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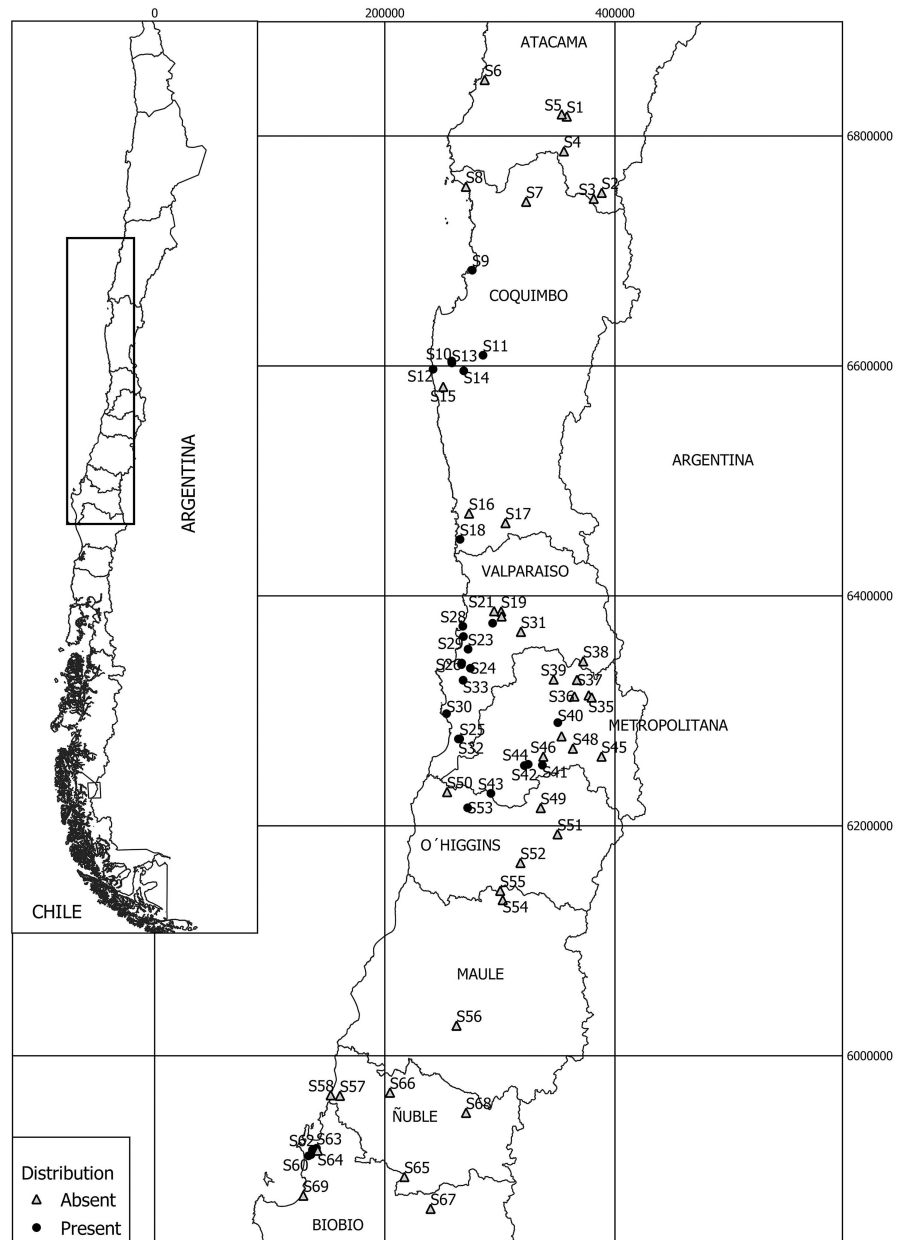
Figure 1. Distribution of *Australoheros facetus* in the Chilean Mediterranean region. The box in the left panel shows the study area in a national context.

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°26.515–71°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 × 40 × 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/m^2 for predator, 8.3 individuals/m^2 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 live-bearer; 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 km^2), 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 km^2) and the southern population 325 km from the central core (occupation area of 16 km^2).

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.
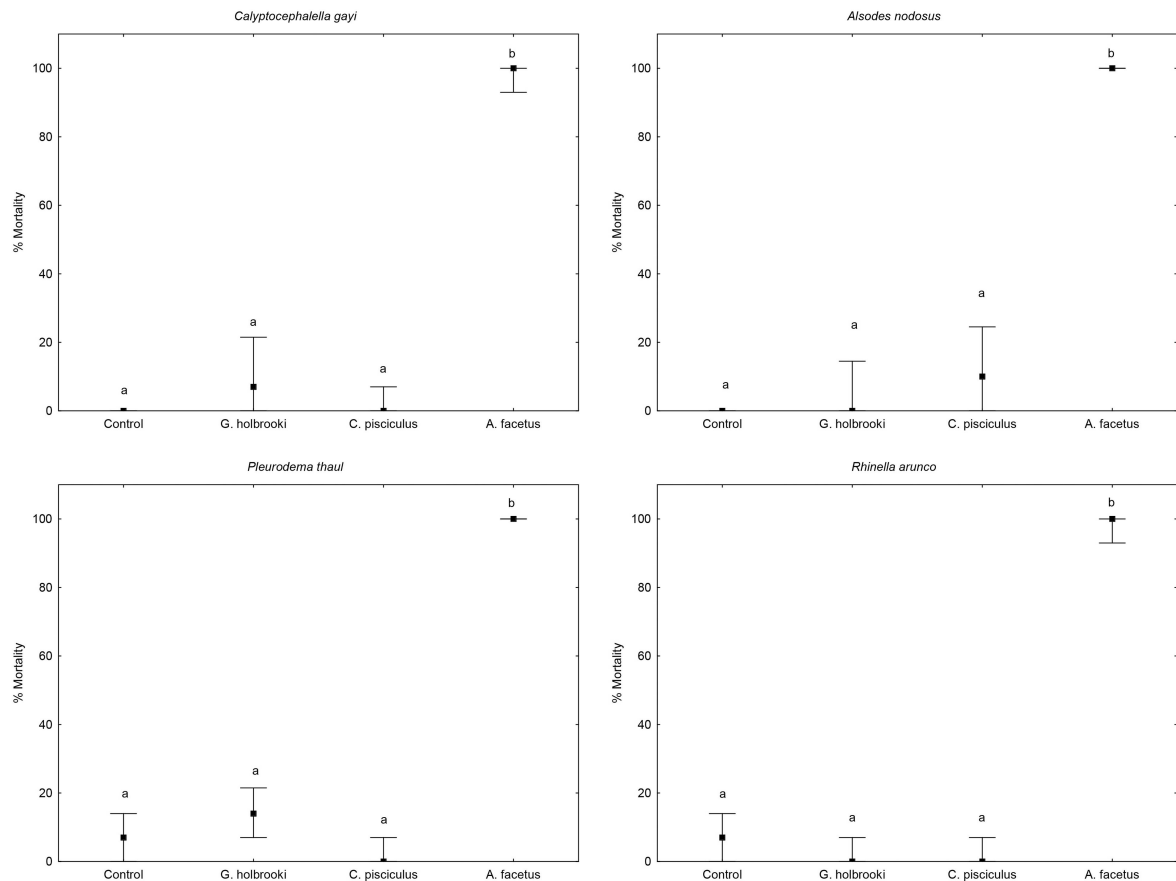
Figure 2. Mortality of anuran larvae by fish expressed as proportion of larvae replaced during the 7-day trial (maximal number of larvae replaced = 7). Median is represented by black square and ± interquartile values by whiskers (n = 4 replicates). Predator fish in the x axis were *Gambusia holbrooki, 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*.
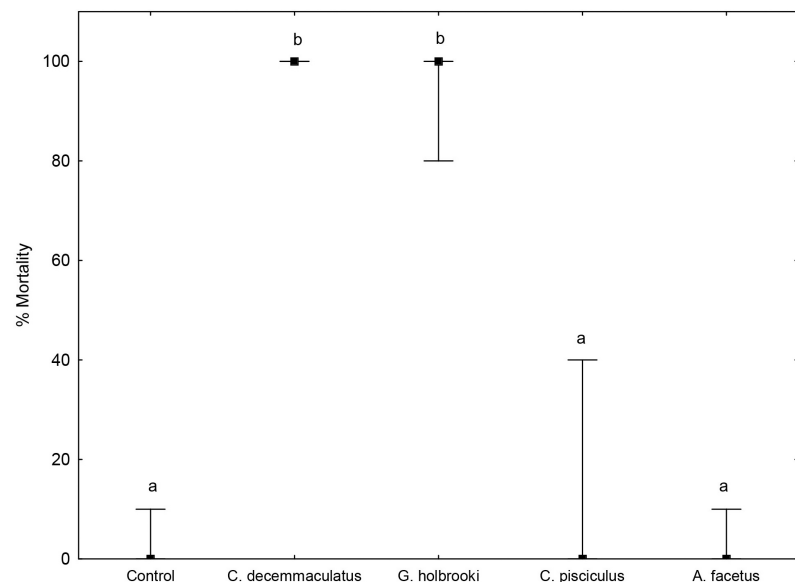

Figure 3. Mortality of eggs of *Xenopus leavis* expressed as proportion of eggs remaining after the 7-day trial (maximal number of eggs remaining = 10). Median is represented by black square and ± interquartile values by whiskers (n = 10 replicates). Predator fish in the x axis were *Cnesterodon decemmaculatus, 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).

References

Álvarez JA, Dunn C, Zuur AF (2002) Response of California red-legged frogs to removal of non-native fish. *Transactions of the western section of The Wildlife Society* 38/39: 9–12

Avilés J, López A, Flores H (2018) Interacciones tróficas de *Australoheros facetus* (Perciformes: Cichlidae), pez exótico en el humedal El Culebrón, Coquimbo, Chile. *Revista de Biología Marina y Oceanografía* 53: 99–105, https://doi.org/10.22370/rbmo.2018.53.0.1261

Baduy F, Saraiva JL, Ribeiro F, Canario AVM, Guerreiro PM (2020) Distribution and risk assessment of potential invasiveness of *Australoheros facetus* (Jenyns, 1842) in Portugal. *Fishes* 5: 1–17, https://doi.org/10.3390/fishes5010003

Barrionuevo JS, Ponssa LM (2008) decline of three species of the genus *Telmatobius* (Anura: Leptodactylidae) from Tucumán province, Argentina. *Herpetologica* 64: 47–62, https://doi.org/10.1655/06-057.1

Blaustein AR, Kiesecker JM (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters* 5: 597–608, https://doi.org/10.1046/j.1461-0248.2002.00352.x

Brodie ED, Formanowicz DR (1987) Antipredator mechanisms of larval anurans: protection of palatable individuals. *Herpetologica* 43: 369–373

Casal C (2006) Global documentation of fish introductions: the growing crisis and recommendations for action. *Biological Invasions* 8: 3–11, https://doi.org/10.1007/s10530-006-1833-0

Chivers D, Mirza R (2001) Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. *Journal of Chemical Ecology* 27: 45–51, https://doi.org/10.1023/A:1005663815856

Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9: 89–98, https://doi.org/10.1046/j.1472-4642.2003.00012.x

Correa C, Cisternas J, Correa-Solís M (2011) Lista comentada de las especies de anfibios de Chile (Amphibia: Anura). *Boletín de Biodiversidad de Chile* 6: 1–21

Correa C, Donoso JP, Ortiz JC (2016) Estado de conocimiento y conservación de los anfibios de Chile: una síntesis de los últimos 10 años de investigación. *Gayana (Concepción)* 80: 103–124, https://doi.org/10.4067/S0717-65382016000100011

Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M (1996) Plant diversity in Mediterranean climate regions. *Trends in Ecology and Evolution* 11: 362–366 https://doi.org/10.1016/0169-5347(96)10044-6

Crossland MR (1998) Ontogenetic variation in the toxicity of tadpoles of the introduced toad *Bufo marinus* to native Australian aquatic invertebrate predators. *Herpetologica* 54: 364–369

Crossland MR, Alford RA (1998) Evaluation of toxicity of eggs, hatchlings and tadpoles of the introduced toad *Bufo marinus* (Anura, Bufonidae) to native Australian aquatic predators. *Australian Journals of Ecology* 23: 129–137, https://doi.org/10.1111/j.1442-9993.1998.tb00711.x

De Buen F (1959) Los peces exóticos en las aguas dulces de Chile. *Investigaciones Zoológicas Chilenas* 5: 103–137

Diamond J, Case TJ (1986) Overview: Introductions, extinctions, exterminations, and invasions. In: Diamond J, Case TJ (eds), Community Ecology. Harper and Row, New York, pp 65–79

Díaz-Páez H, Ortíz JC (2003) Evaluación del estado de conservación de los anfibios de Chile. *Revista Chilena de Historia Natural* 76: 509–525, https://doi.org/10.4067/S0716-078X2003000300014

Dyer B (2000) Systematic review and biogeography of the freshwater fishes of Chile. *Estudios Oceanológicos* 19: 77–98

Fernández-Delgado C, Rossomanno S (1997) Reproductive biology of the mosquitofish in a permanent lagoon in south-west Spain: two tactics for one species. *Journal of Fish Biology* 51: 80–92, https://doi.org/10.1006/jfbi.1997.0413

Ferrari MC, Trowell JJ, Brown GE, Chivers DP (2005) The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Animal Behaviour* 70: 777–784, https://doi.org/10.1016/j.anbehav.2005.01.009

Ferrari MC, Capitania-Kwok T, Chivers DP (2006) The role of learning in the acquisition of threat-sensitive responses to predator odours. *Behavior Ecology Sociobiology* 60: 522–527, https://doi.org/10.1007/s00265-006-0195-z

Ferrari MC, Messier F, Chivers DP (2008) Larval amphibians learn to match antipredator response intensity to temporal patterns of risk. *Behavioral Ecology* 19: 980–983, https://doi.org/10.1093/beheco/arn056

Fischer WJ, Koch WA, Elepfandt A (2000) Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). *Journal of Zoology* 252: 99–107, https://doi.org/10.1111/j.1469-7998.2000.tb00824.x

Gamradt SC, Kats LB (1996) Effect of introduced Crayfish and Mosquitofish on California Newts. *Conservation Biology* 10: 1155–1162, https://doi.org/10.1046/j.1523-1739.1996.10041155.x

Goodsell JA, Kats LB (1999) Effect of introduced mosquitofish on Pacific treefrogs and the role of alternative prey. *Conservation Biology* 13: 921–924, https://doi.org/10.1046/j.1523-1739.1999.98237.x

Gosner KL (1960) A simplified, table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190

Grant EHC, Miller DA, Schmidt BR, Adams MJ, Amburgey SM, Chambert T, Cruickshank SS, Fisher RN, Green DM, Hossack BR, Johnson PTJ, Joseph MB, Rittenhouse TAG, Ryan ME, Waddle JH, Walls SC, Bailey LL, Fellers GM, Gorman TA, Ray AM, Pilliod DS, Price SJ, Saenz D, Sadinski W, Muths E (2016) Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports* 6: 25625, https://doi.org/10.1038/srep25625

Griffin AS, Blumstein DT, Evans CS (2000) Training Captive-Bred or translocated animals to avoid predators. *Conservation Biology* 14: 1317–1326, https://doi.org/10.1046/j.1523-1739.2000.99326.x

Griffiths RA, Schley L, Sharp ET, Dennis JL, Román A (1998) Behavioural responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. *Animal Behaviour* 55: 207–2014, https://doi.org/10.1006/anbe.1997.0596

Habit E, Victoriano P (2005) Peces de agua dulce de la Cordillera de la Costa. In: Smith-Ramírez C, Armesto J, Valdovinos C (eds), Historia, Biodiversidad y Ecología de la Cordillera de la Costa de Chile: Editorial Universitaria, Santiago, Chile, pp 392–406

Habit E, Dyer B, Vila I (2006) Estado de conocimiento de los peces dulceacuícolas de Chile. *Gayana (Concepción)* 70: 100–113, https://doi.org/10.4067/S0717-65382006000100001

Helfman GS (2007) Fish conservation: a guide to understanding and restoring global aquatic biodiversity and fishery resources. Island Press, 600 pp

Harrell FE (2001) Regression modeling strategies: with applications to linear models, logistic regression and survival analysis. Springer-Verlag, New York, 568 pp, https://doi.org/10.1007/978-1-4757-3462-1

Hurlbert S, Zedler J, Fairbanks D (1972) Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science* 175: 639–641, https://doi.org/10.1126/science.175.4022.639

Iriarte JA, Lobos GA, Jaksic FM (2005) Invasive vertebrate species in Chile and their control and monitoring by governmental agencies. *Revista Chilena de Historia Natural* 78: 143–154, https://doi.org/10.4067/S0716-078X2005000100010

Jara FG, Perotti MG (2006) Variación ontogenética en la palatabilidad de los renacuajos de *Bufo spinulosus papillosus*, Philippi, 1902 (Anura, Bufonidae). *Cuadernos de Herpetología* 19: 37–42, https://doi.org/10.4067/S0716-078X2005000100010

Kats LB, Petranka JW, Sih A (1988) Antipredator Defenses and the Persistence of Amphybian larvae With Fishes. *Ecology* 69: 1865–1870, https://doi.org/10.2307/1941163

Kiesecker JM, Chivers D, Blaustein A (1996) The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour* 52: 1237–1245, https://doi.org/10.1006/anbe.1996.0271

Kolar CS, Lodge DM (2000) Freshwater nonindigenous species: interactions with other global changes. 1st ed. Island Press, Washington DC, 384 pp

Komak S, Crossland MR (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* 27: 185–189, https://doi.org/10.1071/WR99028

Kupferberg S (1997) Bullfrog (*Rana catesbeiana*) Invasion of a California River: The Role of Larval Competition. *Ecology* 78: 1736–1751, https://doi.org/10.1890/0012-9658(1997)078[1736:BRCIOA]2.0.CO;2

LaRue MA, Nielsen CK (2008) Modelling potential dispersal corridors for cougars in midwestern North America using least-cost path methods. *Ecological Modelling* 212: 372–381, https://doi.org/10.1016/j.ecolmodel.2007.10.036

Lobos G (2020) Vulnerability of *Xenopus laevis* to *Gambusia holbrooki*: can the larval phase of the African clawed frog be the Achilles heel in its invasive potential? *Aquatic Invasions* 15: 529–541, https://doi.org/10.3391/ai.2020.15.3.11

Lobos G, Vidal M, Correa C, Labra A, Díaz-Páez H, Charrier A, Rabanal F, Díaz S, Tala C (2013) Anfibios de Chile, un desafío para la conservación. Ministerio de Medio Ambiente, Fundación Facultad de Ciencias Veterinarias y Pecuarias de la Universidad de Chile y Red Chilena de Herpetología, Santiago, 104 pp

Lobos G, Hernández J, Jaksic F (2014a) Antropización del paisaje e invasión de vertebrados exóticos en una quebrada de Chile central. In: Jaksic F, Castro S (eds), Invasiones Biológicas en Chile: causas globales e impactos locales. Ediciones Universidad Católica de Chile, pp 393–414

Lobos G, Mendez MA, Cattan P, Jaksic F (2014b) Low genetic diversity of the successful invasive African clawed frog *Xenopus laevis* (Pipidae) in Chile. *Studies on Neotropical Fauna and Environment* 49: 50–60, https://doi.org/10.1080/01650521.2014.912865

Manzur MI (2005) Situación de la Biodiversidad en Chile: Desafíos para la sustentabilidad. Programa Chile Sustentable. Lom Ediciones. Santiago, 208 pp

Marr SM, Marchetti MP, Olden JD, García-Berthou E, Morgan DL, Arismendi I, Day JA, Griffiths CL, Skelton PH (2010) Freshwater fish introductions in mediterranean-climate regions: are there commonalities in the conservation problem? *Diversity and Distributions* 16: 606–619, https://doi.org/10.1111/j.1472-4642.2010.00669.x

Martín-Torrijos L, Sandoval-Sierra JV, Muñoz J, Diéguez-Uribeondo J, Bosch J, Guayasamin JM (2016) Rainbow trout (*Oncorhynchus mykiss*) threaten Andean amphibians. *Neotropical Biodiversity* 2: 26–36, https://doi.org/10.1080/23766808.2016.1151133

Mirza RS, Ferrari MCO, Kiesecker JM, Chivers DP (2006) Responses of American toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour* 143: 877–889, https://doi.org/10.1163/156853906778017926

Morán-López R, Da Silva E, Pérez-Bote JL, Corbacho C (2006) Associations between fish assemblages and environmental factors for Mediterranean-type rivers during summer. *Journal of Fish Biology* 69: 1552–1569, https://doi.org/10.1111/j.1095-8649.2006.01221.x

Morgan DL, Hambleton SJ, Gill HS, Beaty SJ (2002) Distribution, biology and likely impacts of the introduced redfin perch (*Perca fluviatilis*) (Percidae) in Western Australia. *Journal of Marine and Freshwater Research* 53: 1211–1221, https://doi.org/10.1071/MF02047

Morgan DL, Gill HS, Maddern MG, Beaty SJ (2004) Distribution and impacts of introduced freshwater fishes in Western Australia. *New Zealand Journal of Marine and Freshwater Research* 38: 511–523, https://doi.org/10.1080/00288330.2004.9517257

Moyle P, Light T (1996) Fish invasions in California: do abiotic factors determine success? *Ecology* 77: 1666–1670, https://doi.org/10.2307/2265770

Myers JH, Simberloff D, Kuris AM, Carey JR (2000) Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* 15: 316–320, https://doi.org/10.1016/S0169-5347(00)01914-5

Olden JD, Kennard MJ, Pusey BJ (2008) Species invasión and the changing biogeography of Australian freshwater fishes. *Global Ecology and Biogeography* 17: 25–37, https://doi.org/10.1111/j.1466-8238.2007.00340.x

Ortiz-Sandoval JJ, Ortiz N, Cifuentes R, González J, Habit E (2009) Respuesta de la comunidad de peces al dragado de ríos costeros de la región del Biobío (Chile). *Gayana* 73: 64–75, https://doi.org/10.4067/S0717-65382009000100010

Pérez-Bote JL, López MT (2005) Life-history pattern of the introduced eastern mosquitofish, *Gambusia holbrooki* (Baird and Girard, 1854), in a Mediterranean-type river: the River Guadiana (SW Iberian Peninsula). *Italian Journal of Zoology* 72: 241–248, https://doi.org/10.1080/11250000509356678

Pyke GH (2005) A review of the biology of *Gambusia holbrooki* and *G. affinis*. *Reviews in Fish Biology and Fisheries* 15: 339–365, https://doi.org/10.1007/s11160-006-6394-x

R Core Team (2018) R: a language and environment for statistical computing. Version 3.3.1. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rojas P, Vila I, Habit E, Castro SA (2019) Homogenization of the freshwater fish fauna of the biogeographic region of Chile. *Global Ecology and Conservation* 19: e00658, https://doi.org/10.1016/j.gecco.2019.e00658

Ribeiro F, Orjuela RL, Magalhães MF, Collares-Pereira MJ (2007) Variability in feeding ecology of a South American cichlid: a reason for successful invasion in mediterranean-type rivers? *Ecology of Freshwater Fish* 16: 559–569, https://doi.org/10.1111/j.1600-0633.2007.00252.x

Říčan O, Kullander SO (2008) The *Australoheros* (Teleostei: Cichlidae) species of the Uruguay and Paraná River drainages. *Zootaxa* 1724: 1–51, https://doi.org/10.11646/zootaxa.1724.1.1

Ruiz V, Marchant M (2004) Ictiofauna de aguas continentales chilenas, Universidad de Concepción, Chile, 356 pp

Ruiz V, Moyano H, Marchant M (1992) Aspectos biológicos del pez exótico *Cichlasoma facetum* (Jenyns, 1842) (Pisces, Cichlidae) en aguas dulces de Concepción. *Boletín de la Sociedad de Biología de Concepción* 3: 193–201

Russell WMS, Burch RL (1959) The Principles of Humane Experimental Technique. Methuen, London, UK, xiv + 238 pp

Sanderson EW, Jaiteh M, Levy MA, Redford KN, Wannebo AV, Woolmer G (2002) The human footprint and the last of the wild. *BioScience* 52: 891–904, https://doi.org/10.1641/0006-3568(2002)052[0891:THFATL]2.0.CO;2

Santos MJ, Valenzuela F, Michaud P, Téllez R, Núñez S, Lira E, Winkler MI, Boisier ME, Méndez G (2009) Aspectos bioéticos de la experimentación animal: 4to. Taller de Bioética. Comité Asesor de Bioética Fondecyt-Conicyt, Santiago de Chile, 140 pp

Skelly DK (1994) Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour* 47: 465–468, https://doi.org/10.1006/anbe.1994.1063

Skelton PH (2002) An overview of the challenges of conserving freshwater fishes in South Africa. In: Collares-Pereira MJ, Cowx IG, Coelho MN (eds), Conservation of freshwater fishes: options for the future Fishing News Books, Blackwell Science, Oxford, pp 221–236

Soto-Azat C, Valenzuela A, Ortiz JC, Díaz-Páez H, Castro C, Charrier A, Correa C, Cuevas C, Lobos G, Méndez M, Penna M, Peñafiel A, Rabanal F, Vélez CM, Vidal M, Angulo A (2015) ASG Chile Leads Update of the Extinction Risk of Chilean Amphibians for The IUCN Red List of Threatened Species. *FrogLog* 23(4): 6–7

Soto-Azat C, Peñafiel-Ricaurte A, Price SJ, Sallaberry-Pincheira N, Garcíía MP, Alvarado-Rybak M, Cunningham AA (2016) *Xenopus laevis* and Emerging Amphibian Pathogens in Chile. *EcoHealth* 13: 775–783, https://doi.org/10.1007/s10393-016-1186-9

Vargas P, Arismendi I, Gomez-Uchida D (2015) Evaluation taxonomic homogenization of freshwater fish assemblages in Chile. *Revista Chilena de Historia Natural* 88: 16, https://doi.org/10.1186/s40693-015-0046-2

Vila I, Pardo R, Dyer B, Habit E (2006) Peces límnicos: diversidad, origen y estado de conservación. In: Vila I, Veloso A, Schlatter R, Ramírez C (eds), Macrófitas y Vertebrados de los sistemas límnicos de Chile. Santiago, Chile. Editorial Universitaria, pp 73–102

Vila-Gispert A, Alcaraz C, García-Berthou E (2005) Life-history traits of invasive fish in small mediterranean stream. *Biological Invasions* 7: 107–116, https://doi.org/10.1007/s10530-004-9640-y

Wassersug RJ (1971) On the comparative palatability of some dry-season tadpoles from Costa Rica. *American Midland Naturalist* 86: 101–109, https://doi.org/10.2307/2423690

Welsh HH, Pope KL, Boiano D (2006) Sub-alpine amphibian distributions related to species palatability to non-native salmonids in the Klamath mountains of northern California. *Diversity and Distributions* 12: 298–309, https://doi.org/10.1111/j.1366-9516.2006.00254.x

Supplementary material

The following supplementary material is available for this article:

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