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Research Article

# Predatory interactions between two global aquatic invaders beyond their native ranges: An experimental approach

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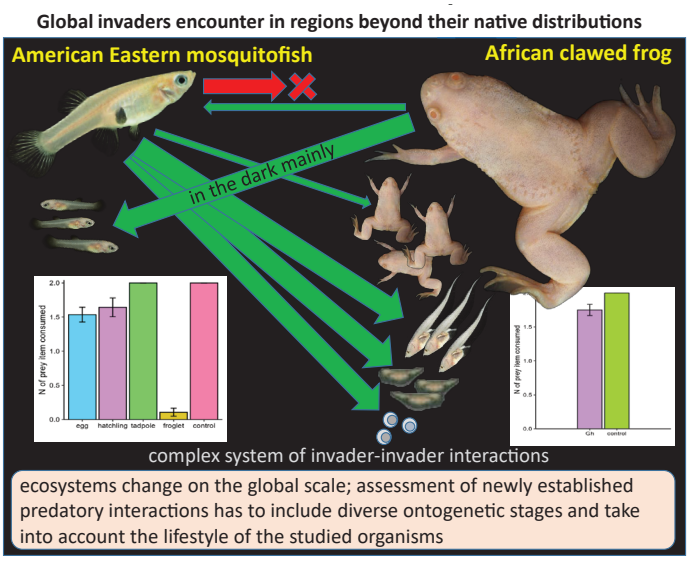
## Abstract

The widespread distribution of invasive species inevitably leads to the emergence of a new category of biotic relationships: interspecific predatory interactions between invasive species. We assessed the vulnerability of different life stages of the clawed frog, *Xenopus laevis* (eggs, hatchlings, tadpoles, newly-metamorphosed froglets, and adults), to predation by the Eastern mosquitofish, *Gambusia holbrooki*, and evaluated whether adult clawed frogs prey upon juvenile and adult mosquitofish. The results confirm differences in the palatability of different ontogenetic stages of the clawed frog by mosquitofish, as well as the low palatability of mosquitofish for adult frogs, with the exception of fish juveniles, which are relatively protected in the light but highly vulnerable under low-light conditions. Therefore, these fish-amphibian interactions are complex and can be defined as unequal bidirectional predation: the mosquitofish readily eliminates its early-stage opponent, but may become prey for its adult individuals. The revealed existence of a vulnerable mosquitofish stage makes it difficult to predict the unconditional suppression of clawed frog populations by this fish species when these two global aquatic invaders become syntopic in new regions. In any case, mosquitofish are likely to dominate in urban water bodies in locations with constant artificial lighting. We emphasise the importance of studying interactions at all ontogenetic stages, as well as taking into account the lifestyle of the organisms studied when analysing the mechanisms of predatory interactions between any pairs of invasive species in new areas of the planet.

## Graphical abstract

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**Key words:** *Gambusia holbrooki*, Global invasions, invasion ecology, predatory interactions, *Xenopus laevis*

## Introduction

The widespread distribution of invasive species inevitably leads to the emergence of a new category of biotic relationships: interspecific interactions between invasive species. Such interactions may be neutral, unidirectional or bidirectional; the establishment of some invaders may facilitate or prevent the invasions of other organisms (Jackson 2015). There are comparatively few studies of interactions between invaders, but their importance is increasing as the biota change (Sheppard et al. 2018; Arismendi et al. 2020).

Some invasive organisms are now globally distributed. One such species is the Eastern mosquitofish (*Gambusia holbrooki* Girard, 1859), which originates from North America (Francis and Hardwick 2012). This fish and a closely related one (*G. affinis* (Baird & Girard, 1853)) were long considered to be a single biological species until 1988 (Wooten et al. 1988). It is assumed that they have very similar biology and behaviour. Many contemporary reviews combine the description of both species into single papers (Pyke 2005; Walton et al. 2012). The main reason for the human-mediated geographical spread of *G. holbrooki*, along with *G. affinis*, is its use for mosquito control (Pyke 2005). This small-sized omnivorous fish has a generalist diet which includes a wide range of animal prey: from ciliates and microcrustaceans to small-sized aquatic vertebrates (Pyke 2008). Invasive mosquitofish populations demonstrate an undesirable impact on native ecosystems (Hurlbert et al. 1972; Hinchliffe et al. 2017). Today, populations of *G. holbrooki* exist on all continents except Antarctica; its invaded range includes at least 92 countries (Reshetnikov et al. 2018). This fish invader ranks fifth among 551 non-native fish species in terms of the number of river basins colonised on a global scale; the most invaded areas are Australia, where *G. holbrooki* ranks 1$^{st}$ among other fish invaders, and the Palearctic, where it ranks 4$^{th}$ (Bernery et al. 2022).

Similar to the fish species presented above, the clawed frog (*Xenopus laevis* (Daudin, 1802)) is a global invader (Measey et al. 2012). This anuran amphibian belongs to the family Pipidae and originates from Southern Africa. After metamorphosis, the adult clawed frogs remain in the water and exhibit an aquatic lifestyle. Its larvae are filtrators whereas adults consume diverse animal prey including comparatively large invertebrates and even vertebrates such as amphibians and fish (Courant et al. 2017). From the 1930s to the 1960s, this amphibian was widely used to test pregnancy (Elkan 1938) and gradually became extremely popular for various scientific laboratory investigations and as a pet. As a result, *X. laevis* is exported outside its native range in Southern Africa (Weldon et al. 2007), bred in captivity and sold in pet shops worldwide (Gurdon 1996). This amphibian species has been introduced into many new countries outside its native distribution (Measey et al. 2012). In new areas, this frog interacts with native biota, affecting local species through competition, predation, and transmission of infectious diseases (Amaral and Rebelo 2012). The clawed frog has been noted as an asymptomatic agent in the spread of the pathogen of the emerging amphibian fungal disease (*Batrachochytrium dendrobatidis*) and ranavirus (Fisher and Garner 2007; Reeder et al. 2012; Peñafiel-Ricaurte et al. 2023). To date, *X. laevis* has been found on all continents except Australia and Antarctica (Rödder et al. 2017).

The climatic niches of this frog and the aforementioned mosquitofish, used for mosquito control, overlap as shown by species distribution models; regions of actual sympatry of these global invaders have already been revealed on all the continents where the frog has naturalised (Ihlow et al. 2016; Jourdan et al. 2021). Furthermore, there is evidence that both the clawed frog and the mosquitofish exhibited climatic niche enlarging as they colonise new areas (Rödder et al. 2017; Jourdan et al. 2021).

Throughout its vast invaded range, the mosquitofish inevitably encounters other aquatic invaders, resulting in the establishment of new trophic interactions. For example, the invasive mosquitofish co-occurs and interacts with the cane toad (*Rhinella marina* (Linnaeus, 1758)) in Australia, the Louisiana crayfish (*Procambarus clarkii* (Girard, 1852)) in Europe, the frog *X. laevis* in South America and other continents (Komak and Crossland 2000; Anastácio et al. 2011; Lobos 2020). Experimental studies have shown that the mosquitofish avoids feeding on the eggs and tadpoles of the cane toad, but the brief hatchling ontogenetic stage has been considered vulnerable to fish predation (Komak and Crossland 2000). The mosquitofish can consume newly hatched Louisiana crayfish, but is eaten by large adult crayfish individuals (Anastácio et al. 2011). Thus, unidirectional and bidirectional predatory interactions between mosquitofish and other invaders are possible. However, many aspects of the alien-alien interactions remain unknown. For example, it has been suggested that mosquitofish in Chile may negatively affect populations of the introduced clawed frog but detailed analyses indicate that the clawed frog population began to decline prior to the date of documented mosquitofish population growth (Lobos 2020). In addition, ontogenetic stages of the clawed frog that are vulnerable to mosquitofish predation and the potential consumption of the mosquitofish by this invasive amphibian remain unknown. Both the mosquitofish and the clawed frog are classic invaders with early sexual maturity, high fecundity, and a broad diet. They are tolerant to a wide range of environmental conditions, and readily colonise heavily modified anthropogenic landscapes (Pyke 2008; Francis and Hardwick 2012). Both the clawed frog and the mosquitofish have similar habitat requirements, preferring shallow, well-vegetated warmed areas of aquatic sites with still or slowly-moving water (Pyke 2008; Francis and Hardwick 2012). However, the possibility of long-term coexistence of populations of these two invaders in the same water body is questionable, as they are active predators with significant potential to impact on syntopic hydrobionts (Lafferty and Page 1997; Hamer 2022).

The aim of this study is to investigate the predatory interactions between two globally invasive species: the Eastern mosquitofish (*Gambusia holbrooki*) and the African clawed frog (*Xenopus laevis*). We hypothesised that different ontogenetic stages of the frog are vulnerable to varying degrees of predation by mosquitofish and that mosquitofish themselves can be preyed upon by adult frogs under certain conditions. To this end, we conducted a series of controlled laboratory experiments to assess the palatability, understood as ability to consume, of *X. laevis* eggs, hatchlings, tadpoles, and metamorphs to mosquitofish. In parallel, we evaluated the palatability of juvenile and adult *G. holbrooki* to adult clawed frogs. Given that *X. laevis* is a predominantly nocturnal predator, we also conducted additional trials under low-light conditions to explore its predation efficiency during the dark phase of the diel cycle.

## Materials and methods

### Animals and their maintenance

The Eastern mosquitofish, *Gambusia holbrooki*, were collected on 13 April 2022 with a deep-net from a pond (43.4263N; 39.9731E) on the Black Sea coast of the Caucasus in Krasnodar Krai at south of the Russian Federation. The collection of *G. holbrooki* is not regulated in Russia as this fish is recognised as invasive (Reshetnikov et al. 2018). The sizes of the fish were: a total length (TL) of 33.7 ± 0.8 (25.0–41.0) mm and a body mass (m) of 0.33 ± 0.03 (0.11–0.63) g; N = 20 (mean values and their standard errors are given throughout the article). The fish were transported to the laboratory. Prior to the experiment, the fish were kept in 45 L aquaria with density 1 fish individual per liter. We used aged tap water with the following values of hydrochemical characteristics: oxidation-reduction potential (ORP) 126.1 ± 1.4 mV; pH 7.14 ± 0.01; concentration of dissolved oxygen (DO) 8.59 ± 0.04 mg/L (Aqua TROLL® 600, the USA); ammonium concentration of < 1.0 $NH_3/NH_4$ mg/l (NILPA®, Russia). Water was changed partially every other day. For preliminary acclimation after transportation to the laboratory, fish were kept in 45 L aquaria at least 10 days before the start of experimental work.

We also used artificially bred clawed frog *X. laevis*. Prior to the experiment, eggs and hatchlings were kept in aquaria with aged tap water (hydrochemical parameters as presented above) at water temperature of 4–5 °C. This low temperature was used to delay the development of eggs and hatchlings in order to have available materials for the experiment by the right date. After a period of keeping at low temperature, eggs and hatchlings remained alive and undamaged, which was confirmed by us: some individuals did not participate in the experiments and later underwent normal development and reached sexual maturity. The metamorphs were kept in 45 L aquaria filled to 35 L (10 individuals per aquarium) and fed with Chironomidae larvae *ad libitum* every other day. Adult clawed frogs were kept under the same conditions but 14 individuals per 45 L aquarium with frequent water changes. Larvae, metamorphs, and adults were kept at a water temperature of 19–21 °C. Sizes (length and mass) of different ontogenetic stages are presented in the Table 1. All the measurements of length were made with an accuracy of 1 mm. The mass was measured with 2 mg accuracy with balances KERN® CM 50-C2N (KERN & Sohn GmbH, Germany), excepting adult frogs, which were measured with balances VK-300.1 (Massa-K®, RF) with 100 mg accuracy. Amphibian sizes were determined for 15 individuals of each of the stages studied.

### Experimental design

Each randomly chosen fish individual was placed in a separate aquarium (N = 28), 39.5 × 28.7 × 24.5 cm, containing 10 L of aged tap water (appr. 9 cm water column). Aquaria with females and males were alternated: 2 aquaria with females, 2 aquaria with males, etc. The experimental fish were visually isolated from each other using white heavy (235 g/m$^2$) paper dividers between aquaria. The artificial photoperiod was 06:00–23:00. Illumination sources (LED lamps, cold light 6500 K) were placed 45 cm above the water and produced 623 ± 34 lx on the water surface. Under these conditions, the fish began to actively consume the food (larvae of Diptera, Chironomidae) within the first day after being

**Table 1.** Size characteristics of different ontogenetic studies of the clawed frog *Xenopus laevis*, used in the experiments: Mean Length ± Standard Error (L, 1 mm), Length Range (minimum-maximum, mm), Mean Weight (W, 2 mg, 10 mg), Weight Range (mm), and number of measured individuals (n).

| Stage | Mean L, mm | Range L, mm | Mean W, Mg | Range W, mg | n |
|---|---|---|---|---|---|
| Egg | 2.0 | 2–2 | 4 | 4–4 | 15 |
| Embryo | 1.5 | 1.5–1.5 | 4 | 4–4 | 15 |
| Hatchling | 7.0 | 7–7 | 4 | 4–4 | 15 |
| Early tadpole | 7.9 ± 0.2 | 7–9 | 8 | 8–8 | 15 |
| Metamorph | 15.8 ± 0.4 | 13–19 | 417 ± 32 | 220–760 | 15 |
| Adult | 36.7 ± 0.5 | 33–43 | 5000 ± 200 | 3500–8100 | 15 |

placed in the aquarium. Nevertheless, the fish were left to acclimatise for five days. Hydrochemical parameters on the second day of acclimation (before partial water change) were: ORP 113.6 ± 1.3 mV; pH 7.28 ± 0.04; DO 8.50 ± 0.04 mg/L (Aqua TROLL® 600, USA); < 1.0 $NH_3$/$NH_4$ mg/l (NILPA®, Russia). All experiments were carried out in a thermostatically controlled laboratory room from December 2022 to November 2023 at a water temperature of 19–21 °C. This temperature is within the temperature range of tolerance and feeding activity of both species studied: mosquitofish and clawed frog (Casterlin and Reynolds 1980; El-Boray 2014; Wilson et al. 2000; our observations). To standardise the level of food motivation, fish were not fed for 24 h prior to the experiment. When a potential food was added to the aquarium, the food was visually isolated from the fish by an opaque plastic screen (9.5 × 25.5 cm) to prevent the fish from responding to the experimenter's hand movements. The screen was then removed from the aquarium with a vertical upward motion.

**i. Palatability of clawed frog to mosquitofish**. In the first experiment, 28 mosquitofish (14 females and 14 males; one individual per aquarium) were offered eggs of the clawed frog. All fish individuals were assigned randomly to two test subgroups. During the first round of the experiment, one half of the experimental fish (first subgroup: odd-numbered aquaria) were offered one amphibian egg (2 mm; 4 mg). Remaining fish (second subgroup: even-numbered aquaria) were offered the control food, two individuals of Chironomidae larvae (each worm was 10.5 ± 0.3 (8–14) mm, data presented from measurements of 20 specimens). The mass of a pair of insect larvae individuals was 6.0 ± 0.0 (6.0–6.0) mg. After 2 h, the uneaten food or its remains were removed and the experiment was continued after another 1 h. In the second round of the experiment, fish of the first subgroup were offered control food whereas fish of the second subgroup were offered amphibian egg. The next day, fish in each group were again offered amphibians and control food twice (first and second round), but in reverse order. Such design of the experiment was applied in order to exclude the potential effect of the sequence of offering the main and control prey species. For statistical analysis we summed the data obtained over two days of an experiment, transforming the results for each certain type of prey to a binary system: consumed / not consumed. During each round, no adjacent aquaria had the same treatment to minimise any bias that might have arisen from aquarium position. No additional food was offered to the fish at the end of the test in this experiment.

In the same way to the same well-acclimated group of fish individuals, we offered embryos, hatchlings, tadpoles (stage 25 according to Gosner 1960; stage 45 following Nieuwkoop and Faber 1994), and newly-metamorphosed froglets of the clawed frog, each in a separate experiment with comparison with control food. The sizes of the used frog stages are presented in Table 1. The sequence of experiments did not correspond to the order, from egg to froglets, listed above. In an experiment with a particular prey species/stage/condition, each individual fish participated once. Testing of individual tadpoles in palatability trials is an appropriate approach, because there are no differences in the survival of clawed frog tadpoles between individual vs. group offering to *G. holbrooki* (Lobos 2020). Beyond the main result of this experiment (assessment of the palatability of different prey types), we had the opportunity to compare the consumption of prey items in the first and second rounds and confirmed no significant differences in prey palatability between rounds (Suppl. material 1: fig. S1).

As part of the above-described series of experiments, a separate trial was used to assess possible differences in the palatability of two close but principally different stages of the clawed frog: early embryo (jelly capsule was removed mechanically by an experimenter) and hatchling (after leaving the capsule on its own). For this purpose, a direct comparison experiment was conducted: embryo vs. hatchling. In this experiment, 2 individuals of chironomid larvae were offered to the fish just after the trial to monitor the presence of food motivation. The presence of food motivation was assumed to be confirmed if chirinomidae larvae were consumed without residue.

As males of mosquitofish are noticeably smaller than females, this could theoretically affect their ability to consume some types of prey. The sizes of males were: TL = 30.1 + 0.6 (25–33) mm; m = 176 + 8 (110–215) mg (80.6 and 38.8% of female length and mass respectively). We used results of the above-described series of the experiments to compare consumption of objects by males and females. For statistical analysis, males and females were coded as 1 and 0 respectively.

**ii. Palatability of mosquitofish to adult clawed frogs.** We also conducted experiments to assess the ability of adult clawed frogs to catch and consume mosquitofish. The sizes of the adult frogs are presented in Table 1. Frogs were placed randomly one per aquarium with 10 L of aged tap water. Hydrochemical parameters of water on the second day of acclimation (before partial water change) were: ORP = 105.4 ± 1.6 mV; pH 7.28 ± 0.06; DO = 8.61 ± 0.03 mg/L (Aqua TROLL® 600, the USA); < 1.0 $NH_3$/$NH_4$ mg/l (NILPA®, Russia). The experiments were conducted at a water temperature of 19–21 °C according to protocol of the main experiment described above, i.e. over two days with two rounds per day. As before, chironomid larvae were used as control prey. First, we tested palatability of adult mosquitofish for frogs (2 h offering of the potential prey).

As consumption of adult fish prey was not revealed in the first experiment, we conducted an additional experiment with prolonged offering during 6 h to test if additional time is important for prey palatability.

To confirm the importance of fish defensive behaviour in escaping frog attacks and exclude version of possible tastelessness, we conducted an additional experiment in which adult frogs were offered live vs. dead adult mosquitofish during 2 h. At the end of the observation period (2 h) and after removal of the remaining fish individuals from the aquaria, the chironomidae larvae were offered to the frogs to confirm their food motivation.

We also tested the assumption that juvenile small fish individuals might be less able to avoid predators than large fish adults. The sizes of the offered juvenile fish were: TL = 19 ± 0.6 (17–23) mm; m = 70 ± 10 (36–136) mg; N = 20. The fish juveniles were offered in comparison with control food.

**iii. Palatability of mosquitofish to adult clawed frogs under low-light conditions.** Considering that the mosquitofish are diurnal and the adult clawed frogs are nocturnal, we have assumed that a frog predator could have an advantage when hunting under low-light conditions. Frogs were left to acclimate in aquaria during 5 days. We conducted an appropriate trial, offering adult mosquitofish vs. control prey during 2 h under low-light conditions (<0.01 lux). Results on prey consumption were recorded at the end of the offering period (2 h).

To test the assumption that frogs will be more successful predators after prolonged low-light period, we also applied modification of the experiment in a separate trial with the 6 h time of offering. Results were recorded at the end of 6 h of offering.

We also hypothesised that small-sized juvenile mosquitofish individuals may have been less protected (comparing to adult fish) from frog predation under low-light conditions. To test this hypothesis, we conducted a separate experiment with 2 h offering juvenile fish vs. control prey under low-light conditions.

### Data analysis

Non-parametric statistic criteria were used. Cochran's Q test (for multiple comparisons) and Sign Z test (for pairwise comparisons) were applied for assessment of comparative palatability. We used package stats from base R (R Core Team 2024) for Spearman correlation test, package coin (Hothorn et al. 2006) for Sign test and Cochran's Q test, package ggplot2 (Wickham 2016) for plots. The level of significance was accepted as 0.05. We present unadjusted p values. All conclusions in the Result section remain valid after applying the Holm-Bonferroni correction for multiple comparisons, except where specifically stated.

## Results

### Palatability of clawed frog for mosquitofish

In all cases, control prey (Chironomidae) was consumed immediately after the first attack, i.e., during the first few seconds after the start of the trial. Mosquitofish readily consumed clawed frog eggs and hatchlings; the mean number of these prey items consumed was 1.5 ± 0.1 (0–2) and 1.6 ± 0.1 (0–2) respectively. The tadpole was the most palatable stage; the fish consumed all tadpoles offered (Fig. 1). Newly-metamorphosed froglet (metamorph) was the least consumed stage: 0.11 ± 0.06 (0–1). The prey item significantly influenced the degree of palatability ($Q = 82.5$; $N = 28$; $df = 4$; $p < 0.001$). When transformed to a binary scale (consumed/not consumed – summary for two experimental rounds), the palatability level of the least edible stage (froglet) was significantly different from the palatability of the egg ($Z = -4.90$; $N = 28$; $p < 0.001$). The differences in palatability between newly-metamorphosed froglets and other stages are even greater (Fig. 1). Except for froglets, differences between other stages are not significant ($Z \leq 1.5$; NS). Fish consumed eggs, hatchlings, and tadpoles as a whole.

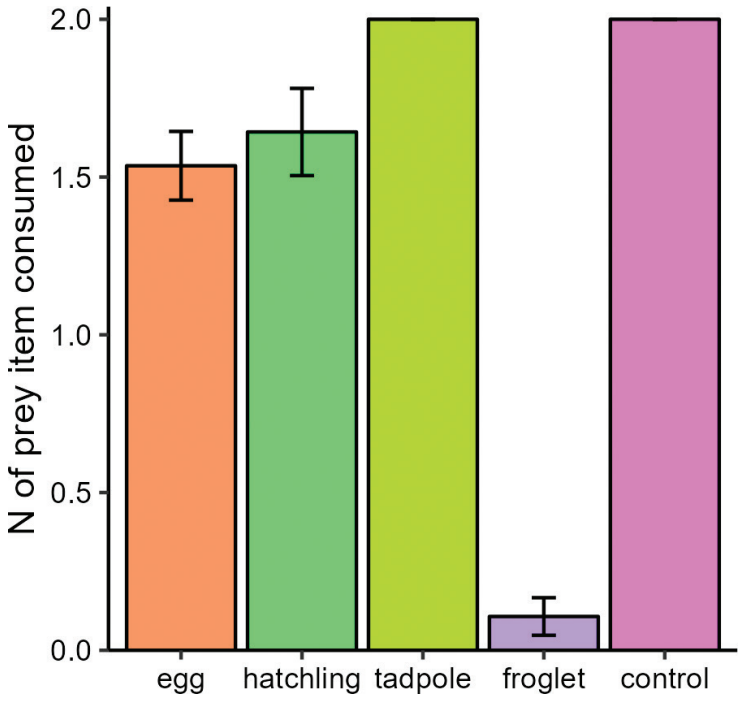


**Figure 1.** Palatability (means ± SE) of different ontogenetic stages of the clawed frog *Xenopus laevis* for the mosquitofish *Gambusia holbrooki*.

Unlike other stages, fish did not consume whole newly-metamorphosed froglets, but some froglets were fatally injured by the fish and died. The injured froglets were partially eaten; their mass was decreased on 12–44 mg or 8% of body mass. It is noteworthy that these newly-metamorphosed froglets did not attempt to attack the adult fish but tried to escape from the fish predator. All fishes that consumed amphibians remained alive with no signs of stress.

Both females and males of mosquitofish readily consumed eggs, hatchlings, and tadpoles of the clawed frog (Suppl. material 1: fig. S2), but females tended to consume a slightly greater number of frog eggs compared to males (Spearman correlation test: rho = -0.44; N = 28; S = 5273.0; p = 0.02; P2crit = 0.017 following Holm-Bonferroni correction). In our experiment, a small number of newly-metamorphosed froglets were injured (Suppl. material 1: fig. S2). Fish males did not injure this type of prey; all froglets were injured and partially consumed by females only (Suppl. material 1: fig. S2).

In a trial with direct testing of palatability of the clawed frog embryo comparing with the hatchling, fish consumed a high number of the offered embryo (Suppl. material 1: fig. S3). The level of palatability was similar to that of the hatchling and did not differ significantly (Z = -1.41; N = 28; p = 0.50).

## Palatability of mosquitofish for adults of the clawed frog

In the experiment with one-year-old adult frogs (as predator) and adult mosquitofish (offered), the fish did not demonstrate any predatory behaviour. In contrast to the fish, each frog performed multiple but unsuccessful attempts to catch the offered fish. Frogs attacked potential prey from a short distance of 1–2 cm. Despite obvious food motivation, no frogs caught the adult fish during 2 h. We repeated this experiment using a longer time period, 6 h offering, and obtained an identical

result: the frogs failed to catch the fish during the prolonged period. The difference in the observed palatability of the control prey (Chironomidae) and the adult mosquitofish is significant ($Z = 5.29$; $N = 28$; $p < 0.001$).

In an additional experiment, adult frogs were offered live vs. dead adult mosquitofish for a period of 2 h. In this experiment, frogs did not eat live adult mosquitofish but consumed all the dead fish offered. The difference in palatability between live and dead fish is significant ($Z = 5.29$; $N = 28$; $p < 0.001$). The sizes of the consumed fish were TL = 33.9 ± 0.6 (27–44) mm, m = 404 ± 27 (142–890) mg, N = 56. These fish were 71–123% in length and 2–18% in mass of the appropriate frog predator. The frogs swallowed fish either from the head or tail end. Parts of large fish individuals were seen sticking out of the frog's mouth. All frogs that consumed fish remained alive with no negative consequences.

In experiments with adult frogs and juvenile mosquitofish, one out of 20 frogs consumed two fish individuals and two frogs consumed one fish each during two rounds. In all cases, the prey was consumed whole. One frog (L = 32 mm, m = 3.3 g) consumed fish prey representing 53% of its length and 2.3% of its mass over two days and the other two frogs consumed fish individuals representing 58 and 62% of frog's length and 1.6 and 2.2% of its mass, respectively. The difference in palatability of test and control prey is significant ($Z = 4.12$; $N = 20$; $p < 0.001$) (Fig. 2b).

### Palatability of mosquitofish for adults of the clawed frog under low-light conditions

No adult mosquitofish were eaten by frogs during a 2-hour trial under low-light conditions; the difference in consumption of test and control prey is significant: $Z = 5.29$; $N = 28$; $p < 0.001$.

In another experiment, during a longer time period (6 h) under low-light conditions, two out of 28 frogs had consumed mosquitofish: one prey fish per amphibian individual (Fig. 2a). The difference between the consumption of test and control prey is also significant: $Z = 5.10$; $N = 28$; $p < 0.001$. In this experiment, there were equal numbers of females and males. Both the consumed fish individuals were males. Their sizes were 29 mm, 252 mg and 28 mm, 238 mg. Their length was 72–83% and their mass 4–5% of a frog predator.

Contrary to the above-described results, the frogs effectively caught and consumed juvenile mosquitofish under low-light conditions: most of the fish were eliminated during 2 h period (Fig. 2c). All adult frogs tested consumed juvenile fish (one or two individuals per frog) as well as control prey during the two test rounds, showing no differences in palatability between these two prey items. All the studied predatory interactions are summarised in the Table 2.

## Discussion

In this way, we have demonstrated reciprocal predation of two studied aquatic invaders of different systematic affiliations. The results confirm differences in palatability of various ontogenetic stages of the clawed frog for mosquitofish, as well as low palatability of adults and high palatability of juveniles of this fish for adult stage of the frog. Vulnerability of juvenile fish to frog predation depends on illumination conditions. These fish-amphibian interactions are complex and their understanding requires more detailed analysis.

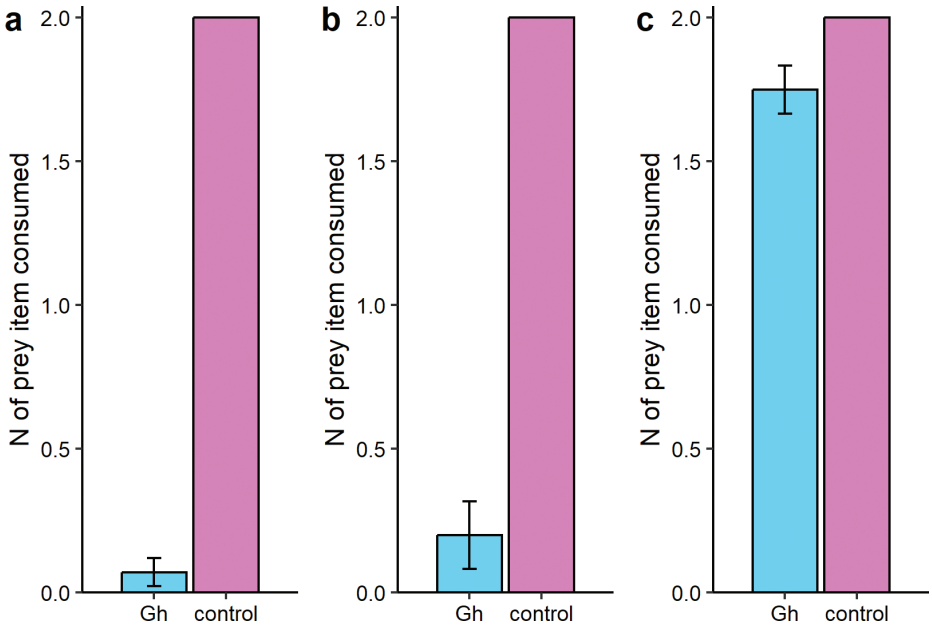


**Figure 2.** Consumption of mosquitofish *Gambusia holbrooki* by adults of the clawed frog *Xenopus laevis* under different conditions. **a.** Consumption of adult mosquitofish by adults of the clawed frog (N = 28) during a long period (6 h) under low-light conditions; **b.** Consumption of juvenile mosquitofish by adults of the clawed frog (N = 20) during a 2 h test in the light; **c** Consumption of juvenile mosquitofish by adults of the clawed frog (N = 28) during a 2 h test under low-light conditions.

**i. Palatability of clawed frog to mosquitofish**. Mosquitofish predation efficiency varies across ontogenetic stages of *X. laevis*. Tadpoles were the most vulnerable stage, while newly-metamorphosed froglets were the least vulnerable. These amphibian stages do not have any toxic or deterrent defense chemicals, as all fish individuals that consumed this amphibian, remained alive with no adverse effects. The fish do not regurgitate this prey. Remarkably, fishes usually ignore or reject amphibian eggs (Azevedo-Ramos and Magnusson 1999; Reshetnikov 2008) due to the protective function of the egg jelly capsule, and *G. holbrooki* often exhibits such behaviour (Komak and Crossland 2000; Reynolds 2009). Nevertheless, mosquitofish can effectively prey on amphibian eggs in some cases (Grubb 1972; Komak and Crossland 2000). Grubb (1972) described differences in vulnerability of amphibian eggs to fish predation. He found that mosquitofish rejected large eggs because of the mechanical defence provided by a thick jelly capsule, but consumed small eggs less than 3 mm. This corresponds well with our results obtained for clawed frog eggs, which are relatively small (2 mm) and have a comparatively thin jelly capsule, which does not protect the embryo from fish predation. Additionally, our results showed that mosquitofish successfully detected this type of prey despite its immobility. We have also revealed that the clawed frog embryo (without a jelly capsule) has no special defensive mechanisms and, similar to hatchling, is edible to the fish. As mentioned above, vulnerability decreases markedly after tadpoles reach the metamorph stage. Then, large-sized adult frogs, i.e., several months after metamorphosis and older, are not vulnerable to mosquitofish predation at all. We have no information on possible chemical protection of adult clawed frogs, but the most likely explanation for their relative (newly-metamorphosed froglets) and absolute (adults) invulnerability is their large size. Therefore, after metamorphosis and after increasing their mass, clawed frogs escape the predation pressure of mosquitofish.

**Table 2.** Predatory interactions between the Eastern mosquitofish *Gambusia holbrooki* and the African clawed frog *Xenopus laevis*. Dominant species/stage is marked in bold. Low palatability (less than 10% of prey consumed) is indicated by [square brackets] around the predator stage name.

| ***Gambusia*, stage** | ***Xenopus*, stage** | **duration, hours** | **illumination** |
|---|---|---|---|
| **adult** | eggs | 2 | light |
| **adult** | embryo | 2 | light |
| **adult** | hatchling | 2 | light |
| **adult** | tadpole | 2 | light |
| **[adult]** | postmetamorph | 2 | light |
| adult | adult | 2 | light |
| adult | adult | 6 | light |
| adult | adult | 2 | low-light |
| adult | **[adult]** | 6 | low-light |
| juvenile | **[adult]** | 2 | light |
| juvenile | **adult** | 2 | low-light |

Predatory abilities of male and female mosquitofish differ slightly. Although both sexes can damage and consume all early ontogenetic stages, females were somewhat more successful in overcoming the protective jelly capsule of frog eggs compared to males, which is obviously explained by the larger size of females. In contrast to the earlier stages listed, newly-metamorphosed froglets are only vulnerable to large-sized mosquitofish females. However, even the female mosquitofish could not swallow the frog metamorphs whole; they bit off parts of the metamorphs, causing fatal damages.

**ii. Palatability of mosquitofish to adult clawed frogs.** Clawed frog predation on mosquitofish is low under bright light. The frogs could not catch adult mosquitofish in the light. Frogs attack potential prey from a short distance and did not act fast enough. Mosquitofish show rapid behavioural responses and are able to, with rare exceptions, successfully escape frog predation. Absence of consumptions of fish by frogs cannot be explained by inactivity of the frogs during daytime, as they immediately consumed the control food offered. This phenomenon also cannot be explained by the toxicity or tastelessness of the fish, since the frogs readily ate immobilised (dead) mosquitofish. Adult clawed frogs ate and consumed juvenile individuals of this fish species under bright light, but the proportion of eaten fish individuals was comparatively low. Juvenile mosquitofish individuals are more vulnerable compared to adults because their size is smaller, hence they move shorter distances when trying to avoid a sudden predator attack. Apparently, these results are specific to the studied fish species under appropriate conditions (bright light) because adult clawed frogs are known to prey on some fish, along with a wide range of invertebrate prey and amphibians, under natural conditions (Lafferty and Page 1997; Amaral and Rebelo 2012; Courant et al. 2017).

**iii. Palatability of mosquitofish to adult clawed frogs under low-light conditions.** Clawed frog predation efficiency depends on illumination level. Contrary to mosquitofish. Adult clawed frogs are predominantly nocturnal (Casterlin and Reynolds 1980) with well-developed orientation abilities under low-light conditions due to olfactory, lateral line and electrical perceptions (Chum et al. 2013).

Contrary to the frog, the mosquitofish is a diurnal animal, as confirmed by our own aquarium observations: during the low-light at night, the fish become inactive and sink to the bottom. Mosquitofish are obviously less able to detect an approaching predator at low-light conditions. The vulnerability of juvenile mosquitofish individuals for predation increases under low-light conditions compared to bright-light conditions. Obviously, as stated above, their small sizes are the main reason. Possibly, the individual experience and limited skills of the juveniles do not matter here, as the frogs also were more successful in eating small-sized males compared to large-sized females in an experiment involving offering adult fish at low-light.

Our findings demonstrate that all early stages of the clawed frog are palatable to mosquitofish and can therefore help predict the vulnerability of this amphibian in natural habitats. Theoretically, additional factors may influence fish-amphibian predatory interactions in open aquatic sites: *e.g.*, the presence of alternative prey, habitat complexity, habitat segregation or geographical isolation.

Some authors emphasise the importance of alternative prey in assessing the fish behavioural responses (Pepin and Shears 1995; Manteifel and Reshetnikov 2002) however experiments focusing on mosquitofish predation on amphibian tadpoles have revealed that the fish readily prey on them even when high densities of alternative prey are present (*e.g.*, Goodsell and Kats 1999; Cabrera-Guzmán et al. 2017).

Although habitat complexity can reduce predation in many aquatic systems, our findings and previous studies (e.g. Baber and Babbitt 2004; Segev et al. 2009) indicate that mosquitofish are capable of foraging effectively even in dense submerged vegetation. This, combined with the pelagic and actively swimming behaviour of *X. laevis* tadpoles, suggests that structural habitat features may offer limited refuge from mosquitofish predation.

In temperate areas and adjacent regions, the reproduction of the clawed frog and development of its tadpoles occur during the warm season. The active feeding of the mosquitofish, a warm-water fish species, is restricted to the same period. Larvae of clawed frog are active during the day and/or around the clock, as confirmed by our observations in laboratory. Therefore, early life stages of the frog, including tadpoles, will be vulnerable to mosquitofish predation in nature despite the limited time of fish activity. Thus, early-life stages of the clawed frog are vulnerable to mosquitofish in areas with temperate climates and adjacent regions despite the period (sometimes long) of mosquitofish seasonal hypobiosis, *i.e.*, without active foraging.

An analysis of literature confirms that the studied species, the mosquitofish and the clawed frog, share the same habitats: mainly lentic shallow warmed-up water bodies, such as lakes, ponds, canals, or shallow-water parts of deep aquatic sites, but lotic environments also contribute, albeit to a lesser extent, to supporting populations and dispersal of these animals (Pyke 2008; Moreira et al. 2017). Without habitat segregation, the two species are doomed to behavioural interactions.

The invaded ranges of the two species studied already overlap on all the continents except Antarctica and Australia (Measey et al. 2012; Jourdan et al. 2021). Australia has been extensively invaded by *G. holbrooki*, but is (for now) free of feral populations of the clawed frog. The expansions of the two studied species are not yet complete. Their potential ranges, based on the climatic niches, partially overlap on all continents, including Australia (Measey et al. 2012; Jourdan et al. 2021). We expect a significant expansion of the geography of predatory interactions between the mosquitofish and the clawed frog on a global scale.

The clawed frog has a greater number of vulnerable stages compared to the mosquitofish and the duration of its vulnerable ontogenetic period is considerable: two to three months (Nieuwkoop and Faber 1994). The population density of mosquitofish is often extremely high, tens of individuals per $m^2$, and is comparable to the density of *Xenopus* larvae in fish-free water bodies (Lobos 2020), posing a significant potential threat to syntopic food items. Nevertheless, given the presence of a vulnerable stage in the mosquitofish, we cannot state unequivocally that the syntopy of these species in new regions of the planet will definitely lead to the decline or destruction of the clawed frog populations by the mosquitofish. Theoretically, the predatory invader that first colonises a certain aquatic site and reaches a high abundance of adult individuals may gain an advantage and, temporarily, or completely, suppress the development of a population of the second invader. Mosquitofish will likely dominate the clawed frog in urban water bodies in locations with 24-hour artificial lighting because in sufficient light, this fish species exhibits rapid defence reactions to effectively avoid predators.

In any case, predatory interactions between these two global aquatic invaders will inevitably serve to reduce the abundance of one or both these species. Hence, the peculiarities of their interactions, which we have revealed, can be considered, in the case of syntopy, as a positive factor for recipient ecosystems located outside the native ranges of the mosquitofish and clawed frog.

## Conclusions

Thus, the interaction between these two invaders is reciprocal (bidirectional) predation. Eastern mosquitofish (a diurnal predator) is a much more active predator compared to adult clawed frogs during the day and consumes all early ontogenetic stages of the frog (eggs, hatchlings, and tadpoles), whereas clawed frog adults (nocturnal predators) eliminate effectively mosquitofish juveniles under low-light conditions. This study highlights the importance of studying interactions at all ontogenetic stages, as well as taking into account the lifestyle of the organisms studied when analysing the mechanisms of predatory interactions between any pairs of invasive species in new areas of the planet.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

Experimental procedures were approved by the Ethics Committee of the A.N. Severtsov Institute of Ecology and Evolution, protocol #11 from 08.07.2017, prolongations from 14.05.2018, 23.04.2021, and 26.10.2022.

## Use of AI

No use of AI was reported.

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## Author contributions

Conceptualisation: ANR, ABP. Data curation: ANR. Formal analysis: ANR. Investigation: ABP, AOR. Methodology: ANR. Project administration: ANR. Resources: AAK, ANR, ABP, DVG. Supervision: ANR. Visualization: NGP. Writing - original draft: ANR. Writing - review and editing: ABP.

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## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Additional images

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Data type: pdf

Explanation note: **fig. S1.** Comparative consumption (means ± SE) of different ontogenetic stages of the clawed frog *Xenopus laevis* by *Gambusia holbrooki* (N = 28) in 1st and 2nd rounds of the experiment. **fig. S2.** Comparative consumption (means ± SE) of different ontogenetic stages of the clawed frog *Xenopus laevis* by females (N = 14) and males (N = 14) of the mosquitofish *Gambusia holbrooki*. **fig. S3.** Comparative consumption (means ± SE) of embryos and hatchlings (direct comparison) of the clawed frog *Xenopus laevis* for the mosquitofish *Gambusia holbrooki* (N = 28).

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