

Research Article

Assessing the threat of a South American cichlid on anurans in the Chilean Mediterranean region

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Abstract

Mediterranean biomes have been recognized as having high value due to their diversity; they are under threat due to anthropic pressures. Both freshwater fish and amphibians show high endemism and vulnerability to several threats; among the latter is the introduction of fishes. In Chile there is scarce documentation of the presence of the non-native *Australoheros facetus* (chameleon cichlid), where it cohabits with other non-native and native fish and anurans. In this study we first sampled for the presence/absence of *A. facetus* in 69 localities in the Chilean Mediterranean region. Secondly, to estimate their potential impact, we evaluated the predatory capacity of *A. facetus* and other two sympatric fish, *Gambusia holbrooki* (mosquitofish; non-native) and *Cheirodon pisciculus* (pocha; native), on larvae of four species of native anurans and on the eggs of a non-native anuran. We report that *A. facetus* has expanded in the entire Chilean Mediterranean region; its presence is related to low altitude areas. We confirmed that lentic environments and irrigation systems of streams increase the probability of successful establishment. The results for predatory capacity showed that *A. facetus* regularly predated larvae of all the tested anurans, being significantly more successful in predating larvae, but also significantly less successful in predating eggs, compared to other fish species. The control of non-native fish is a priority to avoid the invasion of new localities in these ecosystems.

Key words: amphibians, chameleon fish, Chile, invasive species, predation

Introduction

The Mediterranean regions worldwide have been recognized for their rich biodiversity and high threat levels (Myers et al. 2000; Marr et al. 2010). The Chilean area was produced by the uplifting of the Andes Range to the east and the formation of the Atacama Desert in the north and limited by the Pacific Ocean in the west and the cold, wet conditions to the south. It was reinforced by orographic processes such as the Pleistocene glaciations, with profound effects on the aquatic biota (Dyer 2000). The geographic isolation of the Chilean Mediterranean area has favored the development of a unique ecosystem with high levels of endemism, which is one reason for its particular value and importance to conservation (Cowling et al. 1996;

Manzur 2005). One threat in Mediterranean regions is the introduction of exotic fish species (see for Europe: Morán-López et al. 2006; Vila-Gispert et al. 2005; for South Africa: Skelton 2002; Moyle and Light 1996; for California: Morgan et al. 2002, 2004; for Australia: Olden et al. 2008). There is little information for the Mediterranean region of Chile; most of it derives from inventories of the species present in the country (Ruiz and Marchant 2004; Iriarte et al. 2005; Vargas et al. 2015). Amphibians are a relevant biological component of Mediterranean aquatic ecosystems; they are considered a focal group due to their recognized global decline (Collins and Storfer 2003; Grant et al. 2016) as a consequence of complex processes such as global warming, habitat destruction, pollution, emergent diseases and species introductions (Blaustein and Kiesecker 2002; Soto-Azat et al. 2016). Anurans are the only amphibians present in Chile; they include 63 species (Correa et al. 2016), with a high level of endemism (65%) and high degrees of threat (Lobos et al. 2013).

The introduction of species is recognized as one of the main impacts that influence the loss of diversity in freshwater systems (Kolar and Lodge 2000). Non-native fish are a relevant agent in the global decline of freshwater aquatic fauna, particularly in amphibian populations, which can be driven to extinction (Casal 2006; Helfman 2007). Most anurans have defense mechanisms against the predators with which they co-exist, but these may not be effective against introduced predators, in part due to the lack of “evolutionary experience” (Diamond and Case 1986), making them vulnerable to this threat (Griffiths et al. 1998; Griffin et al. 2000). It has been suggested that there is a genetic component to these defense mechanisms (Kats et al. 1988) and an acquired component (Ferrari et al. 2006; Mirza et al. 2006). Defensive mechanisms by amphibian larvae include decreasing mobility (Chivers and Mirza 2001; Ferrari et al. 2005), searching for refuge (Kiesecker et al. 1996; Mirza et al. 2006; Ferrari et al. 2008), development of cryptic coloration (Wassersug 1971) and disagreeable taste (Brodie and Formanowicz 1987).

Australoheros facetus Jenyns, 1842 (chameleon cichlid) is a south American cichlid, native to Argentina, Brazil, Uruguay and Paraguay (Říčan and Kullander 2008). This fish has been introduced to the Iberian Peninsula since 1940 and currently is established in southern Portugal and Spain (Ribeiro et al. 2007). Another invaded area is Chile, where it was reported in 1940 (Iriarte et al. 2005). Its diet is generalist, including fishes and invertebrates, with opportunistic feeding behavior (Ruiz et al. 1992; Ribeiro et al. 2007). It is a benthopelagic freshwater fish that exhibits diurnal activity and bi-parental care of the eggs and fry (Baduy et al. 2020). In Portugal its size reaches up to 20 cm (Ribeiro et al. 2007), while in Chile up to 22 cm (Ruiz et al. 1992). In Chile was reported first in a dam of Central Chile in 1940 (Iriarte et al. 2005). Then Ruiz et al. (1992) indicated their presence in the urban area of Concepción in southern Chile (427 km from

the first report), Iriarte et al. (2005) extended the presence to some localities in central Chile and recently Avilés et al. (2018) reported this fish in an urban wetland in northern Chile (to 232 km north of the first record), showing a discontinuous distribution. No exact reason is known for its introduction in Chile and factors contributing to the invasiveness of *A. facetus* remain unclear. In this way, recently the use of two risk assessment kits, indicates to this fish as a species with high invasiveness potential in a Mediterranean biome (Baduy et al. 2020).

There are few studies in South America that explore the effect of non-native fish on amphibians; two of these are evaluations of the predatory impact of *Oncorhynchus mykiss* Walbaum, 1792 (rainbow trout) on Andean amphibians (Barrios and Ponssa 2008; Martín-Torrijos et al. 2016). Up to now there has been little information on the role that exotic fish play in the community of Mediterranean amphibians (Goodsell and Kats 1999; Álvarez et al. 2002), and no information is available for the Chilean Mediterranean region.

Objectives of this study were to (i) update the distribution of *A. facetus* and determine environmental variables facilitating its establishment, and (ii) evaluate the predation of amphibian eggs and larvae by *A. facetus* in comparison with other native and non-native fish.

Materials and methods

Chameleon fish field surveys

The study was performed in the entire Chilean Mediterranean region, which includes approximately 956 km latitudinally between the Atacama and Biobío administrative regions (Figure 1). We sampled in 62 localities and a few cases (7), we report other studies (Supplementary material Table S1). At each of the sampling stations we performed transects of 100 m length along the riparian zone, where fish were collected with a SAMUS 725 MD electrofishing device. Sites surveyed (29 positive or presences and 40 negative or absences), were represented in a geographic information system in UTM coordinates, zone 19 south, datum WGS 84 (Qgis 3.1°). To determine the factors explaining the presence/absence of the species, we recorded in the field the type of environment in each site (running streams, slow flow streams, streams with seasonal flow and stagnant water bodies as ponds, dams and lakes). We defined a buffer area of 3000 m around each site, in order to measure density of water courses as the sum of the length of water channels (m) within each site divided by the area of its polygon (LaRue and Nielsen 2008; maps are available at <http://sigweb.sag.gob.cl/sagmaps/>) and human impact as mean value of an index of human impact (between 0 = naturalness, to 100 = disturbed) (Sanderson et al. 2002). To explore which variables were correlated with the presence of *A. facetus*, we constructed a generalized linear model (GLM) with a binomial distribution (multiple

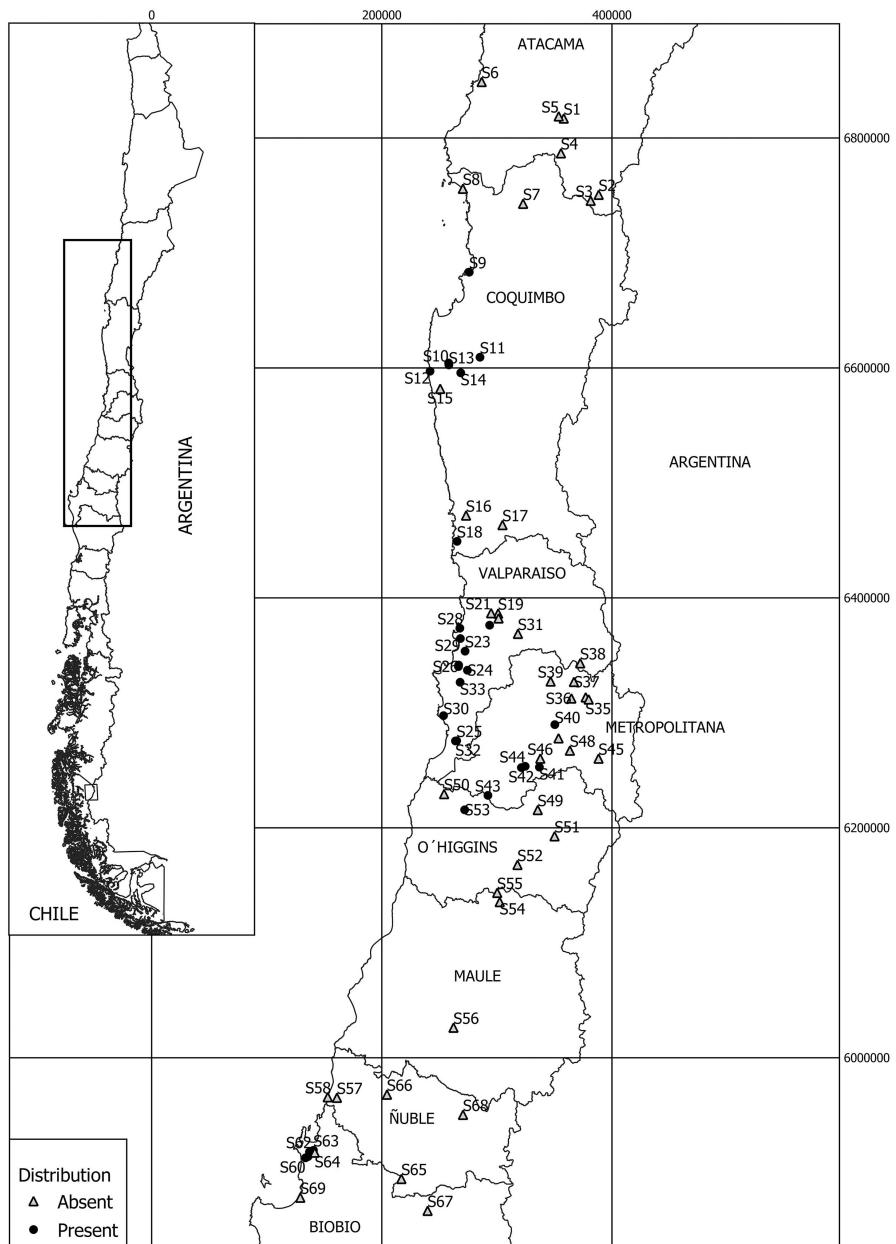


Figure 1. Distribution of *Australoheros facetus* in the Chilean Mediterranean region. The box in the left panel shows the study area in a national context.

logistic regressions). The presence/absence data for the species were utilized as a response variable. Due to the imbalance between types of environment by altitudinal categories (low altitude sites being mostly slow flow streams and stagnant water bodies), we did not include the altitude to our model. We compared all the candidate models (i.e. all possible predictor combinations, Table 1) and used Akaike Information Criterion (Harrell 2001) to select the best model. All statistical analyses were carried out in the R environment (R Core Team 2018).

Evaluation of the predatory role of fish on anuran larvae

We performed trials in a mesocosm experiment to evaluate the predatory role of *A. facetus*; smaller-scale experiments have been proposed to analyze

Table 1. Comparison of generalized lineal models for the evaluation of the determining factors of the presence/absence of *Australoheros facetus* in Chile's area of invasion. Model with lowest Akaike information criterion (AIC) was chosen as the best model and is shown in bold.

Models	Variables	AIC
Model 1	Type of environment + density of water course	43.18
Model 2	Type of environment + density of water course + human impact	44.19
Model 3	Type of environment	48.17
Model 4	Type of environment + human impact	48.23
Model 5	Density of water course + human impact	82.74
Model 6	Density of water course	90.93

competition and predation relationships on anuran larvae (Kupferberg 1997; Komak and Crossland 2000). We compared the predatory capacity of *A. facetus* with two predator fish; the non-native *G. holbrooki* Girard, 1859 (mosquitofish; native to USA), present in Chile for at least 88 years (widely distributed in the country, along a latitudinal gradient of 2481 km) (Lobos 2020) and the native *Cheirodon pisciculus* Girard, 1855 (pocha), a shallow water fish that cohabits with amphibians. The average sizes of each species of fish were: *A. facetus* = 5.7 ± 0.6 cm; *G. holbrooki* = 3.4 ± 0.6 cm and *C. pisciculus* = 4.3 ± 0.9 cm. We standardized fish sizes by using larger individuals of *G. holbrooki* and *C. pisciculus* and smaller individuals of *A. facetus*. We used larvae of four amphibian species as prey; two species with larvae with prolonged aquatic development, *Calyptocephalella gayi* Duméril and Bibron, 1841 (helmeted water frog; aquatic; endemic) and *Alsodes nodosus* Duméril and Bibron, 1841 (black spiny-chest toad; terrestrial; endemic), and two with larvae with a short aquatic development period; *Pleurodema thaul* Lesson, 1826 (four-eyed frog; semi-terrestrial) and *Rhinella arunco* Guichenot, 1848 (Chilean common toad; terrestrial; endemic). To standardize the trials, all larvae used were in Gosner stages 26–27 (Gosner 1960). The average sizes of the larvae of each species of amphibian were: *C. gayi* = 3.1 ± 0.5 cm; *A. nodosus* = 3.0 ± 0.5 cm; *P. thaul* = 2.7 ± 0.6 cm and *R. arunco* = 2.6 ± 0.5 cm.

All the fish and amphibian species used are sympatric in the Chilean Mediterranean region; they were collected in the Santuario de la Naturaleza Quebrada de Córdova ($33^{\circ}26.515\text{--}71^{\circ}39.662$), region of Valparaíso, about 120 km from the city of Santiago. Since all the anuran species tested experience some degree of threat according to the current rules for classification of wild species in Chile, (Species Classification Regulation, Ministry Environment of Chile), the number of individuals used was minimized, to comply both with the capture permits obtained and the bioethical principle of reducing the number of animals sacrificed (Russell and Burch 1959).

Fish and larvae were fed daily with commercial food, which was suspended 24 hours before the beginning of each trial. We performed trials in plastic containers ($30 \times 40 \times 35$ cm) in 20 l of dechlorinated water (Skelly 1994; Kupferberg 1997). Four different treatments were used to

evaluate possible interactions between fish and anuran larvae: one anuran larva alone as a control group, one anuran larva and one individual of *A. facetus*, one larva and one individual of *G. holbrooki* and one larva and one individual of *C. pisciculus*. The densities of anurans and fish (8.3 individuals/m² for predator, 8.3 individuals/m² for prey) were derived from densities estimated in previous studies (Lobos et al. 2014a; Lobos 2020). Predators and treatments were randomly assigned to different mesocosms; each treatment was replicated four times and evaluated every 24 hours for a period of 7 days, replacing preyed larvae. Dead individuals were replaced by others with similar characteristics (Gosner stages and average size) to maintain the density relation in all treatments. Mortality was estimated as the total number of larvae eaten (replaced) at the end of the trial (ranging from 0 to 7). The values of the treatments were compared with a general lineal model (GLM) followed by an *a posteriori* GLM comparisons for each pair. All statistical analyses were done with the software R (R Core Team 2018), using a significance level of $p < 0.05$.

*Evaluation of the predatory role of fish on *Xenopus laevis* eggs*

We evaluated fish predation on eggs of the non-native *Xenopus laevis* Daudin, 1802 (African clawed frog), given the ease of obtaining them in the laboratory, using the protocol of Fischer et al. (2000). We used the same fish species as in the previous experiment, plus the non-native fish *Cnesterodon decemmaculatus* Jenyns, 1842 = 3.2 ± 0.5 cm (ten-spotted livebearer; native to Argentina and Uruguay), which is a small fish sympatric with the others mentioned above. This species was not used in the previous experiments due to the small number collected when we performed the trials. The same container conditions were used as in the previous experiment. Each trial used five treatments: 1) 10 eggs of *X. laevis* as a control; 2) 10 eggs and one *A. facetus*; 3) 10 eggs and one *G. holbrooki*; 4) 10 eggs and one *C. pisciculus*; 5) 10 eggs and one *C. decemmaculatus*; each treatment was replicated ten times. Mortality was estimated for each essay based on the percentage of eggs eaten after seven days. We followed the same statistical analysis mentioned in the previous larval experiments.

Results

Chameleon fish field surveys

The Chilean Mediterranean region has been widely invaded by *Australoheros facetus* (791 km extension). There is a core population in central Chile and two isolated populations; one in the north (Coquimbo) and another in the south (Concepción), all within the limits of the Mediterranean region (Figure 1). The chameleon fish extends for 235 km latitudinally in central Chile (occupation area of 8851 km²), the population of the northern area being 145 km from the central core (occupation area

Table 2. The best model determining factors of the presence/absence of *Australoheros facetus* in Chile's area of invasion. B is the coefficient of multiple regression, Z is the Z-test value, p is probability under the null hypothesis, and AIC is the Akaike information criterion. Values in bold indicate significance.

Variables	B	Z	p
Intercept	-5.74	-3.08	0.0020
Density of water courses	7.33	2.08	0.0370
Slow flow stream	5.82	3.60	0.0003
Stagnant water bodies	5.56	3.56	0.0004
Seasonal flow streams	-14.88	-0.01	0.9940

of 2109 km²) and the southern population 325 km from the central core (occupation area of 16 km²).

We sampled 69 sites (Table S1), with an elevation range from 4 to 3078 m (mean = 522); the human impact index ranged from 7 to 93 (mean = 43) with lentic and lotic environments (24.64% stagnant water bodies, 24.64% slow flow streams, 37.68% running streams and 13.04% seasonal flow streams). The species was present in 29 localities, with preference for low elevations (mean = 119 m, minimum 5 m, maximum 490 m), median values of the human impact index (mean = 54, minimum = 25, maximum = 93) and lentic environments (48.3% stagnant water bodies, 48.3% slow flow streams and 3.4% running streams). Our final logistic regression model showed the type of environment and density of water courses as the variables that determine the presence of *A. facetus* at a given site (Table 2). There was no significant effect of the index of human impact (not present in the final model).

Evaluation of the predatory role of fish on anuran larvae

The mesocosm experiments showed significant differences between the treatments in all species of anuran larvae; *C. gayi* (GLM, n = 16, p < 0.0001), *A. nodosus* (GLM, n = 16, p < 0.0001), *P. thaul* (GLM, n = 16, p < 0.0001) and *R. arunco* (GLM, n = 16, p < 0.0001). The predation by *A. facetus* on all species of anuran larvae was significantly greater than predation by other fish species (Figure 2, GLM, all p < 0.05). Chameleon cichlid consumed the anuran larva each day of a trial in almost each cases, while the mortality rate in treatment with other fish species rarely reached one larva per seven days, being not significantly greater than that in control group (Figure 2, GLM, all p < 0.05). The mortality in control assays was low, ruling out other causes of mortality.

Evaluation of the predatory role of fish on anuran eggs

There were differences in eggs mortality among the four fish species (GLM, n = 50, p = 0.04; Figure 3). *G. holbrooki* and *C. decemmaculatus* predated significantly more eggs of *X. laevis* than *A. facetus* and *C. pisciculus* (Figure 3, GLM, all p < 0.05). Egg mortality in the control group was low and was due to the presence of fungal contamination.

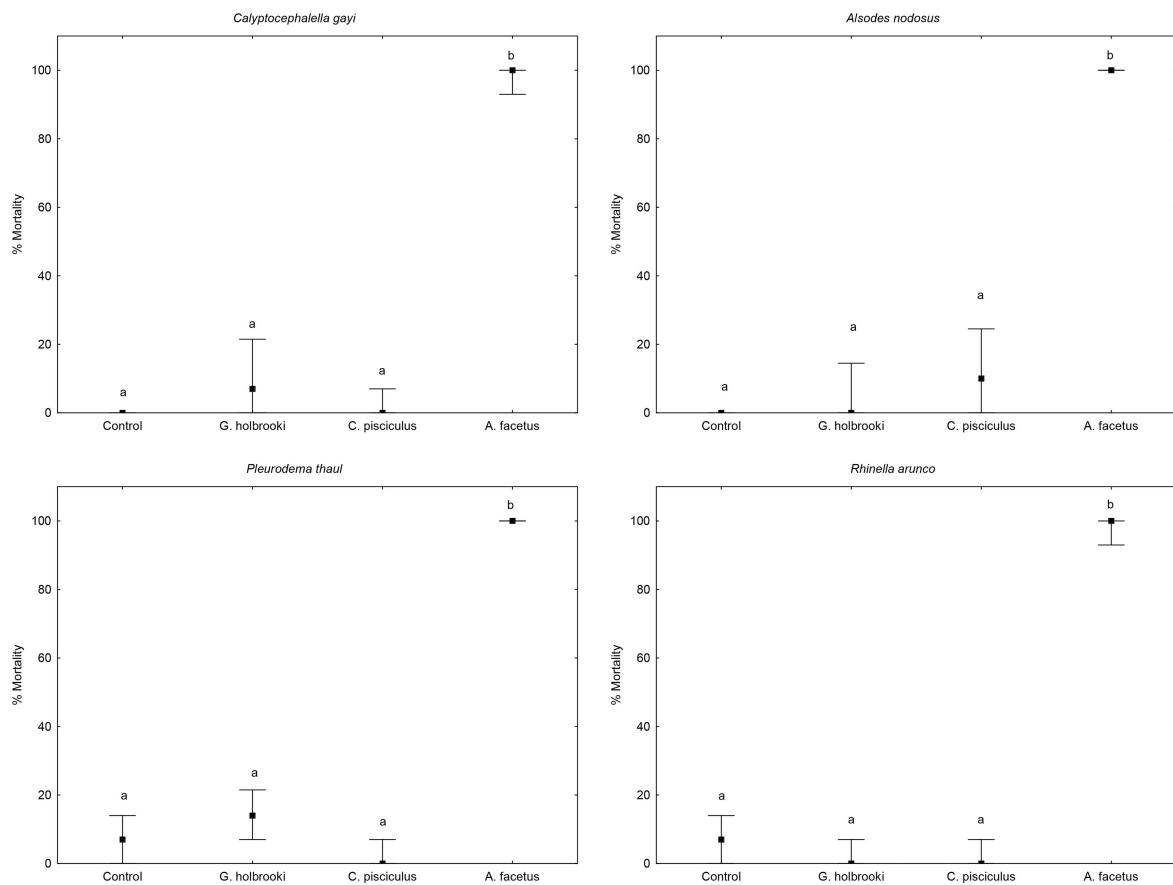


Figure 2. Mortality of anuran larvae by fish expressed as proportion of larvae replaced during the 7-day trial (maximal number of larvae replaced = 7). Median is represented by black square and \pm interquartile values by whiskers ($n = 4$ replicates). Predator fish in the x axis were *Gambusia holbrooki*, *Cheirodon pisciculus* and *Australoheros facetus*. Control group was one anuran larva alone. A general lineal model (GLM) with an a posteriori comparisons for each pair indicate significant difference (letters a and b) in the rate predation of all anuran species by *A. facetus*.

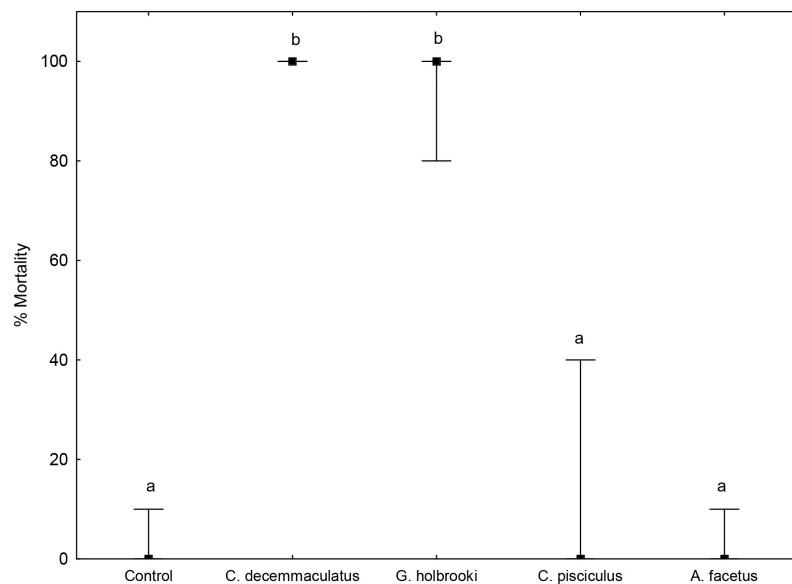


Figure 3. Mortality of eggs of *Xenopus laevis* expressed as proportion of eggs remaining after the 7-day trial (maximal number of eggs remaining = 10). Median is represented by black square and \pm interquartile values by whiskers ($n = 10$ replicates). Predator fish in the x axis were *Cnesterodon decemmaculatus*, *Gambusia holbrooki*, *Cheirodon pisciculus* and *Australoheros facetus*. The control group had 10 eggs of *Xenopus laevis*. A general lineal model (GLM) with an a posteriori comparisons for each pair indicate significant difference (letters a and b) in the rate predation by *C. decemmaculatus* and *G. holbrooki*.

Discussion

We found a low richness of fish fauna in the Chilean Mediterranean area (15 species in central Chile), with predominance of small-sized fish (39% have lengths less than 20 cm) and high endemism (Vila et al. 2006). The same pattern is found for amphibians (Correa et al. 2011). There is no native freshwater fish species in Chile that have parental care like *A. facetus* or that are viviparous like *G. holbrooki* (De Buen 1959).

Although *A. facetus* was introduced in 1940 (Iriarte et al. 2005), its expansion in the country occurred only beginning in the 1990s (Ruiz et al. 1992; Iriarte et al. 2005; Ortiz-Sandoval et al. 2009) and its presence in Mediterranean Chile has been little documented. Our study found that the distribution of *A. facetus* is continuous in the central part of Chile, with two gaps in the northern and southern limits, suggesting the presence of different foci of introduction. This long-distance movement implies a high risk of propagation of chameleon fish and has been suggested as one of the factors involved in the successful invasion of African clawed frog in Mediterranean Chile (Lobos et al. 2014b). Based on results of our model, we predict that lentic aquatic environments (slow flow streams and stagnant water bodies) and the presence of a network of streams and canals facilitate successful establishment. The lack of correlation with the index of human impact (Sanderson et al. 2002) reflects its presence both in natural (wetlands, streams) and artificial (irrigation ponds, reservoirs) environments. The localities with presence of *A. facetus* are at low altitudes, where the water courses have less runoff. Some of these variables closely resemble the conditions that *A. facetus* faces in its native range (Říčan and Kullander 2008) and other Mediterranean regions (Ribeiro et al. 2007).

Exotic fish may affect native anurans by predating eggs, larvae and adults (Welsh et al. 2006; Jara and Perotti 2006). Although the number of replicates we used to evaluate predation of larvae was low due to bioethical (Russell and Burch 1959; Santos et al. 2009) and legal considerations (Soto-Azat et al. 2015), there was a clear tendency; *A. facetus* indistinctly predated larvae of all anuran species tested, with much less predatory activity by smaller fish (both native *C. pisciculus* and non-native *G. holbrooki*). It is probable that smaller fish had a physical limitation for eating larvae (mean length 30–40 mm, to predate on larvae with mean lengths of 26–31 mm), although it has been suggested that some species such as those of the genus *Rhinella* may have developed toxins that make them unpalatable (Kiesecker et al. 1996; Jara and Perotti 2006). We observed that *G. holbrooki* nibbled the tails of *R. arunco* larvae but did not kill them during the evaluation period, only *A. facetus* predated these larvae (Figure 2). It has been indicated that bufotenins may act as a defense mechanism in the family Bufonidae (Crossland 1998; Crossland and Alford 1998). *Australoheros facetus* consumed all species of larvae tested without difficulty. We therefore suggest that *A. facetus* represent a serious threat to the native anurans. Ruiz et al. (1992)

reported the predation of *A. facetus* on small native fish in Chile and Avilés et al. (2018) of a native and threatened crayfish. The size of *A. facetus* appears to be main factor in its possible impact, considering also that parental care and aggressive behavior have been identified as key elements of successful invasion (Baduy et al. 2020). Notably, the largest native fish species in central Chile, *Basilichthys australis* Eigenmann, 1928 (Chilean silverside) and *Percichthys trucha* Valenciennes, 1833 (creole perch), inhabit deep water, while *A. facetus* forages in shallow water where the tested amphibians reproduce.

The opposite tendency was found for egg predation, in which the smaller fish of our study showed greater voracity. Both *G. holbrooki* and *C. decemmaculatus* do not reach lengths greater than 4 cm (Fernández-Delgado and Rossomanno 1997; Pérez-Bote and López 2005); they are recognized as voracious species that invade water which is shallow, has low runoff and abundant refuges (Habit and Victoriano 2005; Pyke 2005), which are the habitats of choice for amphibian egg laying. Some studies in California, USA have indicated *G. holbrooki* as responsible for the decline in some amphibian species by predation of their eggs and larvae (Gamradt and Kats 1996; Goodsell and Kats 1999; Álvarez et al. 2002) and probably due to habitat modification (Hurlbert et al. 1972). The native anurans thus appear to be exposed to predation pressure by small fish in their earliest development stages and by larger fish in later larval stages. Note, however, that we did not test for effectiveness of smaller, juvenile stages of *A. facetus*. If these stages were as efficient in consuming eggs or early larval stages as were *G. holbrooki* and *C. decemmaculatus*, *A. facetus* can represent even more complex threat to the native anurans.

The vulnerability of Chilean anurans to exotic fish is worrying, since all the anurans tested have been recognized as having some degree of threat (Díaz-Páez and Ortiz 2003; Soto-Azat et al. 2015). This study recognizes that the exotic fish are a high-risk factor for the Chilean Mediterranean zone (which may also occur in other Mediterranean areas), especially due the isolation in the evolution of its biota. This is alarming given that Ruiz and Marchant (2004) listed 26 exotic fish species in Chile, compared to 44 native species (De Buen 1959; Iriarte et al. 2005; Habit et al. 2006; Rojas et al. 2019). The future is worrying, since in Chile the danger associated with anuran enemies has not been recognized in spite of the long time since some of them were introduced.

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Ethics and Permits

This project was performed with number permit 4495 Chilean Agricultural and Livestock Service and permits for capture of fishes numbers 1947 and 3199 (National Fishing Service).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sites of samples for *Australoheros facetus* in Chile.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Alzamora_Lobos_SupplementaryMaterial.xlsx