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Feral frogs, native newts, and chemical cues: identifying threats from and management opportunities for invasive African Clawed Frogs in Washington state

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Abstract

Invasive species threaten biodiversity globally. Amphibians are one of the most threatened vertebrate taxa and are particularly sensitive to invasive species, including other amphibians. African clawed frogs (*Xenopus laevis*, ACF) are native to ~~southern~~ Southern Africa but have subsequently become invasive on multiple continents - including multiple parts of North America - due to releases from the pet and biomedical trades. Despite their prevalence as a global invader, the impact of ACF remains understudied. This includes the Pacific Northwest of the USA which now hosts multiple expanding ACF populations. For many amphibians, chemical cues communicate important information, including the presence of predators. Here, we tested the role chemical cues may play in mediating interactions between feral ACF and native amphibians in the Pacific Northwest. We tested whether native red-legged frog (*Rana aurora*) tadpoles display an antipredator response to non-native frog (ACF) or native newt (rough-skinned newts, *Taricha granulosa*) predator chemical stimuli. We found that *R. aurora* tadpoles exhibited pronounced anti-predator responses when exposed to chemical cues from *T. granulosa*~~native newts (*Taricha granulosa*)~~ but did not display anti-predator response to invasive ACF chemical cues. We also began experimentally testing whether *T. granulosa* ~~newts~~ - which produce the powerful neurotoxin tetrodotoxin (TTX) – may elicit an anti-predator response in ACF by deterring co-~~occupation .~~occupation. However, our short-duration experiments found that ACF were attracted to newt chemical stimuli rather than deterred. Our findings show that ACF likely ~~pose~~poses a threat to native amphibians which may be particularly vulnerable to this invasive predator compared to native predators and because toxic native newts may not help limit ACF invasions. Our research provides some of the first indications that native Pacific Northwest species may be threatened by invasive ACF and provides a foundation for future experiments testing potential management techniques for ACF.

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Introduction

Invasive species threaten biodiversity globally (Didham *et al*., 2005; Didham *et al*., 2007; Pyšek and Richardson, 2010; Ahmed *et al*., 2022). While some effects of invasive species on native species and ecosystems are easily recognizable, other effects are challenging to identify. In some cases, native species responses that are behaviorally mediated may not be recognizable through consumptive measures (Simberloff *et al*., 2013). Understanding the impacts of invasive species on particular species or ecosystems is essential for appropriately allocating resources and coordinating management efforts (Epanchin-Niell *et al*., 2009).

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Amphibians globally have experienced tremendous losses and an estimated 41% of amphibian species are listed as threatened on the International Union for Conservation of Nature Red List (IUCN, 2023). Invasive species have contributed greatly to these declines as roughly 16% of threatened amphibian declines and approximately 30% of amphibian extinctions are at least partially attributed to invasive species (Falaschi *et al*., 2020). The threat of invasive species to amphibians may be greatest from aquatic invasive predators (Kats and Ferrer, 2003) due to predation, competition, hybridization, and disease (Falaschi *et al*., 2020). In North America, for example, native amphibians are not only threatened by invasive species in general but by competition with and predation by invasive amphibians (Meshaka *et al*., 2022).

Management approaches for aquatic invaders include diverse techniques including trapping and removal or euthanization, habitat modification, and chemical poisoning (Adams and Pearl, 2007; Lorrain-Soligon *et al*., 2021; Ojala-Barbour *et al*., 2021). One technique used with varying success for a range of invasive species includes the use of biocontrols. Biocontrols are living organisms that are introduced to an area or whose populations are enhanced to reduce an invasive species' population or impact (Stoner, 2023). While some biocontrol management plans have introduced new problems to ecosystems, the use of native biocontrols has been a successful approach in others (Messing and Wright, 2006). For example, large-bodied groupers (*Epinephelus striatus* and *Mycteroperca tigris*) have been found to actively consume invasive lionfish (*Pterios volitans/miles)* in the Caribbean (Mumby *et al*., 2011). Amplifying native species in some locations may help bolster biocontrol efforts.

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Animal behavior analyses have become essential tools for conservation and have aided in identifying the impacts of invasive species and effective management techniques (Holway and Suarez, 1999, Berger-Tal *et al*., 2011). Amphibians are a model species for understanding the role of chemical cues in mediating predator-prey relationships and various non-consumptive interactions (Kiesecker *et al*., 1996, Grayson *et al*., 2012). For instance, when presented with a visual cue, Western toad (*Bufo boreas*) tadpoles did not exhibit antipredator behavior, however in the presence of a predator chemical cue they display avoidance behaviors (Kiesecker *et al*., 1996). These same types of analyses can be informative for understanding invasive species impacts as well. For example, Pacific chorus frog (*Pseudacris regilla*) tadpoles exhibit avoidance behavior when exposed to chemical cues of invasive bullfrogs (*Rana catesbeiana*; Chivers *et al*., 2001). Further, red-legged frog (*Rana aurora*) tadpoles exhibited high anti-predator refuge use behavior in response to both native and invasive fish and crayfish predator chemical cues, whereas chorus frog (*Pseudacris regilla*) tadpoles only responded to native fish predators but not invasive fish or crayfish chemical cues (Pearl *et al*., 2003). *R~~ed legged frogs~~. aurora* also showed an increase in antipredator behavior when introduced to chemical cues for metabolic waste of tadpoles, showing a reduction in

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movement as a main response (Kiesecker et al. 1999). -Additionally, ~~red-legged frogs'~~ *R. aurora* behavioral responses to introduced bullfrogs varied by population (Kiesecker and Blaustein 1997). Further, non-native amphibians sometimes cannot recognize cues from native predators (Garcia et al. 2012, Marcheti and Beard 2021).

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The African clawed frog (*Xenopus laevis*, ACF) is a species native to ~~southern~~ Southern Africa (Sittert and Measey, 2016), but has been introduced to many countries around the world (Measey *et al*., 2012). ACF prey voraciously on a diversity of invertebrate and vertebrate animals in freshwater ecosystems (Fibla *et al*., 2020, Lillo *et al.*, 2011). ACF are likely successful invaders due to their generalist diet (Courant *et al.*, 2017) and fast maturation times and there is concern that invasive ACF may outcompete native species for shared prey items or directly consume and extirpate native species (Rödder *et al*.,2017). In the United States, ACF has become well established in Florida, Arizona, California, and Washington. ACF in Washington are particularly troublesome because they have spread across multiple cities and counties in the south Puget Sound area and the frogs seem to persist in ponds that freeze in winter (Ojala-Barbour *et al*., 2021).

Although ACF were first discovered in Washington in 2015, the threat of ACF to native species in Washington or the broader Pacific Northwest region is largely unknown nor is the degree of its spread beyond the three known regions where it currently occurs. Determining the threat to native aquatic species could help identify and refine management targets (Ojala-Barbour *et al*., 2021). However, current management tools for ACF in Washington are also sparse as prior eradication efforts using trapping and poisoning have failed (Ojala-Barbour *et al*., 2021). Thus, there is an urgent need to understand how much of a threat ACF poses to native species, particularly in this ~~region, and~~region, and what tools might be available to manage ACF.

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To address the knowledge gaps in our understanding of the degree of threat AFC pose to native amphibians ~~We~~ we used chemical behavioral analyses to explore the threat of and management options for ~~ACF~~this invasive frog. First, we tested whether larvae of a native amphibian species ~~(red-legged frogs;~~, *R. aurora*~~)~~ , respond to chemical cues from feral ACF differently than to native amphibian predator chemical cues. This goal emerged from observations showing that ponds without ACF have diverse native amphibian communities whereas adjacent ponds with ACF are devoid of native amphibian species (Figure 1; Friesen *et al*., unpubl). Second, we assessed whether native rough-skinned newts (*Taricha granulosa*) could be an effective biocontrol against ACF by testing whether feral ACF responded to newt chemical cues (including toxins). This goal emerged from two observations in early 2022. First, students at Saint Martin's University began assisting the ~~migrating~~ migration of newts across fence barriers that were meant to stop ACF from spreading (Figure 2). Although we were regularly catching, marking, and releasing ACF in the preceding fall, our trapping in Lacey, WA yielded no ACF once additional newts were added to the pond, despite concurrent trapping effort in Issaquah (similar latitude, ~ 100 km east) that yielded hundreds of ACF in similar sized ponds over the same timeframe.

Second, we temporarily housed an ACF with a newt in our husbandry facilities which resulted in the ACF dying in less than 24 h. These two observations led to the hypothesis that ACF avoided and/ or were harmed by newt toxins or other cutaneous chemicals.

The newt, *T. granulosa*, is native to Western Washington and is commonly found in and around rivers, creeks, and wetlands. This species must reproduce in water and can either live permanently in water or migrate upland after breeding. These newts are predators of amphibian larvae and so ACF may compete with newts for food. Further, the newts and other members of the genus *Taricha* have been the subject of intense study due to their robust cutaneous toxins, particularly tetrodotoxin (TTX; Vaelli *et al*., 2020). Research has shown that aqueous toxins exuded from these newts can elicit an antipredator behavioral response in larval amphibians, reduce the predatory success of dragonfly larvae, and cause invasive snails to migrate away (Zimmer *et al*., 2006; Bucciarelli and Kats, 2015; Ota *et al*., 2018). Accordingly, we predicted that native amphibian larvae would elicit an anti-predator response to a native newt but not an ACF and that ACF would be deterred by newt chemical cues.

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Methods ~~–~~

Species and sites

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We studied feral ACF captured from stormwater ponds in Lacey and Issaquah, WA and housed in a captive facility at the Saint Martin's University campus in Lacey (animal ethics SMUAE 22_1). Native species were captured from stormwater ponds (Ponds 1, 2, and 3) also in Lacey, WA ~~but,~~, where ACF are not present (Figure 1), with permissions from Washington Department of Fish and Wildlife. On 24 March 2022, two partial ~~native red-legged frog (~~*R. aurora*~~)~~ egg masses (~ 50 embryos each) were collected from the Ecology (ECY) stormwater pond ~ 1km northeast of the Lacey stormwater ponds where ACF inhabit (Figure 1). ACF are not known to inhabit the ECY pond. ACF were collected from ~~pond~~ Pond 1, and newts collected between ~~pond~~ Pond 1 and 2. ACF and 5 native newts were housed in small groups in 38 L tanks and fed dehydrated and frozen blood worms during the duration of our trials, with tanks cleaned daily or every other day. Tadpoles were housed independently in 0.47 L plastic containers and fed ground up fish flakes (Omega One Super Color Flakes) every other day. Animals were housed in the lab (not euthanized) after trials for future research. Newts were captive for at least two weeks prior to any trials.

Predator cues

We housed both partial egg masses together and ~~-~~*R. aurora* embryos hatched in aged tap water at room temperature from 25 May - 22 June 2022. We exposed tadpoles (Gosner stages 24 - 42) to chemical cues from *T. granulosa* ~~newts~~ (a native newt predator) and feral ACF. The tadpoles developed during the trials. *R. aurora* tadpoles were collected from an adjacent pond where no ACF were present. We made a chemical stimulus solution by soaking an adult newt or ACF in 300mL of aged tap water for two hours in separate 0 .47L containers (Figure 3). Untreated aged tap water was used as a control. After two hours, the adult amphibians were returned to the housing enclosures. We pipetted 2mL of the ACF cue, newt cue, or control water into *R. aurora* tadpole experimental containers containing 200 mL of aged tap water. The tadpoles were allowed two minutes of acclimation prior to recording behaviors. After these two acclimation minutes, we recorded tadpole behaviors for 10 minutes. At least three trials of each treatment were conducted each day. We completed 90 trials - 28 ACF cues and 31 each for newt cues and controls - using a total of 17 *R. aurora* tadpoles. Over the duration of our study, we exposed most tadpoles~~-~~ to all three treatments (control and two cue treatments), although some tadpoles were only exposed to two different treatment types across the study due to logistical constraints~~.~~. Three replicates of each treatment were done each day and tadpoles were assigned to treatments to ensure they were exposed to different treatments in subsequent trials. Experiments occurred at room temperature and no refugia were added given the small size of the experimental containers. We scored *R. aurora* larval behaviors into four behavior categories and recorded duration of each: nothing, foraging, swimming, and frantic swimming. We defined “nothing” as sedentary tadpoles displaying no movement, “foraging” as tadpoles exhibiting mouth movements and pecking at the bottom of the experimental containers, “swimming” as constant, slow movements in circular patterns around the containers, and “frantic swimming” (sometimes ~~also called~~referred to as~~-~~ rapid swimming in other studies; cite) as rapid, erratic movements in variable directions.

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Native newt biocontrol

Between 9 June 2022 and 8 September 2022, we performed behavioral choice tests on ACF exposed to newts to test whether ACF responded to newt cues. Adult ACF and newts were used in the biocontrol experiment and each animal was randomly selected from our husbandry facility. Each choice test was conducted in 2 L of aged tap water inside of a rectangular 38 L aquarium. The aquarium was divided into five sections along the long axis (Figure 4). Mesh pouches made of black window screening were placed inside of the aquarium, adjacent and parallel to each of the two short sides (Figure 4). One pouch was empty (control) and the other contained a newt (treatment). At the initiation of the experiment, we manually agitated each newt for one minute, by gently stroking the anterior and posterior sides to promote the production of tetrodotoxin (Bucciarelli and Kats, 2015). Newt~~s~~ movement and direct interaction were 180 constrained by the use of sealed pouches but still allowed ACF to be exposed to chemical and visual stimuli. An ACF was placed in the center of the tank, parallel to the mesh pouches and facing out of the aquarium. For ten minutes post-release we observed ACF behavior and the duration spent at various positions within the enclosure. We record ACF positions based on where they occurred across the five sections in the enclosure and the total amount of time spent in each section. When the ACF was on the section with the newt or the section adjacent to the newt, its position was recorded as “Newt” (Figure 4). When the ACF were in the middle fifth section, the time was recorded as “Center”. When the ACF was on the section with the empty mesh bag or the section adjacent to the empty mesh bag, the ACF’s position was recorded as “Away” from the newt. We performed a total of 50 ACF behavioral choice tests: 25 with the newt on the southwest side of the aquarium and 25 with the newt on the northeast side of the aquarium. We switched which side of the aquarium that newts were placed to ensure ACF were not responding to other confounding cues in the laboratory.

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TTX Analysis: We collected toxin samples from newts used in trials by repeatedly stroking the dorsal region of a newt anterior to posterior for one minute and then soaking it in 100 mL of aged tap water for one hour. After soaking the water solution was ~~aloquated~~aliquoted into 1.5 mL screw cap microtubules. The samples were prepared for TTX analysis following methods outlined in Ota et al. (2018). All samples were analyzed using a Shimadzu high-performance liquid chromatography system with fluorescence detection. The detection limit of the system is below femtomolar concentrations. We evaluated peak area of chromatograms against known TTX standards to determine whether TTX was present in solutions and if so, the approximate molar concentrations.

Statistical Analyses

Predator cues: For the predator cue data, we used linear mixed effects model (lmer function, ‘lme4’ package) and likelihood ratio tests (anova function) to test whether *R. aurora* tadpole behavior differed between the three treatments (Newt or ACF cues and Controls). We performed models for each of the three active behaviors separately (excluding ‘Nothing’). For random effects, we used tadpole identity as well as day-of-year (DOY) as a proxy for tadpole ontogeny and because tadpoles were used for the same treatment type on different days. We visually checked model fit.

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Native newt biocontrol: We used linear mixed effects models (*lmer* function, ‘lme4’ package) and likelihood ratio tests (*anova* function) to test whether feral ACF spent disproportionately more time near or away toxic native newts. We used trial day as a random effect in these models and our global model included the two fixed effects of Choice and Side. “Choice” included the three categories - Newt, Center, or Away - which represent the three regions of the experimental tanks where ACF spent time. The “Center” category was indicative of a frozen behavior, while movement towards the newt was considered “Newt,” and movement opposite was classified as “Away.” The “Side” category reflected the northeast or southwest orientation of the experimental tanks where newts were placed on each side for half of the trials. We used likelihood ratio tests to compare the global model to two reduced models containing only one variable and to compare the univariate models to a null model. If Choice was significant, we used Tukey’s post hoc tests (*glht* function, ‘multcomp’ package) to assess pairwise differences among Newt, Center, or Away choices. We performed all statistical analyses in R version 4.0.4 (R Core Team 2020).

Results

Predator cues: Our models on individual behaviors found differences in *R. aurora* ~~Swimming~~ swimming behavior (p = 0.03). Tukey’s post-hoc tests found that *R. aurora* tadpole Swimming rates were reduced in the newt treatment compared to Control treatments (p = 0.05). Tadpole ~~Swimming~~ swimming rates in response to ACF were statistically indistinguishable from both the Control (p = 0.87) and Newt treatments (p = 0.15). For f~~F~~rantic s~~S~~wimming (p = 0.09) and ~~Foraging~~ foraging (p = 0.89), our models found no differences in *R. aurora* behavior among treatments.

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Native biocontrol: Linear mixed effects models and likelihood ratio tests supported a model containing only the variable Choice (Figure 5; p =7.99 e -14). Tukey's post hoc tests found that all pairwise comparisons were significant (Center vs Away p = 1.0 e -0.4, Newt vs Away p = 0.003, Newt vs Center p = 1.0 e -04) such that feral ACF spent the least time in the Center third of the tanks (mean = 8.9 seconds, ± 1.0 seconds SE), intermediate amounts of time Away from newts (mean = 210.6 seconds, ± 4.9 seconds SE), and the most time next to the newts (mean = 368.5 seconds, ± 5.1 seconds SE).

TTX Analysis: We did not detect TTX in the sample solutions. Chromatograms showed no peak at the standard-derived elution time for TTX (Appendix Figure). It is possible that there are TTX analogues in the sample, but without commercially available standards, identification in the scope of this study is not possible. In general, the lack of a TTX peaks in the chromatogram indicates that TTX was at concentrations lower than 10x-15 moles/liter or possibly not present.

Discussion

We add to the growing body of evidence that feral ACF pose a threat to native aquatic species (Kruger *et al.*, 2019, Lafferty & Page 1997, Lillo *et al.*, 2011). Feral ACF may be a concerning, hard-to-manage invasive predator in the Pacific ~~northwest~~Northwest. Our results show that a native species may not recognize ACF as a predator~~s~~ and that toxic *T. granulosa*~~newts~~ may be challenging to use as native biocontrols against ACF; at least in the short term. In our experiments, native *R. aurora* tadpoles exhibited strong anti-predator responses to native newt chemical cues by decreasing foraging and increasing frantic swimming, but did not respond to ACF chemical cues. Interestingly, despite native tadpoles responding strongly to newt chemical cues, feral ACF did not respond to newts. These results underscore the threats that ACF poses to native species as a predator with few effective management options (Ojala-Barbour *et al*., 2021).

R. aurora tadpoles exhibit more antipredator behavior towards native newts than to invasive ACF. Newts elicited a classic anti-predator behavioral syndrome in tadpoles by causing tadpoles to be sedentary with bouts of frantic swimming compared to more typical cruising swimming and foraging behaviors (Watkins 1996, Laurila *et al*., 1997; Van Buskirk 2001; Bridges, 2002; Gabor *et al*., 2019,). ACF cues elicited no such response in *R. aurora* tadpoles. These findings suggest that native Pacific Northwest amphibians have evolved to exhibit antipredator behavior towards native predators but are unable to recognize invasive amphibian predator cues. This indicates *R. aurora* tadpoles are potentially vulnerable to ACF predation. However, the overall predation risk from ACF to *R. aurora* remains unclear as we did not perform feeding trials. Further research could clarify whether invasive ACF consume native amphibian larvae at high enough rates to cause population-level impacts. Additionally, because continued exposure

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to a predator cue can change the response of the cue receiver, it is possible that responses could have changed over the course of the trials (Kruger *et al.*, 2019).

For this work, we focused on antipredator behaviors in *R. aurora*~~red-legged frog~~ tadpoles – a species that has a relatively stable population status, despite ~~-~~experiencing declines primarily due to forest loss (Washington herp atlas, 2009). Beyond the direct potential impacts to *R. aurora*, this work highlights how ACF may be a threat to other native species - including more sensitive species - which may not recognize it as a predator. For instance, our source ACF population in Lacey, WA is less than 35 km away from known populations of federally threatened Oregon spotted frogs (*R. pretiosa*) in Thurston County. Given the close proximity of invasive ACF to federally listed amphibians, there is a need to proactively manage the spread of ACF and understand impacts to sensitive species, particularly if these species are naive to ACF predator cues.

Beyond impacts to amphibians, there is also a need to understand potential impacts to native fishes. For instance, the same nearby habitats that host federally threatened Oregon Spotted Frogs also are home to Olympic Mudminnows (*Novumbra hubbsi*), a state-sensitive species that is small (< 80 mm long) and potentially vulnerable to ACF predation. Further, diverse salmonid species occur near invasive ACF populations in Washington (Ojala-Barbour *et al*., 2021). Salmonids in the Pacific Northwest are culturally, ecologically, and economically important and several are listed under the U.S. Endangered Species Act (Quinn, 2018). Invasive ACF have been repeatedly detected in and adjacent to water bodies with various salmon species including kokanee (*Oncorhynchus nerka*). Although adult salmon are too large for ACF to consume, embryonic and fry life stages may be vulnerable to ACF predation, particularly if salmon are naive to ACF predator cues.

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We anticipated that native newts might serve as a potential biocontrol agent against ACF. Although the neurotoxin TTX has been extensively studied in *Taricha* newts for its anti-predatory properties (Zimmer *et al.*, 2006; Bucciarelli and Kats, 2015; Ota *et al.*, 2018), to our knowledge it has not been studied for potential biocontrol purposes. We were motivated to test whether newts might be an effective biocontrol because several casual observations suggested that ACF may be sensitive to newt toxins. In particular, we anticipated that newts would be so toxic as to elicit a relatively rapid behavioral response in ACF. However, the presence of newts in our study appeared to attract rather than deter ACF. There are multiple reasons for this. First, we conducted relatively short-duration trials to assess ACF behavior. Longer trials may reveal different patterns if aqueous TTX takes longer than 10 minutes to influence ACF physiology. Second, additional work may benefit from testing different densities of newts as higher doses of TTX may be needed to influence ACF. Third, our experiments did not allow ACF to directly interact with newts. Although we attempted to digitally stimulate TTX in the newts, our experimental design limited interspecific interactions that could have produced ecologically relevant exposures. Regardless, the potential utility of newts as a biocontrol is probably greater through passive toxicity rather than through consumption. Other types of biocontrols could include large invertebrates, which ACF have been shown to exhibit antipredator behavior to (Kruger *et al.*, 2019). ~~-~~Finally, ACF may be attracted to visual cues more so than chemical ones. One study found that removing ACF was most successful when traps were baited with conspecifics (Lorrain-Soligon *et al*., 2021). This result in tandem with our findings suggests that ACF may generally respond to visual cues like movement. Future studies may benefit from testing the response of ACF strictly to chemical cues. Although the ability to produce a powerful neurotoxin

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makes *Taricha* newts a tantalizing potential candidate for ACF biocontrol, additional research is needed to assess if this is a viable and ecologically neutral management option.

While our research indicated that invasive ACF are chemically cryptic predators that could pose a risk to native species and which are not readily deterred by newt chemical cues (including toxins), the chemical mechanisms underlying the relationships we explored warrants further attention. The newts used in our research were collected at our field site and kept in a laboratory setting for 1-2 months prior to our experiments. Because of the conflicting observations that motivated our experiment and our experimental findings, we analyzed aqueous newt extracts to determine if TTX was present and estimated concentrations. This analysis found no detectable TTX in the solutions which may have affected chemical cues between the newts and ACF in this study. Even so, this analysis found possible TTX analogues and/or relevant metabolites. While some research has indicated that TTX may increase in captive newts (Hanifin *et al*., 2002), other research shows lower TTX levels in newts compared to wild individuals (Gall *et al*., 2022). There is also evidence that TTX is linked to the newt microbiome, potentially indicating our captive setting did not allow for proper microbe growth (Vaelli *et al*., 2020; Gall *et al*., 2022). Further, TTX concentrations vary and fluctuate within and among newt populations (Bucciarelli et al. 2016; Reimche et al. 2020) and so our population may inherently maintain low amounts of TTX or at the time of sampling, possessed relatively low toxin concentrations. Interestingly, our results clearly show that native larvae respond to newt chemical cues, regardless of whether TTX or some other possible analogue was the constituent molecule of the solution. These findings highlight new opportunities for understanding the chemical ecology of newts and their interactions with other species.

Conclusion

We aimed to identify the roles that chemical cues play in mediating the relationships between invasive ACF and native amphibian prey and toxic newts. We found that: (1) native *R. aurora* tadpoles show strong anti-predator responses to newts but do not recognize ACF as predators and (2) ACF were attracted rather than deterred by newt chemical cues in short-duration trials. The lack of anti-predator responses to invasive ACF may provide a foraging advantage over native amphibian predators and suggest ACF have potential to have detrimental effects on native species populations. It is also possible that introduced ACF do not have a response to the defenses of native species because they have not co-evolved with the mechanism. Our work has begun to uncover some of the mechanisms that may allow ACF to threaten native species and highlights new areas of research to improve management of this global invader.

Acknowledgments

We would like to thank the Washington Department of Fish and Wildlife. We are also grateful to Jacie Fabela and Quin Butler for their assistance in animal care during our trials. Additionally, Rebecca Lavier, Hannah Dotterweich, Panos Stratis helped with animal trapping. This research was done under Saint Martin's University animal ethics permit SMUAE 22_1. State permissions were under the programmatic permit issued to WDFW employees for capturing and handling wildlife.

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