0) population (km)

upstream ⟷ downstream

Fig. 3

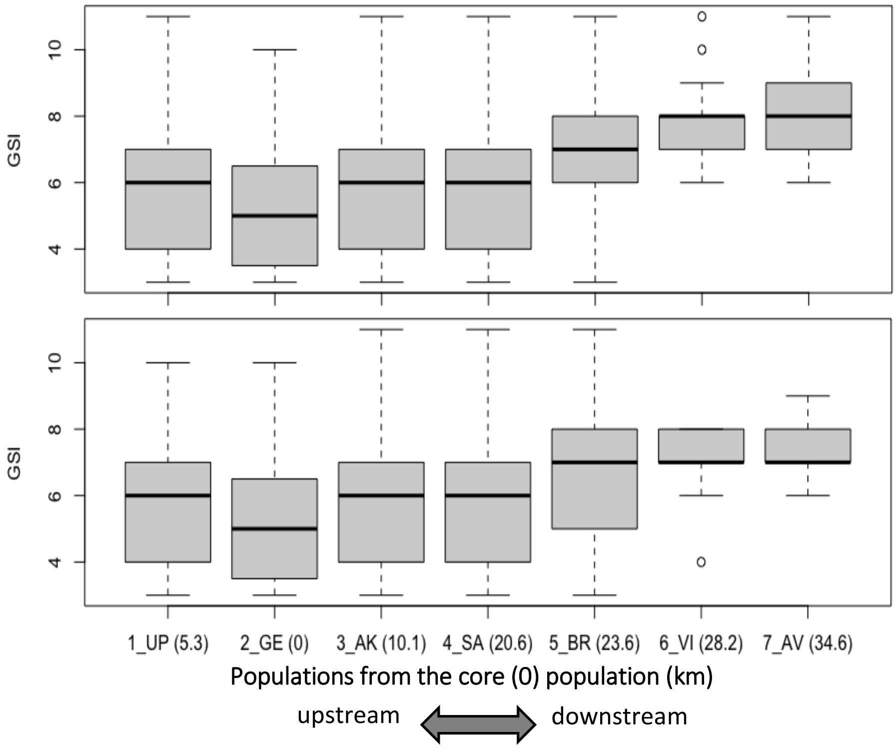


Fig. 4

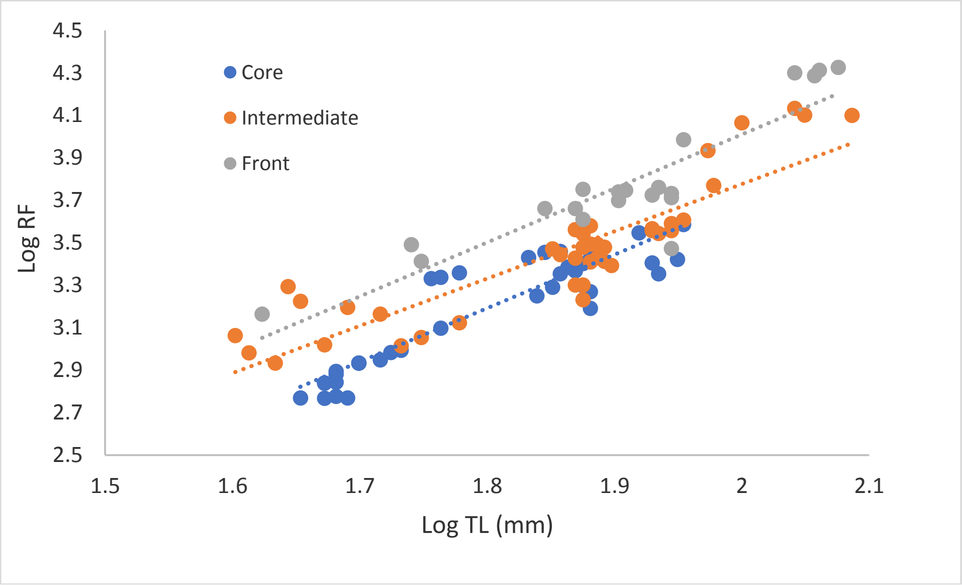

4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
Log RF
● Core
● Intermediate
● Front
1.5
1.6
1.7
1.8
1.9
2
2.1
Log TL (mm)

Fig. 5