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ORIGINAL ARTICLE

Does non-native pumpkinseed *Lepomis gibbosus* affect endemic algae-scraping *Capoeta aydinensis* in case of introduction to a small stream? An ex situ growth experiment

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Abstract

The survival and establishment of alien fishes in novel environments can result in resource partitioning with native fishes. This can cause ecological impact and suppression of native populations. However, quantifying the impact of novel interactions between alien and native species remains highly challenging in the wild. Consequently, to determine the ecological risk of *Lepomis gibbosus* in case of introduction to a small stream, experimental approach was used to predict its competitive interaction with a poorly studied endemic fish, *Capoeta aydinensis*. The aim was to test whether *L. gibbosus* has an adverse effect on native species using an experimental design under allopatric and sympatric context with temperature regimes of 15 and 24°C. The results indicated that temperature and fish proportion had effects on the growth of *C. aydinensis* while these factors were not important for *L. gibbosus*. These results provided little evidence of *L. gibbosus* presence being detrimental for endemic *C. aydinensis*, but nevertheless steps should be taken to avoid their further co-habitation in the wild.

KEYWORDS

alien species, *Capoeta aydinensis*, competition, experimental design, impact, native species

1 | INTRODUCTION

Non-native fish introductions can cause problems, which occur when fish interact with native species and may result in community- and ecosystem-level impacts (Cucherousset & Olden, 2011). These impact rise from both intentional and accidental releases of species that are associated with, for example, fish stocking or the release of unwanted ornamental and bait fishes (Copp et al., 2005; Winfield et al., 2011; Jackson & Grey, 2013; Gaygusuz et al., 2015). Indeed, when competition for resources occurs and increases, food-web structure may be altered (Britton et al., 2010; Vander Zanden et al., 1999), which disrupts natural habitat integrity via direct trophic links (Witte et al., 1992). However, the species-specific effects of these introductions depend, in particular, on the inherent traits of both the native and introduced fishes (Paterson et al., 2015; Jackson et al., 2016). For example, introduction of predator species undermines the balance in native species' populations (Li & Moyle, 1981). They may cause shifts in habitat use (Brabrand & Faafeng, 1993), changes on growth and reproductive characteristics (Fraser & Gilliam, 1992) or shifts to a more specialised diet of native/resident species (Copp et al., 2017).

Despite large number of introductions followed by deteriorations, impact on endemic species is relatively poorly studied except for a few of the more notorious species, such as common carp *Cyprinus carpio* and *Pseudorasbora parva* topmouth gudgeon (Koehn, 2004; Gozlan et al., 2005; Matsuzaki et al., 2009; Britton et al., 2011).

The North American centrarchid, pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), is a small-bodied, warm-water fish that has been introduced firstly to Europe as an ornamental fish in the 19th century from North America (Copp & Fox, 2007), and it is one of the species that have the highest establishment success (Garcia-Berthou, 2007). Pumpkinseed inhabits both lentic and lotic environments and most studies have been conducted in Europe (Copp et al., 2002; Villeneuve et al., 2005; Fox et al., 2011; Vilizzi et al., 2012). Investigations of populations in Turkey have focused on their dispersal (Özcan, 2007; Reis et al., 2018), colonisation success (Ağdamar et al., 2015), habitat use (Top et al., 2016) and morphological variation (Mangıt et al., 2018). Albeit the increasing expansion of *L. gibbosus* within Turkey (Ağdamar et al., 2015), there is limited information on their interactions with, and consequences for native fishes (Top et al., 2016; Tarkan et al., 2021).

Capoeta aydinensis is a poorly known endemic fish species in Western Anatolia (Şaşı, 2009; Akbaş et al., 2019). Their distribution as a recently re-described endemic fish is restricted to few moderate sized streams in the region (Turan et al., 2017). Also, they co-occur with many non-native fish in a large catchment in the same region (Büyük Menderes river; 550 km river long) (Özcan, 2008; Güçlü et al., 2013). *C. aydinensis* populations have been exposed to several detrimental factors, such as introduction of non-native species (Özcan, 2008, Karakuş et al., 2013) and being hunted by local people (Şaşı, 2009; Akbaş et al., 2019). Gaygusuz et al., (2013) assessed the potential impact of non-native fishes on some native fishes including a *Capoeta* species from inner Anatolia and reported that the relative condition was higher in allopatry than sympatry. Currently, there are no data on the population trends of scrapers but it is suspected to be slowly declining (Freyhof, 2014b, Freyhof, 2014c). There have been no conservation actions in place for this endemic species so far (Emiroğlu et al., 2020). Although the species are reported as abundant within the distribution area (Akbaş et al., 2019), monitoring of the populations should be considered to watch over declining populations. Growth features of *C. aydinensis* in natural habitats specifically on Tersakan stream were determined as lowest among their congeners in Anatolia (Akbaş et al., 2019), and they have low tolerance levels to the different environmental factors (Özdemir et al., 2015). Moreover, their sexual maturity is late and their relative fecundity is quite low (Akbaş, 2015). Therefore, this endemic species seems quite vulnerable, especially in co-habitation with a potential invader.

Predicting the consequences of fish introductions is necessary for understanding their situation and likely impact in new habitats. The information provided from *in situ* studies is often not enough to explain the impacts quantitatively (i.e., Top et al., 2016). Experimental approaches can be used to predict the outcome of the interactions for this purpose (Britton et al., 2011; Busst & Britton, 2014). Thus, the objective of this study was to test interactions between *L. gibbosus* and *C. aydinensis* in case of co-occurrence under experimental conditions. Our specific aim was to compare the growth rates between the controls and treatments with different proportion of fish at different temperature regimes using a small recirculating system.

2 | MATERIALS AND METHODS

The study was conducted in an indoor research unit and model fishes were collected by backpack electrofishing. Endemic fish were collected from Tersakan stream, which is a shallow and muddy stream located in southern Muğla (36.791667, 28.829167). Beside most abundant *C. aydinensis*, several native (*Squalius fellowesii*, *Alburnus carianorum*, *Barbus xanthos*), non-native fishes (*Carassius gibelio*, *Coptodon zillii*) and several bivalves (*Unio crassus*, *U. pictorum*, *Anodonta anatina*) reside the stream. Water temperature changes between 12–27°C across a year (Akbaş et al., 2019). Non-native *L. gibbosus* was sourced from Sarıçay stream (37.343889, 27.729167) on December 2016 after the breeding season (Top, 2012), thus avoiding bias due to nest-guarding aggressive behaviours. Fish were transferred without loss to Muğla Sıtkı Koçman University Research Unit using 25 L buckets with simple air pumps. In the unit, they were transferred into larger tanks and medicated for any parasitic infection using Praziquantel solution.

A series of co-habitation aquaria experiments were used to examine the growth rates of *C. aydinensis* (C) when placed in contact with *L. gibbosus* (L). The experimental design used controls and treatments with three simultaneous replications. Allopatric context was applied in the control groups. Three specimens of each species (n = 3) were added separately to the aquarium (with three replicates, nine fish of each species used totally in control groups). The treatments were in sympatric context, with different proportion of both species (L+CCC, LL+CC, LLL+C). All fish were measured (fork length, FL, nearest cm), weighed (TW, to 0.01 g) and individually tagged with different colour tags under anaesthesia. Initial fish were selected in adult sizes (L: 8.9–12.3 cm, C: 15.8–22 cm, FL) according to their length at maturity sizes (Top, 2012; Akbaş, 2015). Treatments and controls were completed in aquariums of 100 L volume each, arranged in columns of four shelves (one aquarium per shelf) on recirculating systems. Two different temperatures, 15°C and 24°C, were determined to observe experiment relative to natural conditions in Tersakan stream. The experiment was conducted specifically in early December 2016 to pursue the 15°C treatments by ambient temperature, and air conditioner was used to obtain 24°C. Bottom aquariums of the 4-shelved recirculating system were filled up with biological filter (sponges, gravels and bio balls), and water was sanitised with UV filters, which were connected to the water pipes of the system. Water parameters were measured with a multiprobe (YSI 556 MPS) each morning for the temperature and dissolved oxygen. Regarding the nitrite, nitrate and phosphate concentrations, comparator kits were applied weekly (Lovibond CHECKIT) and 2/3 of water was changed when necessary. Lighting was provided both naturally from large windows and with electricity (artificial lighting synchronised with the natural one using time adjustable sockets during 9 h (light)/15 h (darkness)). All fish were adapted to experimental conditions for two weeks before experiment. Fish were fed below *ad libitum* to quantify possible competitive interactions (Busst & Britton, 2014) with pelletised food (Skretting Perla MP) during 40 days. Black curtains were used between the columns to avoid visual interaction, and feeding was accomplished by simple automatic food dropper. Pipes (15 cm diameter, 30 cm long) were added as shelters to each aquarium to reflect natural nesting holes in the stream. *Capoeta aydinensis* is a generally benthic feeder; nevertheless, it was observed that they had high capability to feed from the surface, even though the food was sinking type. At the end of the experiment, all of the fish were identified by their tag colour, re-measured and weighed. After the experiment, native fishes were returned to the same stream, and the non-native fishes were sacrificed with an overdose of 2-phenoxyethanol solution due to their non-native status.

To quantify the consequences for growth in the controls and treatments at both temperatures, specific growth rate (SGR) was determined from [(lnW_{t+1}) lnW_t)/$_t$]* 100, where W_t = starting weight of fish, W_{t+1} = end weight and t = number of days. Values were determined for each fish per treatment and the mean per treatment with 95% confidence limits. Growth comparisons among different fish proportions (control vs. treatment) and between temperatures (15℃ vs. 24℃) were made with general linear model with univariate analysis. Between subject effects of temperature and treatments were analysed for each species independently. Significant terms were explored using Tukey HSD post hoc tests. All statistics were completed in SPSS v.20.0 with significance evaluated at α = 0.05.

3 | RESULTS

Our study revealed that temperature had a significant effect on the growth of *C. aydinensis* (Figure 1, Table 1). SGR of *C. aydinensis* in control groups was highest at 15℃ and was lowest when number of *L. gibbosus* increased (LLL+C) (Table 2). On the other hand, growth rate was higher, when three individuals of *C. aydinensis* were co-habitated (L+CCC), or when the proportion was equal (LL+CC), yet it was still lower compared to control group ($F_{3,26}$ = 3.046, p = .061). Besides, with multiple comparison (Tukey HSD) test, only the interaction between three *L. gibbosus* and single *C. aydinensis* was found significant (p = .039). The growth rate of *C. aydinensis* had significant reductions at the temperature of 24℃ in all experiments ($F_{1,47}$ = 36.67, p < .05), while fish proportion did not affect the growth ($F_{3,26}$ = 0.356, p = .785).

In contrast, proportion of fishes and temperature were not important for *L. gibbosus* (Figure 1, Table 1). At 15℃, growth rate was slightly higher in control groups and was highest at equal proportion (LL+CC) (Table 2), whereas it was lowest in the single *L. gibbosus* experiment (L+CCC). Conversely, SGRs of *L. gibbosus* at 24℃ decreased in control groups while faster growth was observed in sympatric groups. Nevertheless, the differences between control and treatments were not significant ($F_{3,26}$ = 1.048, p = .414).

With the overall analysis, the results showed no significant growth difference for the endemic species in terms of allopatry and sympatry ($F_{1,89}$ = 0.063, p = .803).

4 | DISCUSSION

We found that there was negligible interaction between *C. aydinensis* and *L. gibbosus*, with little change in *C. aydinensis* growth in the presence of the alien (Table 2); however, there was an effect

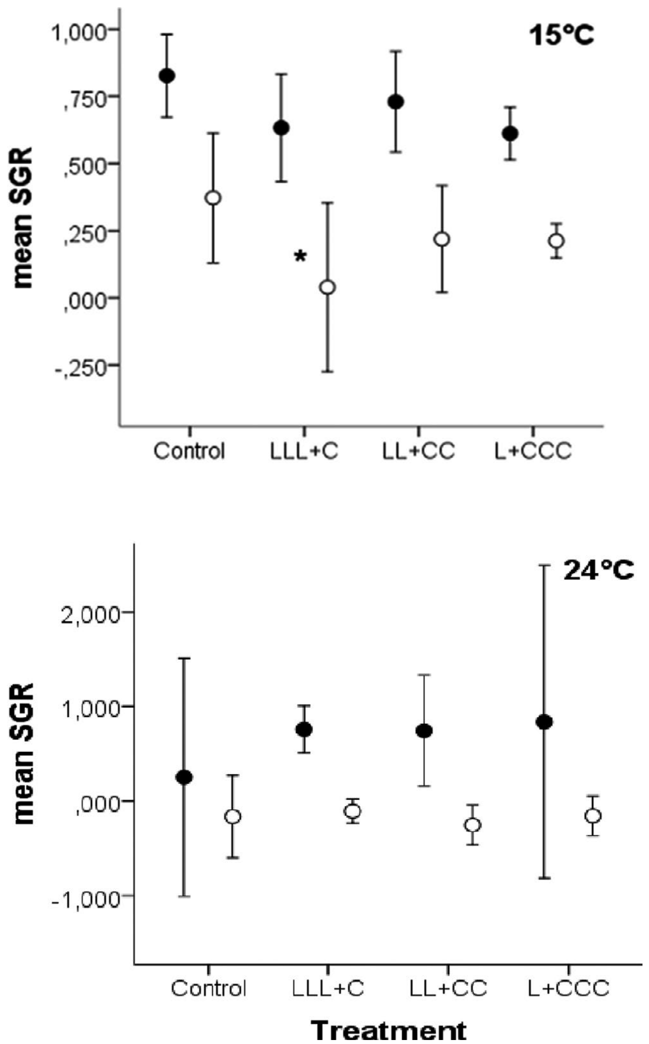

FIGURE 1 Mean specific growth rate (SGR) of *Capoeta aydinensis* (C) and *Lepomis gibbosus* (L) in the control and treatments with different water temperatures (15 and 24℃). Treatment with star (*) demonstrates significant fish proportion. Black circles indicate *L. gibbosus*, and open circles indicate *C. aydinensis*. *Error bars* represent 95% confidence intervals

of temperature for both fish species that cannot to be neglected (Figure 1, Table 1). Single individuals were suppressed by the other fish in both cases (i.e., endemic fish was stressed due to pumpkinseed specimens or *vice versa*). This situation might be a result of an exploitation that arises from consuming majority of existing food resources in the environment (Britton et al., 2011). At warmer water temperature, growth of *C. aydinensis* was apparently decreasing. This result suggests an evident that *C. aydinensis* generally inhabits cooler temperate areas in the wild (Akbaş et al., 2019), which may be a competitive disadvantage for the future climate change scenarios. However, it has also been observed that different feeding strategy such as obtaining food from the surface or adapting to pelletised food, even feed on chironomids as well as scraping algae (personal observations) was displayed by *C. aydinensis*. Nevertheless, *C. aydinensis* has low tolerance levels to the environmental factors (Özdemir et al., 2015).

TABLE 1 Effect of temperature and fish proportion on the mean specific growth rates of *Capoeta aydinensis* and *Lepomis gibbosus* (α = 0.05) from univariate analysis

	Specific growth rate	
Effect	*C. aydinensis*	*L. gibbosus*
Temperature	$F_{1,47} = 36.67$, $\mathbf{p < .05}$	$F_{1,47} = 0.17$, $p = .683$
Fish proportion	$F_{3,47} = 0.77$, $p = .517$	$F_{3,47} = 0.42$, $p = .734$
Tukey HSD		
15°C	$F_{3,26} = 3.046$, $p = .061$	$F_{3,26} = 0.822$, $p = .502$
24°C	$F_{3,26} = 0.356$, $p = .785$	$F_{3,26} = 1.015$, $p = .414$

Note: Bold value indicates significant factors of the experiment

TABLE 2 Mean specific growth rates (SGR) of fishes in each treatment for different temperatures.

Species	Temperature	Fish proportion	Mean SGR
C	**15**°C	Control	0.372
		LLL+C	**0.039**
		LL+CC	0.219
		L+CCC	0.212
	24°C	Control	−0.163
		LLL+C	−0.106
		LL+CC	−0.251
		L+CCC	−0.156
L	15°C	Control	0.827
		LLL+C	0.633
		LL+CC	0.73
		L+CCC	0.612
	24°C	Control	0.253
		LLL+C	0.759
		LL+CC	0.744
		L+CCC	0.84

Note: Bold value shows significant treatment. (C = *C. aydinensis*, L = *L. gibbosus*).

The growth rate of *L. gibbosus* at elevated temperature decreased in controls while faster growth was observed in sympatric groups. Altered thermal regimes can elevate the growth of non-native species through competitive superiority (Rahel & Olden, 2008). For example, in laboratory experiments, brook trout and brown trout were equal competitors for food at cold temperatures, but brown trout were superior competitor at warm temperatures (Taniguchi et al., 1998). Hence, this may be the case for faster growth of *L. gibbosus* through interspecific competition on sympatric treatments. However, this interaction did not affect the growth of *C. aydinensis* (Figure 1).

The present study revealed that the ecological consequences of *L. gibbosus* on *C. aydinensis* were minimal, with *C. aydinensis* responding more to natural mechanisms than the presence of non-native fishes. On account of the positive correlation between water temperature and *L. gibbosus* (Özdemir et al., 2015), the results recorded in the present study should not be disregarded, with increasing temperatures impact on the recipient communities may be more likely (Rahel & Olden, 2008). Interactions between native and non-native fishes may arise indirectly without sharing same resources (Britton et al., 2010) or may be profitable for natives (Gozlan, 2008). For instance, high abundance of *L. gibbosus* may cause shifts in food webs, such as consuming invertebrates which may result in increased baseline resources such as algae (Van Kleef et al., 2008) and this may favour *C. aydinensis* as an algae-scraping species (Levin et al., 2012).

Spread of non-native fishes by fish stocking is a well-known fact in Turkey (Gaygusuz et al., 2015). At present, several non-native fishes inhabit in the Tersakan stream. Considering the distribution rate (Reis et al., 2018) and presence of *L. gibbosus* in a relatively close basin (i.e., Büyük Menderes; Özcan, 2008; Güçlü et al., 2013), an introduction may happen to Tersakan any moment, which suits the ecological and biological requirements of *L. gibbosus* perfectly (Top, 2012). From a conservation perspective, as a re-described endemic species (Turan et al., 2017), IUCN status is currently not evaluated (NE) for *C. aydinensis* (IUCN, 2020), yet it was listed as vulnerable (VU) with previous taxonomy (*Capoeta bergamae*), and this may point out that it is more vulnerable under current circumstances by occupying smaller spatial range than before.

Consequently, although present case study highlights a benign consequence of a non-native species on a native species, both may interact in natural conditions, depending on available food or habitat resources. Further, available literature would not be enough to suggest an invasion of *L. gibbosus* in Turkey with lack of studies on the subject. It is therefore important to report even experimental cases especially for prioritisation of management plans for invaders (Britton et al., 2011; Jackson et al., 2016).

On the whole, *ex situ* studies can be used as a tool to quantify potential consequences of introduced fishes (Britton, 2018) as well as effect of changing environmental factors (e.g., temperature). Here, we showed that it can be valuable in predicting whether or not aliens will impact co-existing natives even without co-occurrence.

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NTK designed and performed the experiments, derived the models and analysed the data. NTK wrote the manuscript in consultation with AST.

AUTHOR CONTRIBUTIONS

NTK designed and performed the experiments, derived the models and analysed the data. NTK wrote the manuscript in consultation with AST.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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