Warming affects the feeding success of invader and native fish in Iberian streams

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Abstract

We experimentally tested the effects of anticipated warmer temperatures on the feeding success of invasive pumpkinseed and chameleon cichlid and the functionally similar Southern Iberian chub. Singly, prey captures by chub and the cichlid were constant but those by pumpkinseed were affected by warming. Moreover, at warmer temperatures chub captured much less prey in the presence of the cichlid and prey faster in the presence of pumpkinseed than with conspecifics. Warming effects are likely species dependent, and asymmetries in feeding success between species may be enhanced at warmer temperatures to the benefit of warm-water invaders.

Keywords: biological invasions; chameleon cichlid; climate change; pumpkinseed sunfish; southern Iberian chub.

1. Introduction

The implications of global warming for biological invasions in freshwater ecosystems remain poorly understood (Radinger and García Berthou 2020). Freshwater ecosystems are among the most threatened in the world, and invasive species are repeatedly implicated in biota declines (Reid et al. 2019 and references therein). Warming is expected to affect bioenergetics, physiology, behaviour, and distribution of freshwater ectotherms and pose further challenges to biodiversity (Jesus et al. 2018; Spence and Tingley 2020; Neubauer and Andersen 2020). Ultimately, the way warming will affect invasions will depend on its relative effects on invasive and native comparators (Robinson et al. 2020). Understanding how warmer temperatures may shape interactions between native and invasive species is thus of major interest.

Here we explored the effects of warming in the feeding success of native and invasive fish in Iberian streams. Iberian fish are highly threatened and expected to decline further due to continued new introductions and spread of established invaders (Muñoz-Mas and García-Berthou 2020), and to the anticipated rise in air temperature in the region which may reach circa 5°C under scenario RCP8.5 and bring ambient conditions outside the thermal optima of locally adapted species (Guiot and Cramer 2016).

We compared experimentally the feeding success of three species coexisting in the wild, the invasive pumpkinseed *Lepomis gibbosus* and chameleon cichlid *Australoheros facetus* and the functionally similar and endangered Southern Iberian chub *Squalius* (Rogado et al. 2005). We focused on feeding success because this is a common proxy of fitness for fish, assuming food is more plentiful and energy gain is higher (Hazelton and Grossman 2009; Grossman 2014). We analysed the feeding success of each species singly and of chub partnered with conspecifics and invasive species under current and anticipated warmer temperatures.

Chub is an invertivore that is widespread in southern Iberian Peninsula (Collares-Pereira et al. 2021) but has unknown thermal limits. Pumpkinseed is a North American invertivore that has spread worldwide (Yavno et al. 2020) and shows high thermal tolerance, stopping feeding below 8.5 °C (Keast 1968) and continuing spawning at 27.8 °C (Wismer and Christie 1987). Chameleon cichlid is a South

American omnivore that tolerates temperatures between 4.5 °C and 39.1 °C (Baduy 2018). This would be expected to benefit the later invader under anticipated warming.

2. Methods

2.1. Fish collection, maintenance, and acclimation

Using electrofishing, we collected 43 pumpkinseed (mean±SD: 63.0±7.1; minimum-maximum: 50-76 cm TL) and 44 cichlid (59.0±7.4; 48-74 cm) in the Corona stream (38°01´36´´ N, 8°25´52´´W), and 97 chub (63.8 ± 8; 45-80 cm) in the São Martinho stream (38°10´09´´N, 8°34´10´´W) where the invaders are absent. Most fish were collected in 2016 (175 out of 184), thought six chub, one pumpkinseed and two cichlids were collected in 2017 to balance replicates among experimental treatments. Mean monthly air temperature was similar between streams, ranging between 15.0 and 31.0°C in Corona and 14.8 and 30.3°C in S. Martinho (http://www.worldclim.org/; 1990-2017). Water temperature at fish collection showed no variation between streams (z=0.630, p=0.528) and years (z=0.267, p=0.789), and ranged between 14°C and 24°C.

Fish were transported to the laboratory in aerated vats separated by species and kept in quarantine for two weeks in 120 L aquaria (99x40 x30 cm) and 60 L aquaria (62x33x30 cm) provided with gravel and air supply, at the temperature they experienced in the wild, and 12/12-h light: dark cycle. The number of fish per temperature reflected variations in the size of collections in the wild. After quarantine, fish were moved into holding aquaria with 18 L (35x 25x20 cm) to 39 L (52x29x26 cm), acclimated to experimental temperatures at the rate of 2 °C per day, and kept in the test temperatures for at least seven days.

During quarantine and acclimation, fish were separated by species, kept at similar densities among aquaria, fed to satiation once daily with defrosted frozen bloodworm (*Chironomus* spp), and fasted for 48 hrs before experiments.

2.2. Feeding experiments

Experiments were conducted at 19°C, 24°C, and 29°C, covering a 5°C rise in current water temperature in the collection streams, as anticipated for the Iberian Peninsula (Guiot and Cramer 2016). Feeding trials were conducted for each species singly and for chub partnered with conspecifics and invaders. Only one individual per species was used in the pairs, matched by size (<8mm difference in TL). The 18 treatments were replicated 6 to 9 times, contingent on fish collections in the wild (Figures 1 and 2). Each fish was used in only one trial.

Experiments were conducted in a 39 L aquarium (52x29x26 cm), divided in two compartments by a removable mesh barrier, and enclosed with black plastic at the back and sides, to minimize fish disturbance. Fish were randomly allocated to compartments and allowed ten minutes to recover from handling. Feeding trials began two minutes after removal of the mesh barrier. Each trial included ten releases of single defrosted bloodworm, at two minutes intervals, through one of five randomly selected tubes at the corners and back of the aquarium. Bloodworm were readily visible and consumed by fish. Each 20-min trial was recorded from behind the aquarium, using a Sony Handycam video camera fixed to a tripod.

We used a focal approach in video footage analysis, focusing on single fish at a time. Fish in pair trails were randomly selected and watched sequentially. For each fish we recorded (i) the number of prey that were captured (henceforth prey captures), and ii) the time taken to capture each prey after delivery, and the corresponding average (henceforth time to capture). A capture represented a fish grasping a prey independently of whether the prey was consumed (Hazelton and Grossman 2009).

2.3. Data analysis

We used non-parametric Kruskal-Wallis tests with post-hoc Dunn's Multiple Comparisons and Mann-Whitney tests (Zar 1996) to analyse variation in prey captures and in time to capture by each species among temperatures and among species at each temperature. Separated sets of analyses were conducted for single and pair trials because prey capture probabilities were inherently variable between these treatments. Pair trial analysis focused on chub. The significance of statistical testing was assessed at $p<0.05$ and adjusted for multiple testing using the Bonferroni sequential correction. Analyses were run with R Software (v.4.0.0, R Core Team 2020).

3. Results

In total, 66 single and 59 pair trials were analysed. There were no differences in the size of fish among temperatures nor species treatments (Supplementary Table S1), and neither there were year effects on prey captures nor in time to capture (Supplementary Table S2).

3.1 Single species trials

Temperature had no influence on the feeding success of chub and the cichlid but affected that of pumpkinseed. Similar prey numbers were captured among temperatures by chub (4.1±3.2) and the cichlid (8.6±2.6), but pumpkinseed captured about twice less prey at 24°C than at 29°C (5.6±2.9 vs 9.3±1.5) (Figure 1a; Supplementary Table S3). Conversely, time to capture remained constant among temperatures for chub (30.1±21.5 sec), pumpkinseed (7.7±9.4 sec) and the cichlid (5.9±6.0 sec) (Figure 1b; Supplementary Table S4).

At warmer temperatures feeding success varied significantly among species. Prey captures were similar at 19°C, but chub captured twice less prey than the cichlid (4.1±2.8 vs 9.5±0.9 vs) at 24 °C and that both pumpkinseed (9.3±1.5) and the cichlid (9.6±0.7) at 29°C (4.7±2.8) (Figure 1a; Supplementary Table S3). Conversely, there was no variation in time to capture at 29°C, but chub was four times slower than pumpkinseed (33.2±1.9 vs 8.3±8.1 sec) at 19°C and twelve times slower than the cichlid (39.7±26.8 vs 3.2±3.4 sec) at 24°C (Figure 1b; Supplementary Table S4).

3.2. Pair species trials

Feeding success of chub partnered with conspecifics and invaders was not affected by temperature. Prey captures were similar among temperatures when chub was partnered with conspecifics (4.5±1.8), pumpkinseed (2.5±2.2), and the cichlid (0.7±1.5) (Figure 2a; Supplementary Table S3). Likewise, time to capture remained constant among temperatures when chub was with conspecifics (23.3±16.0) and with pumpkinseed (6.2±9.3) (Figure 2b; Supplementary Table S4). No comparisons could be made for chub partnered with the cichlid due to few observations.

At warmer temperatures, there were significant differences in feeding success between chub partnered with conspecifics and the invaders. At 19°C prey captures were similar among species pairs, but chub captured less prey with the cichlid than with conspecifics at both 24°C (0.0 ± 0.0 vs 4.3 ± 2.4) and at 29°C (p=0.002; 0.6±0.5 vs. 4.3±1.4) (Figure 2a, Supplementary Table S3). Moreover, chub captured prey nine times faster with pumpkinseed than with conspecifics at 19°C (2.1±0.7 vs 17.9±14.4 sec) and seven times faster at 29°C (3.0±2.4 vs 22.3±9.9 sec) (Figure 2b, Supplementary Table S3). No comparisons could be made for chub partnered with the cichlid due to few observations.

4. Discussion

Warmer temperatures simulating a projected climate change scenario affected the feeding success of native and invasive fish. Prey captures by the cichlid and by chub singly and partnered with conspecifics and the invaders were similar over temperatures, but singly pumpkinseed prey less at 24°C than 29°C. Moreover, at warmer temperatures chub prey less and much slower than single cichlid and pumpkinseed, and also captured less prey partnered with the cichlid than with conspecifics. Conversely, at warmer temperatures chub prey faster in the presence of pumpkinseed than conspecifics. These results highlight that warming effects are species dependent and suggest that warmer temperatures may enhance between species asymmetries in feeding success to the benefit of warm-water invaders.

The apparent resistance to warming by single chub and the cichlid likely reflected their evolutionary acclimatization to temperature (*sensu* Penk et al. 2016). Similar to other species endemic to Southern Iberian streams, chub is probably adapted to high water temperature and great temperature fluctuations (Jesus et al. 2018), and may be able to capture prey over a broad thermal window and cope with warming at least to some extent. Likewise, the cichlid may be well suited to projected warmed temperatures in Iberian streams which will approach conditions in its native distribution range (http://www.worldclim.org/) and are still well within its thermal tolerance (Baduy 2018).

At warmer temperatures, feeding success of chub was much inferior in the presence of the cichlid than conspecifics. This finding corroborates evidence indicating that the Iberian toothcarp *Aphanius iberus* becomes an inferior forager than invasive mosquito fish *Gambusia holbrooki* with warming (Carmona-Catot et al. 2013), and temperature-mediated shifts in competitive dominance found for cutthroat trout *Oncorhynchus clarkii* competing against brook trout *Salvelinus fontinalis* at warmer temperatures (Nakano et al. 1998). Characteristic differences in prey acquisition ability (*sensu*

Alexander et al 2014) may be more evident with warming and likely lead to differences in native-invader when compared to native-native pairings.

Some divergence in feeding success may also occur between chub partnered with pumpkinseed as opposed to conspecifics. Faster prey capture by chub in the presence of the invader may reflect an increase in activity with temperature, which generally occurs up to an optimum, beyond which warming may become stressful and limiting (Neubauer and Andersen 2020). However, this may also be related to higher susceptibility to temperature fluctuations by pumpkinseed which showed some latency in prey capture at 24ºC, that is close to the thermal optimum for reproduction when feeding activity is decreased (Wismer and Christie 1987). Moreover, reduction in prey handling time in native-invader pairings compared with conspecifics may be associated with changes in aggression levels (Lopez et al. 2018; Clemmer and Rettig 2019). Because faster prey capture has not been translated into more captures, the extent to which warming may affect interactions between chub and pumpkinseed is still unclear and warrant further analysis.

Taken together our results indicate that under warmer temperatures warm-water invasive fish such as the cichlid may outcompete native fish. However, the feeding success of native and invasive species should be further analysed at densities that watch those found in the wild, provided fish may seldom occur alone or in pairs. Outcomes of species interactions are often context dependent, and the frequency and strength of interactions between conspecifics as opposed to heterospecifics change with population density (Lopez et al. 2018). Experimental studies addressing interactive effects of warming and density will help predicting ecological impacts of invaders and design conservation-management for freshwater biodiversity under future climates.

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Figure captions

Fig. 1. Boxplots of prey captures (a) and mean time to capture (b) for single chub, pumpkinseed, and the cichlid, at 19°C, 24°C, and 29°C.

Fig. 1. Boxplots of prey captures (a) and mean time to capture (b) for chub partnered with conspecifics, pumpkinseed, and the cichlid, at 19°C, 24°C and 29°C.

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Ethics approval

Fish capture, handling, housing, acclimation, feeding, experimentation, final disposition and euthanasia procedures conformed to Portuguese legislation and international guidelines on animal treatment and welfare in scientific research (htpps://doi.org/10.1016/j.anbehav.2017.10.001). All efforts were made to minimize accidental deaths and stress on fish throughout the study. At the end of experiments, pumpkinseed and the cichlid were euthanized using MS222 and chub were acclimated to ambient temperature and released at the site of collection. All fish released were in good conditions and showed no injuries or contamination signs. The study was carried out under the permits of Instituto da Conservação da Natureza e das Florestas (ICNF, IP; 402/2016/CAPT and 219/2017/CAPT).

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Availability of data and material

Data may be available upon request.

Code availability

Not applicable.

Conflicts of interest/Competing interests

All the authors declare no conflict of interests nor competing interests.

Consent to participate

All the authors consent to participation.

Consent for publication

All the authors consent to publication.

Authors' contributions

CG, FR and MFM defined the field sampling and experimental protocols. FR led fish collection and AK conducted the experiments and gathered the data. CG and MFM defined the statistical approach and CG performed the analysis. CG and MFM led the writing of the manuscript. All authors contributed to the interpretation of results and editing of the manuscript.