

Abstract

PREDATOR OUT OF PLACE: THE DIFFERENTIAL IMPACTS OF NATIVE AND NON-NATIVE CRAYFISH ON BUFO TADPOLES

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Predators have been shown to alter important fitness components (larval period, mass at metamorphosis, and overall survivorship) of larval anurans. Uncommonly studied predators of tadpoles include crayfish. Both native and introduced crayfish occur in NC. To gain a better understanding of the effect of introduced crayfish in NC, we examined the impact of native (*Procambarus acutus*) and introduced (*P. clarkii*) crayfish on Fowler's toad larvae (*Bufo fowleri*). We hypothesized *P. clarkii* would have a greater effect on fitness components of *Bufo fowleri* because it is larger, more aggressive and invasive in many parts of the world. Because these two species can co-occur, we conducted an experiment that allowed us to evaluate the independent and interactive effects of the two crayfish species at different densities (0, 1 or 2 individuals present).

We found that crayfish did not alter toad larval period, but reduced toad survival and caused toads to metamorphose at larger masses. The effect of crayfish on toad survival and mass at metamorphosis increased with crayfish density. Interestingly, native crayfish caused a stronger reduction in survival and a stronger enhancement in mass at metamorphosis than did introduced

crayfish when present at similar densities. Although toad survivorship in ponds with both crayfish species was comparable to survivorship in ponds containing only native crayfish, toads metamorphosed at larger sizes when both crayfish species were present. We caution, however, that *P. clarkii* can achieve higher densities than native species in some situations and likely have a more important effect under high-density conditions.

PREDATOR OUT OF PLACE: THE DIFFERENTIAL IMPACTS OF NATIVE AND NON-
NATIVE CRAYFISH ON BUFO TADPOLES

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CHAPTER 1: Review of Species Invasions

Humans have been moving species around the planet for as long as they themselves have been moving around the planet. In the beginning, when we were constrained by technology, the species we moved were few and portable: our diseases, our parasites, our domesticated livelihoods. As the human race developed, so did our ingenuity. While we were busy exploring the planet by land and water, the organisms we transported (with or without our knowledge) were exploring the places we took them. They lived and thrived or died out, according to their ecological needs and what was available in their new homes.

The evidence of this global transplantation is sometimes apparent, but in some cases the immigrants fit in so well that they go unnoticed. Species such as dandelions, redbuds, daylilies, daffodils, and English ivy are so common in neighborhoods and along roadways that we may forget that they escaped someone's garden and proliferated. As benign as these species seem, there are many organisms that can cause much damage when transplanted. Organisms that have been transplanted out of their natural range are known as nonindigenous, nonnative, exotic, or introduced species (Mac, Opler et al. 1998). When introduced species become pests they are called invasive species (Wonham 2006).

Although the wholesale demonization of all introduced species seems to be the norm in both the scientific community and the media, consideration should be given to other aspects of non-native species. For instance, the addition of a new species in an area can potentially increase the local biodiversity area by adding a species (Sax and Gaines 2003). On a sociopolitical level, the vilification of species from foreign countries may

cause or perpetuate xenophobia and prejudice of “others” and is commonly spoken of in militaristic terminology (Larson, Nerlich et al. 2005). The “war against invasive species” may have an attention-getting ring to it and may point accusatory fingers elsewhere, but it should be recognized that species introductions are generally either accidental or the result of myopia. However species become introduced, their impacts in a new area may or may not be detrimental.

Introduced species take time to become invasive, and the threshold between a nonindigenous species and an invasive one are often blurry. In 1876 at the Philadelphia Centennial Exposition, a Japanese vine called kudzu (*Pueraria montana*) was introduced to the United States as an ornamental plant. The plant was explored for its use as an ornamental, a forage crop, and as a means to prevent erosion. In fact, for a time the U.S. Soil Conservation Service paid farmers \$8.00 an acre to grow kudzu. Over 1 million acres of kudzu were planted under this program. Unfortunately, kudzu was difficult to bale as forage, and direct grazing was limited by trampling of the vines by hoofed livestock. Kudzu was finally recognized as a weed in the 1950’s and was listed as a Federal Noxious Weed by Congress in 1998 (Britton, Orr et al. 2002). Kudzu now grows wild in around 30 states in the U.S. and costs power companies an estimated \$1.5 million dollars a year in control costs (Britton, Orr et al. 2002; USDA 2010). This does not take into account the loss by timber companies, homeowners, and the ecological damage to our ecosystems. This vine, due to its ability to displace native species, its ecological and environmental costs, and its pervasiveness, has become an invasive species (USDA 2010).

Invasive species are not limited to plants. Species like zebra mussels, nutria, and fire ants are commonly cited invasives that wreak havoc on ecosystems in the United States. We transport these species both consciously, as in the example of kudzu, and unconsciously. Both zebra mussels and fire ants likely arrived accidentally in the ballast used to keep cargo ships upright (APHIS 2009; Benson and Raikow 2010). Some species, such as the woolly adelgid (an insect that destroys eastern hemlock trees), have been introduced through contaminated cargo shipments from other countries (USDA 2010). Damaging species introductions are not limited to intercontinental exchanges. In fact, it is thought that intracontinental introductions can be as damaging as intercontinental introductions, especially in freshwater environments where the species are isolated by dispersal ability (Cox and Lima 2006). In the United States, bullfrogs (*Rana catesbeiana*) that are so common and relatively benign in the southeast are competing with and in many cases are extirpating local frog species (*Rana aurora*) on the west coast of the U.S. (Kiesecker and Blaustein 1998; Kats and Ferrer 2003).

The result of species introductions is not always ecological devastation. Individuals must first be hardy enough to survive the method of transit and then only establish if ecological conditions of the new environment are suitable. Competition from native species, changes in season and inability to disperse can all prevent an introduced species from proliferating in a new habitat. Introduced species may become naturalized (non-invasive residents) in an area if competition from natives and niche-availability allows. Williamson and Fitter (Williamson and Fitter 1996) suggested that a “tens rule” applies to the escape, establishment, and invasion by nonnative species. In other words, only 10% of species introduced appear in the wild, only 10% of those in the wild will

establish and only 10% of those that establish will become invasive. However, this is only a heuristic rule and there are several exceptions (Williamson and Fitter 1996).

It is conceivable that any species, if given the proper conditions, could become invasive outside of its natural habitat. Nevertheless, it has been posited that certain traits may contribute to a species' invasiveness. Among these traits are life history strategies such as early maturation, high fecundity, r-selected population growth, and the ability to reproduce both sexually and asexually (Gherardi 2006; Wonham 2006; Grabowski, Bacela et al. 2007).

In addition to life history strategies, ecological needs can be indicators of whether a species will persist outside of its native range. Species that have broad ranges of ecological tolerances (temperature, water, nutrient availability, salinity) tend to have larger ranges in which they can establish than those with narrow tolerance ranges (Mac, Opler et al. 1998). It might be assumed that species that evolved in colder climates may do well in any climate whereas species from tropical areas may be limited by temperature. One exception to this rule is *Caulerpa taxifolia*, a tropical seaweed commonly used as an aquarium plant that was transplanted into the Mediterranean Sea. It was assumed that the colder waters would slow the growth of the tropical alga, but instead it grew very quickly and has become a major pest in the areas where it has been introduced (Williams 2006).

Habitat and diet generalists, good competitors and dispersers, and novelty in the invaded territory are also indicators of species that are likely to become invasive (Mac, Opler et al. 1998; Wonham 2006). A classic example of species novelty is that of the invasive brown treesnake (*Boiga irregularis*) and its decimation of the native species of

Guam. Because Guam is so isolated (2000 km from another large landmass) the only animal species native to Guam are those that can fly (birds and bats) and those which are small enough so survive on rafts of vegetation for several weeks (lizards and a few small mammals (Gordon, Fritts et al. 1997). Before the accidental introduction of the brown treesnake, the only snake on the island was a small insectivorous blind snake (*Ramphotyphlops braminus*) which posed no threat to vertebrates (Fritts and Leasman-Tanner 2001). It is likely that the absence of archetypical snake predators on Guam contributed to predation on naïve fauna. The novelty of the treesnake combined with its willingness to eat varied prey, has resulted in the extinction or local extirpation of nine bird species, two lizard species, and two of the three native bat species on Guam (Rodda, Fritts et al. 1997; Colvin, Fall et al. 2005).

In addition to invasive species having traits in common, highly invaded (or invasable) habitats also have a suite of general characteristics. Habitats with mild climates, open niches, low biodiversity, high levels of geographic isolation, absence of predators (of the invading species) and areas with high levels of disturbance are generally more likely to be invaded (Lodge 1993; Mac, Opler et al. 1998; Wonham 2006). This last trait, disturbance, is often mediated by humans.

A close association with humans is generally found with both invaded habitats and invasive species. Invasive species are generally transplanted by humans (Jeschke and Strayer 2006; USDA 2010) and invaded areas are usually occupied (if only briefly) by humans. In addition, regions that have been disturbed by humans provide new areas for invasives to colonize (Mac, Opler et al. 1998). Fields left fallow or areas where ground

has been broken for new construction are quite frequently areas that are invaded by non-native species.

Another key component in the ability of a species to become established is the propagule pressure (Jeschke and Strayer 2006). Propagule pressure of a nonnative species is the number of individuals, the quality of those individuals, and the number of times the species is introduced into a new environment. If too few individuals or poor quality individuals are introduced, the species will have the same kinds of problems as those experiencing a severe population decline: Allee effects and genetic bottlenecks (Wonham 2006). Interestingly, it has been the case with some introduced species that a genetic bottleneck has actually contributed to a species' invasiveness. One such case is the Argentine ant (*Iridomyrmex humilis*) which displays high levels of intraspecific aggression in native populations but not introduced populations. It is likely that this difference in populations is genetically based (Snyder and Evans 2006; Wonham 2006). Propagule pressure is mediated by humans in the case of nonnative species; the more individuals we move and the more frequently we move them, the less likely it is that introduced species will experience Allee effects or genetic bottlenecks. In addition, the organisms that humans choose to move are likely to be the healthiest and best specimens, further increasing the chances of future propagation.

The closer a species is associated with humans, the more likely we are to introduce it to new areas. In the case of animals, those we use for companionship and food are frequently transplanted. Feral pigs (escaped domesticated livestock) are highly invasive in several parts of the United States, including Texas, Florida, California and Hawaii (Van Driesche and Van Driesche 2000; Pimentel, Zuniga et al. 2005). Feral cats

are considered to be a nuisance in many areas of the world, including North and South America, Europe and Australia (Veitch 2009). This example of our domesticated pets gone wild is having severe predatory effects on Australian ground-breeding species such as the cockatoo, golden bandicoot, and burrowing bettong (Veitch 2009).

If association with humans is an important factor in determining whether or not a species will become invasive (Mac, Opler et al. 1998) it follows that a species associated with humans in more than one way might have a better chance for becoming invasive. The red swamp crayfish (*Procambarus clarkii*) is invasive in over 30 countries, including the United States (Global Invasive Species Database 2006). It may owe part of its success as an invader its multiple roles in the lives of humans. This crawfish, *P. clarkii*, is first and foremost a food source (crayfish is the scientific term, crawfish implies consumption). Ninety percent of the total U.S. crawfish production (over 112 million pounds of crawfish per year) occurs in Louisiana through both aquaculture and wild collection. Of this, 70% is consumed in Louisiana to the tune of approximately 10lbs of crawfish per person per year (Lutz, Sambidi et al. 2009). The other 10% of U.S. crawfish production occurs mainly in Alabama, Texas, and Florida (Dunning 2001). Crawfish production in the U.S. consists primarily of *P. clarkii* and another species *P. acutus*; however the vast majority of the production is *P. clarkii* (Brown and Gunderson 1997). Crayfish aquaculture occurs in shallow ponds that generally have no fencing to prevent the crayfish from escaping into nearby waterways (Steve Gabel, NC Cooperative Extension, personal comm.); this is despite the fact that crayfish are known to travel over land in order to colonize new habitat (Holdich and Lowery 1988; Kerby, Riley et al.

2005). It has been found that *P. clarkii* is able to escape aquaculture and colonize nearby waterways (Kilian, Frentress et al. 2009).

Although crayfish are primarily a food source, they are also often kept as pets (Peters and Lodge 2009). Specifically, white and blue morphs of *P. clarkii* are prized among enthusiasts and garner high prices in online auctions. Crayfish are active when kept in captivity and their antics and hunting make them an entertaining addition to fish tanks. However, irresponsible release of pets is fairly common in the U.S., as is evidenced by the proliferation of red-eared sliders, Burmese pythons, iguanas, and housecats in the wild. Although little data on pet crayfish release is currently available, it likely occurs even if only on a limited basis.

Crayfish are also frequently used in biology classes for dissections, observational studies and as class pets. In fact, *Procambarus clarkii* is available for live transport from Carolina Biological Supply in quantities of up to 50 per order. Although some states require permits to obtain these crayfish and some states forbid it altogether, most states allow anyone to order these potential invaders. Carolina Biological provides no warnings against releasing these possibly invasive crayfish in local ponds to the intrepid schoolteacher (personal observation).

Procambarus clarkii are also popular recreationally as fish bait (Benson, Jacono et al. 2004) and are sold as bait for carp, striped bass, trout, walleye and several other sport fish. Many crayfish introductions are presumed to be anglers emptying bait bucket leftovers into ponds, lakes and streams (Benson, Jacono et al. 2004; Peters and Lodge 2009). The four aforementioned pathways to introduction (aquaculture, pet trade, classrooms, bait industry) probably provide both a significant propagule pressure and

high genetic diversity of introduced *P. clarkii* which may contribute to the likelihood of successful establishment of this species in new locales.

Procambarus clarkii has been introduced extensively around the world. In many cases, *P. clarkii* seems to do the most damage in places with no other crayfish species. For instance, *P. clarkii* has been introduced into the Nile River in Egypt, where it does extensive damage to the river banks and kills many of the fish in fishermen's nets before they can be removed (Van Marsh 2008). This crayfish has no natural predator in Egypt so populations go largely unchecked (Van Marsh 2008). The Egyptian people have begrudgingly learned to appreciate the crayfish because they have a tendency to consume snails, an intermediate host for schistosomiasis (*Bilharzia*) (Fishar 2006). People that live near areas of the Nile that are highly invaded by crayfish have a lower incidence of schistosomiasis (Van Marsh 2008). Crayfish are so adept at reducing snail populations that introductions have been intentionally made in Kenya to help reduce snail populations (Hofkin, Mkoji et al. 1991). These introductions have had unforeseen detrimental effects on the fishing industry in Kenya but have provided a new industry in the form of 500 tons of exported crayfish per year (Hofkin, Mkoji et al. 1991).

Procambarus clarkii's introduction into Spain's Iberian Peninsula is very well-documented. Demand for crayfish as a food source is high in Europe, and when indigenous crayfish populations began to shrink due to crayfish plague (*Aphanomyces astaci*) alternatives were explored. Unlike Spanish species, *P. clarkii* and other North American crayfish are resistant to crayfish plague (Gherardi 2006). Ironically, it is thought that the crayfish plague was introduced into Spain by American crayfish caught in ballast water (Gherardi 2006). In 1973, after consultation with scientists in the U.S.

and permission of the Spanish government, 500kg of crayfish from Louisiana (mostly *P. clarkii*) were introduced to a farm in the Spanish province of Badajoz (Gherardi 2006; Global Invasive Species Database 2006). From this first introduction and many smaller subsequent ones, *P. clarkii* has spread through all of Spain (Gherardi 2006). The detrimental effects of *P. clarkii* have been numerous in Spain. *P. clarkii* is documented to severely reduce native aquatic macrophytes and gastropods, have devastating effects on rice agriculture, exclude Natterjack toads (*Bufo calamita*) from breeding ponds, and prevent the recovery of native crayfish populations through competition and disease-transmission (Gil-Sánchez and Alba-Tercedor 2002; Cruz, Pascoal et al. 2006; Cruz and Rebelo 2007).

Although *Procambarus clarkii* is indigenous to the United States, it has been introduced in 15 states outside of its native range (Benson and Fuller 1999). In 14 of the 15 states with introductions (including North Carolina), *P. clarkii* is considered invasive (GISD 2006; Cooper and Cooper 2009). In California, *P. clarkii* has been introduced into streams that contain the California newt (*Taricha torosa*). Despite the high toxicity of this newt, *P. clarkii* consumes and drastically reduces the number of eggs of *T. torosa*. In addition, *P. clarkii* feeds on the larvae of the newt and have been observed to chase adult newts (also highly toxic) out of the water (Gamradt and Kats 1996). Because there is no evolutionary history with crayfish, it is possible that *Taricha* larvae are captured more easily due to naïveté.

Behavioral traits and ecological needs of *P. clarkii* may also contribute to its invasiveness. Part of what makes crayfish good invaders is their generalist feeding habits (Snyder and Evans 2006). In invaded ranges, *Procambarus clarkii* has been found to

switch more readily to new prey items, possibly giving it a predatory advantage over native crayfish (Gherardi, Renai et al. 2001; Renai and Gherardi 2004). This behavioral adaptation in addition to the fact that crayfish are omnivorous likely facilitates *P. clarkii* in persisting in new habitats. *P. clarkii*, unlike some species of crayfish, is fairly well adapted to many types of environments. In its natural range *P. clarkii* generally inhabits seasonally flooded wetlands, but can be found in habitats ranging from streams to rice paddies, lakes, and ditches. The burrowing response in *P. clarkii* has been shown to occur during breeding seasons and dry seasons and is also likely to serve as a means of protection from predation (Holdich and Lowery 1988; Correia and Ferreira 1995; Ilheu, Acquistapace et al. 2003). *P. clarkii*'s ability to tolerate many types of environment, including those that dry down seasonally, in conjunction with its ability to exploit many kinds of resources and its close association with humans in many ways make it an ideal candidate for an invasive species.

Although *Procambarus clarkii* is probably the most widely introduced crayfish species due to its suitability for aquaculture, it is not the only invasive crayfish species. Three other North American crayfish species are known to be invasive, the rusty crayfish (*Orconectes rusticus*), virile crayfish (*Orconectes virilis*), and the signal crayfish (*Pacifastacus leniusculus*) and one Australian species is thought to be on its way to invasiveness, the Australian red claw crayfish (*Cherax quadricarinatus*) (Global Invasive Species Database 2006; Ahyong and Yeo 2007). Of these, *Orconectes rusticus* is perhaps the most widespread and the most studied. *O. rusticus* has been found to hybridize with native crayfish in invaded areas, is behaviorally more aggressive than natives, and grows faster and survives more frequently than native congeners (Hill, Sinars et al. 1993; Perry,

Feder et al. 2001; Pintor and Sih 2009). However, *O. rusticus* is only known to have invasive populations in North America (Global Invasive Species Database 2006).

Much of the literature about *P. clarkii* as an invasive concerns its detrimental effects on species (including other crayfish) that have no innate defenses against a Procambriid crayfish. Less information is available about *P. clarkii* and how its behavior differs from similar species (but see (Romaine and Lutz 1989; Acquistapace, Daniels et al. 2004; Mazlum and Eversole 2005; Mazlum 2007; Mazlum and Eversole 2008)).

Within the native range of *P. clarkii* there are two other very similar Procambriid species, *P. acutus* and *P. zonangulus*, neither of which are known to be invasive (Global Invasive Species Database 2006). Interestingly, in mixed-species aquaculture situations, it has been found that *P. clarkii* will gradually replace *P. zonangulus*, but will gradually be replaced by *P. acutus* (Blank and Figler 1996; Mazlum and Eversole 2008). The shift of populations from *P. acutus* to *P. clarkii* thought to be mediated by longer chelae (claws) and larger body size of *P. acutus*, which is also known to have faster growth rates than *P. clarkii* (Holdich and Lowery 1988; Eversole, Mazlum et al. 2004; Mazlum and Eversole 2005). However, *P. clarkii* reproduces more rapidly and earlier in the season (Holdich and Lowery 1988; Mazlum and Eversole 2008). These two species overlap both in and outside of the native range of *P. clarkii* (USGS 2010). Previous studies with crayfish have shown that a species can act more aggressively (foraging activity, bait-stealing) outside of its native habitat (Pintor and Sih 2009) but this behavior has not yet been recorded in *P. clarkii*. Little is known about the behavioral and predatory differences in *P. clarkii* with respect to congeners that share both a native and introduced range with this species.

Predatory effects of one species on another are fairly simple to measure. However, as the complexity of a system increases, prey and predator behavior can change causing changes in predator efficiency. A simple sample scenario is fish in a lake. If a prey fish is faced with a snapping turtle that lives at the bottom of the lake, it may alter its behavior and swim more often in the top of the water column. However, if this same fish is also hunted by an eagle, it may choose to swim in the middle of the water column to reduce the chances of being consumed by either the eagle or the turtle. Alternatively, the fish could reduce its activity greatly so as to go unnoticed by both predators. This change in behavior by the prey fish could increase or decrease the predatory efficiency of both the turtle and the eagle. In this way, the presence of the eagle may indirectly alter the predation efficiency of the turtle (or vice versa).

To calculate what the expected combined effects of predators are the multiplicative risk model can be used (Soluk and Collins 1988). This model can be used to calculate the expected number of prey remaining in a multiple predator system based on the per capita consumption of individual predators. The multiplicative risk model is more biologically accurate than an additive model (in which the proportion consumed are simply added together) because it takes into account that a prey item cannot be killed twice. To use the multiplicative risk model, the proportion of the prey consumed by each individual predator must be known. This data can be used to calculate what predation should be with both predators in the system using the multiplicative risk model:

$$\text{Predicted prey consumption} = (P_1 + P_2 - P_1P_2)N_P$$

In this model, P_1 is the proportion of prey killed by predator 1, P_2 is the proportion of prey killed by predator 2 and N_P is the total number of prey. The P_1P_2 term is the proportion of the prey that cannot be killed by one predator because it has already been killed by the other. If this term were absent, it would be possible to calculate a predatory reduction of over 100% of the prey in a multiple predator system (Sih, Englund et al. 1998). If the number of prey killed is significantly above or below what is calculated with the multiplicative risk model, then there is possibly an “emergent multiple predator effect.” (Sih, Englund et al. 1998), i.e. the presence of the predator has risk-enhancing or risk-reducing effects on the prey species (Soluk and Collins 1988; Sih, Englund et al. 1998).

Risk-enhancement by predators suggests that either the predators interact in some way so as to assist each other in consuming more prey or the prey react to the combination of the predators in some manner so as to make themselves more vulnerable to predation. Risk-reduction in a multiple predator system suggests that prey may react more strongly to the presence of more than one predator in the system and may therefore increase whatever predator-avoidance mechanism they use. It is also possible that predator species may interact in some way (intraguild aggression or predation) so as to consume fewer prey than expected.

A system in which emergent multiple predator effects have been recorded is that of snails, fish and crayfish (DeWitt and Langerhans 2003). Fish induce snails to spend more time in covered habitats which are also frequented by crayfish, essentially chasing the snails into the claws of the crayfish (DeWitt and Langerhans 2003). This has direct effects on the snail populations in that the reduction of the snails by the two predators

together is higher than the additive effect of predators is calculated to be (a risk-enhancing emergent multiple predator effect) (DeWitt and Langerhans 2003).

Risk-reduction in multiple predator systems is perhaps more common than risk-enhancement. In a study of 10 combinations of a two-predator system, Vance-Chalcraft and Soluk found that risk-reduction occurred more frequently than risk-enhancement, such that 27% fewer prey were killed overall than were expected (Vance-Chalcraft and Soluk 2005). This was postulated to be due to predation-reducing behaviors exhibited by the prey species. In their system, the prey (mayfly larvae) avoids predation by reducing movement and relying on cryptic coloration. Furthermore, they found that the magnitude of the risk-reduction was marginally significantly higher for intraspecific risk-reduction rather than interspecific risk-reduction (Vance-Chalcraft and Soluk 2005). This may have been due to intraspecific interference between the two predators in the system.

A change in prey population is not the only consequence of multiple predators; prey morphology can also be altered depending on the predator present. In a study by Lakowitz et al, fish predators were found to induce thicker and more rounded shell in snails, whereas crayfish had no effect on shell morphology (Lakowitz, Bronmark et al. 2008). When the two predators were combined in the same system, snails developed an intermediate shell shape (less rounded but with more shell material) (Lakowitz, Bronmark et al. 2008).

Larval anurans alter behaviors, morphology, and life history strategies in response to their predators (Bridges 2002; Relyea 2002; Pearl, Adams et al. 2003; Relyea 2003; Saenz, Johnson et al. 2003). Because anuran larvae are phenotypically plastic, they provide a unique opportunity to examine the effects of predation not only on

survivorship, but also on growth and development (Wilbur and Collins 1973; McCollum and VanBuskirk 1996; Van Buskirk, McCollum et al. 1997). As such, they have been used in numerous experiments examining predator-prey interactions (Peacor and Werner 1997; Babbitt and Tanner 1998; Van Buskirk and Relyea 1998; Relyea 2003; Baber and Babbitt 2004). In multiple predator situations, wood frog (*Rana sylvatica*) tadpoles were found to alter their morphology differently depending on the predator in the system. Interestingly, when more than one type of predator was present, 90% of the time tadpoles exhibited the same morphology as they would for the more risky of the two predators (Relyea 2003).

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CHAPTER 2: Experimental Evaluation of Two Crayfish Species on Fowler's Toads

Introduction

When species are transplanted out of their native ranges, there may be unintended negative effects in the new habitat. When the introduced species is an omnivorous generalist predator such as crayfish, both flora and fauna in the new habitat are likely to be affected (Holdich and Lowery 1988). Red Swamp crayfish (*Procambarus clarkii*) are introduced and known to be invasive in many parts of the world, including North America (Global Invasive Species Database 2006). *P. clarkii* is an easily cultivated source of protein and a delicacy, and is the crayfish most commonly used in aquaculture and found on your dinner plate (Brown and Gunderson 1997). Because of its wide range of ecological tolerances, *P. clarkii* can garner a foothold in most climates in which it is introduced (Holdich and Lowery 1988; Fullerton 2002). If *P. clarkii* escapes aquaculture or is released it is likely to become established, as is evidenced by self-sustaining populations on every continent except Australia and Antarctica (Global Invasive Species Database 2006; Kilian, Frentress et al. 2009).

Invasive species are thought to be a major factor influencing global amphibian declines, mostly in amphibian species that have no natural history with the invasive predator/competitor (Kats and Ferrer 2003). This is not the case for crayfish and amphibians in North America. There are nearly 400 species of crayfish in North America, the vast majority of which are found in the Eastern U.S. (Lodge, Taylor et al. 2000) which is also home to a high diversity of amphibians (Martof, Palmer et al. 1980). However, for a group of organisms that are already globally threatened, such as

amphibians, new predators may tip the balance toward extirpation or extinction (Wilcove, Rothstein et al. 1998).

Crayfish are predators of amphibian eggs and larvae (Gamradt and Kats 1996; Lodge, Stein et al. 1998; Gherardi, Renai et al. 2001; Dorn and Wojdak 2004; Cruz, Pascoal et al. 2006). In salamanders, it was found that the invasive crayfish *Procambarus clarkii* not only consumes the eggs and larvae of highly toxic California newts (*Taricha torosa*), but also aggressively chase adult newts from waters where they are attempting to oviposit (Gamradt and Kats 1996; Gamradt, Kats et al. 1997). Past studies have found that crayfish can also affect frog species through reducing survival of larvae, inducing accelerated hatching of eggs, influencing distribution, and may also affect egg deposition of anurans (Saenz, Johnson et al. 2003; Dorn and Wojdak 2004; Renai and Gherardi 2004; Cruz and Rebelo 2005; Cruz, Pascoal et al. 2006).

In North Carolina alone, there are approximately 40 species of crayfish, 13 of which may occur in the coastal plain region (Fullerton 2002) which is also the region of North Carolina with the highest diversity of anurans (Martof, Palmer et al. 1980). Although different crayfish species represent the same predator archetype and possibly produce similar water-borne cues, differences in behavior between species may have dissimilar effects on prey. For instance, in previous studies it was found that *P. clarkii* has a higher background activity level than another similar species, *Procambarus acutus* (Acquistapace, Daniels et al. 2004). In addition, *P. clarkii* appeared to have a lower latency time in capturing and consuming tadpole prey (Gherardi, Renai et al. 2001) and was more likely to switch to naïve prey (Renai and Gherardi 2004) in comparison with European crayfish species (*Austropotamobius pallipes* and *A. italicus*, respectively).

Differences between invasive and non-invasive crayfish may be common; in a comparison of two crayfish known to act invasively (*P. clarkii* and *Orconectes rusticus*), to two crayfish without invasive tendencies (*O. virilis* and *A. pallipes*) it was found that the invasive crayfish retained memory of predatory threat longer than native species (Hazlett, Acquistapace et al. 2002).

In its introduced range in North Carolina, *P. clarkii* interacts with some of the same amphibian species that are found in its native environment (Lannoo 2005; USGS 2010). However, the activities of species outside of their native range are not necessarily the same as inside of their native range, and mechanisms that allow for the co-existence of *P. clarkii* with amphibian species in its native range may differ in the non-native populations. Because of this possible change in behavior, non-indigenous species may have alternate effects on their communities in introduced and native ranges. For instance, behavioral differences have been noted for Argentine ants (*Linepithema humile*), which are intraspecifically aggressive in their native habitat, but not in their invaded ranges (Snyder and Evans 2006; Wonham 2006). This lack of intraspecific aggression has allowed non-indigenous Argentine ants to reach higher densities than they would in their native range (Snyder and Evans 2006). In crayfish, an invasive population of *Orconectes rusticus* (the Rusty crayfish) was found to have higher growth rates than a native population of *O. rusticus* (Pintor and Sih 2009). The invasive population also aggressively stole food, another trait not found in native *O. rusticus*. The differences in growth and aggression seemed to be due to a distinction in the population of invasive *O. rusticus* and not because of differences in the behavior of conspecific crayfish in and outside the invaded ranges (Pintor and Sih 2009).

The two crayfish species chosen for this study were *Procambarus clarkii* and *P. acutus* (Plate 1). These are the two species most commonly used in aquaculture for the food and bait industries (Brown and Gunderson 1997). *P. acutus* is a native to the Eastern half of the U.S. and can be found in the piedmont and coastal plain of North Carolina (Fullerton 2002). The native range of *Procambarus clarkii* includes all of the Gulf Coast states, northward into Illinois and Missouri, and west into Texas, Oklahoma and Mexico (USGS 2010). *P. clarkii* has been introduced into 15 states outside of its range, including North Carolina (Benson and Fuller 1999). The native range of *P. acutus* overlaps with the native range of *P. clarkii*, but not in North Carolina where *P. clarkii* has been introduced (USGS 2010).

I sought to quantify the per capita effects of *Procambarus clarkii* on larval anurans in its introduced range in North Carolina. I chose larval anurans so that I could examine not only the lethal effects of *P. clarkii*, but also how it may alter growth and development of native freshwater fauna as compared to a native crayfish *Procambarus acutus* (a species also known to consume larval anurans) (Figiel and Semlitsch 1991). *P. acutus* and *P. clarkii* are commonly found in bodies of water which are also breeding sites for anurans, and may influence life history traits and survival of larval anurans via predation and competition for algal and detrital resources. In this study, I assess the net impact of native and non-native crayfish on survival and growth of *Bufo fowleri* larvae, a toad species that shares ranges with both *P. acutus* and *P. clarkii* (Plate 3 and 4).

Bufo fowleri toads oviposit in most types of freshwater habitats, from temporary pools and ditches to lakes and river shallows (Lannoo 2005). Most of these habitats would place their larvae in locations that are suitable for both *Procambarus acutus* and *P.*

clarkii (Holdich and Lowery 1988). Tadpoles of the genus *Bufo* are known to school in shallow waters (Lannoo 2005) which in conjunction with the habits of crayfish makes them susceptible to crayfish predators. Compared to other North Carolina toad species, such as *B. americanus* and *B. terrestris*, *B. fowleri* is less frequently studied in predator-prey experiments (personal observation, literature search). Additionally, *B. fowleri* are thought to be more tolerant of human disturbance than other toad species, as is evidenced by their presence on roadsides and near homes (Lannoo 2005; Wells 2007). This may be important because introduced species are often associated with disturbed and human-associated areas and would therefore encounter species associated with these habitats more frequently. It is also interesting to note that *Bufo* tadpoles have been assumed to be unpalatable to fish predators because of their higher survival rate in permanent ponds than amphibians that are more commonly found in semi-permanent or temporary aquatic environments (reviewed in (Gunzburger and Travis 2005)). However, the unpalatability of toad larvae to crayfish has not been well-studied in North American species, both of which (like fish) can inhabit permanent waters.

Effects of *Procambarus acutus* on tadpoles have only been studied previously in their predatory capacity and were found to be efficient predators of tadpoles only when portions of the tadpole's tail were removed (Figiel and Semlitsch 1991). Although *P. acutus* is not the only crayfish found in conjunction with *P. clarkii*, it is a very common denizen of the coastal plain in eastern North Carolina. The effects of *P. clarkii* on anurans in its invaded range in North Carolina have not been studied.

Comparing the per capita effects of *P. clarkii* to the effects of *P. acutus* will help to identify the impacts of a common native and non-native invasive crayfish on anuran

survival and life history traits. Because introduced species inevitably come into contact with native species, *P. acutus* and *P. clarkii*'s combined effects should be studied. Multiple predator species may have differing effects on prey than one species alone, as was found for snails in the presence of both fish and crayfish (DeWitt and Langerhans 2003). However, to my knowledge, the impacts of multiple congeneric species of crayfish predators on tadpoles have not been studied.

To examine the effects of *Procambarus acutus* and *P. clarkii* on larval anurans, I designed an experiment that will look at both the differential effects of each predator separately in high and low density and both predators together in high density. This will allow us to examine the different effects of crayfish density and diversity in these two species as well as the per capita effects of each species. Looking at the effect of both crayfish species together is a vital component of this experiment in that the introduction of a new species causes new and unknown interactions with established native species. The combined predatory effects of *P. acutus* and *P. clarkii* have not been previously studied.

I believe that *P. clarkii* is likely to be the more voracious of the two crayfish species and will reduce survival in larval anurans over *P. acutus* in the single-species treatments. This will likely be due to *P. clarkii*'s propensity for invasiveness (*P. acutus* is not known to act invasively) and higher aggression levels compared to *P. acutus* (Gherardi and Daniels 2004). I also believe that higher densities of crayfish will cause a greater reduction in the survival of larval anurans. I posit that larval period will decrease and that mass of metamorphs will increase in response to crayfish predators and that this response will be more pronounced in treatments with a high density of crayfish or

treatments containing *P. clarkii*. The increased body mass is hypothesized to be due to higher levels of resources in treatments in which intraspecific competitors have been thinned by predators whereas the shorter larval period is hypothesized to be a response to escape an environment with a high predation risk as early as possible.

I hypothesize that risk- reduction (hindered predation) will occur in treatments containing only one species of crayfish whereas risk-enhancement will occur in treatments containing two crayfish species. I believe that risk-reduction will occur for the single-species treatments because risk-reduction is a general trend seen in multiple predator studies that may be due to generalized defenses of prey items (Vance-Chalcraft and Soluk 2005). For instance, if prey reduce movement as a generalized response to a predator, they may reduce movement to a higher degree in response to a higher density of predators. I further posit that survival of larvae will be lower when the two crayfish species are combined than for either species alone. Despite the likelihood that tadpoles may have a generalized anti-predator response to crayfish, I believe the higher activity levels of the *P. clarkii* will scare the tadpoles into movement. This may cause lower survival by improving the efficiency of *P. acutus* in the presence of *P. clarkii* because higher activity level of *P. clarkii* would scare tadpoles toward the other predator. This study will further knowledge of species interactions that affect anurans and may help to inform management needs for *P. clarkii* in North Carolina.

Methods

To test my predictions I conducted an experiment in 30 mesocosms (stock tanks modified to resemble a natural pond) at East Carolina University's West Research Campus in Greenville, NC. Mesocosms are an ideal venue for studying predator-prey

dynamics in pond communities. Natural pond characteristics (water, leaf litter, zooplankton, phytoplankton) can be mimicked, all other organisms except those of interest can be excluded. Covering mesocosms with mesh screening ensures that flying predators (or the offspring of flying predators, such as dragonfly larvae) cannot enter or oviposit. Additionally, mesh screening prevents oviposition by other frog species and the escape of experimental animals. Another venue for studying pond organisms is through the use of enclosures placed in ponds. However, this method does not allow for the complete isolation of experimental organisms because chemical cues can be exchanged between animals in different enclosures and between animals in enclosures and animals inside the pond. Consequently, enclosures placed in the same pond are not independent of each other (Chalcraft, Binckley et al. 2005).

To examine the independent and interactive effects of each crayfish species on larval anurans I developed four treatments where I independently manipulated the abundance (0 versus 1 individual) of each crayfish species. Although these treatments allow me to compare the per capita effects of each crayfish species at low density and to evaluate risk reduction and risk enhancement when both crayfish species are in the same environment, it is possible that predation may be higher in the treatment with two crayfish species than one crayfish species because there are more crayfish present (2 versus 1). Consequently, I included two additional treatments where each crayfish species occurred alone at a higher density (2 individuals). For a pictorial representation of treatments, see Plate 5. These treatments allow me to not only examine density-dependent changes in consumption rates of crayfish but they also allow me to determine whether the combined effect of both crayfish species is really any different than having more crayfish

of a single species present. If anuran response is found to be similar in all treatments containing two crayfish (regardless of species) and the effects are greater than those in the single predator treatments, then predator density may be the driving influence rather than predator identity. Densities of crayfish were within ranges found in nature (Figiel and Semlitsch 1991).

Experimental mesocosms consisted of 190 L cattle tanks containing aged well water, 200g of mixed pine/hardwood leaf litter and 470 ml of homogenized zooplankton. Each mesocosm was covered tightly with shade cloth to prevent colonization by flying insects and treefrogs and to contain experimental animals. Each was also equipped with two 15 cm lengths of PVC pipe to provide shelter for crayfish. Experimental units were arranged in randomized blocks, in which one replicate of each treatment is clustered together in order to minimize the effects of any spatial gradients across the array of the experimental mesocosms. Treatments were randomly assigned to mesocosms within each block. Utilizing a randomized block design allows for statistical accounting of any differences due to spatial arrangement of the mesocosms. Additionally, because experimental animals are often differently sized, they can be blocked by size and the size difference statistically accounted for. Each of the six treatments was replicated in five blocks for a total of 30 experimental units.

Although *Procambarus acutus* and *P. clarkii* are known to burrow as ponds dry (Holdich and Lowery 1988; Loughman 2007), they can be found foraging at depths of only a few inches even when summer water temperatures get high (personal observation). *Procambarus acutus* were collected from an area that is not known to have *P. clarkii*, however, *P. clarkii* were collected in an area that is known to have *P. acutus* but in very

low abundance. The two species are easily distinguished by the prominent claw nodules, red coloration and a dark vein under the tail of *P. clarkii* (Benson and Fuller 1999).

Crayfish were collected by the use of unbaited minnow traps and dip nets on 1 and 2 June, 2009. Crayfish were size-matched and assigned to blocks according to size, to avoid intraguild predation and to help account for any variation in predation due to predator size.

I identified a chorus of *Bufo fowleri* on the evenings of 30 and 31 May 2009. Four resultant egg clutches were collected in single-clutch containers on 31 May and 1 June 2009. Hatchlings from the three viable clutches were allowed to progress to the free-swimming stage before use in the experiment. To help account for any genetic differences in the *B. fowleri* clutches, each experimental cohort contained equal proportions of tadpoles from each of the three clutches, so that all experimental cohorts contained the same genetic diversity. Each experimental unit received one cohort of 150 tadpoles on 8 June 2009. This density of tadpoles is within the natural range of similar species (Wilbur 1977). Tadpoles were allowed to acclimate to the mesocosms overnight and crayfish treatments were applied on 9 June 2009.

Mesocosms were checked daily and toads were collected as metamorphs as soon as one forelimb had emerged. Metamorphs were held in small plastic containers until their tail was completely resorbed. Toads were then weighed, the dates noted for the calculation of larval period, and then subsequently returned to their natal pond. At the close of the experiment, mesocosms were drained and the remaining leaf litter was carefully sorted through for any residual tadpoles. Any remaining tadpoles were included

in survivorship data only. Experimental crayfish were transported back to the laboratory at East Carolina University, weighed, and released.

Statistics

Response variables included mass at metamorphosis, survivorship and larval period of *Bufo fowleri* toad metamorphs. Larval period was considered to be the number of days between the start of the experiment and the time at which a metamorphs' tail was completely resorbed. Survivorship was calculated as the natural log of the proportion of surviving anurans per experimental unit. Using the natural log for survivorship data is appropriate as it reflects instantaneous per capita mortality rates for populations experiencing no births or immigration. To avoid pseudoreplication, mean values for larval period and mass at metamorphosis were calculated for each experimental unit and were used for subsequent statistical analyses.

Using SAS statistical software, ANOVA were utilized to determine the effect of crayfish presence, density, and identity on the three response variables. Block effects were included in all analyses. To find the effect of crayfish presence on anurans an ANOVA was used to compare the control treatment (no crayfish present) to all treatments containing crayfish. Similarly, to examine the effects of density, treatments containing two crayfish were compared with treatments containing one crayfish with no regard to species differences. I separated the treatments into low and high density and performed ANOVA on each to determine the effect of the different species at either density. In two of the experimental units, crayfish predators did not survive to the completion of the experiment, and in those cases the data are excluded.

I used two-tailed paired t-tests to compare actual prey mortality in the high density treatments to a predicted prey mortality to determine if risk-enhancement or risk-reduction were occurring in my treatments. Predicted prey mortality was calculated using the multiplicative risk model (Soluk and Collins 1988):

$$\text{Predicted prey killed} = P_a + P_b - P_a P_b$$

where P_a is the proportion of prey killed by species a in the low density treatment and P_b is the proportion of prey killed by species b in its low density treatment. Subtracting the term $P_a P_b$ indicates that the same prey item cannot be killed twice. P_a and P_b were considered to be equal to each other when predicting the combined impact of two individuals of the same species.

Results

Crayfish presence caused a significant reduction in *Bufo fowleri* survival and larval period but enhanced mass at metamorphosis ($F_{1,18} = 23.22$, $p = 0.0001$, $F_{1,18} = 18.83$, $p = 0.0004$, and $F_{1,18} = 27.51$, $p = 0.0001$, respectively, Plate 6, Figure 1). Similarly, as density of crayfish increased, mass increased ($F_{2,13} = 18.59$, $p = 0.0002$) and survivorship decreased ($F_{2,13} = 26.75$, $p < 0.0001$, Plate 7, Figure 2). However, no difference could be found between low and high crayfish density for larval period although toads in both of these sets of treatments had shorter larval periods than the predator-free controls ($F_{2,13} = 13.23$, $p = 0.0007$, Figure 2). Whether a treatment held one or two *Procambarus clarkii* had no effect on survivorship ($F_{1,3} = 4.87$, $p = 0.114$), mass ($F_{1,3} = 1.34$, $p = 0.331$), or larval period ($F_{1,3} = 0.24$, $p = 0.655$, Plate 8, Figure 3) of *Bufo*

in treatments containing only *P. clarkii*. Although density of *P. acutus* did not significantly affect mass ($F_{1,4} = 3.24$, $p = 0.146$), treatments with a high density of *P. acutus* caused a significant reduction in survivorship of anuran larvae ($F_{1,4} = 10.23$, $p = 0.033$) and a strong trend for reduction in larval period ($F_{1,4} = 6.32$, $p = 0.066$, see Plate 9, Figure 4). Crayfish species did not differ in their effects on *Bufo* (for survivorship, $F_{1,4} = 6.40$, $p = 0.0647$; for mass, $F_{1,4} = 0.22$, $p = 0.6657$; for larval period, $F_{1,4} = 2.00$, $p = 0.2305$, Plate 10, Figure 5) when crayfish were present in low densities. Tadpole survival, however, tended to be lower in treatments with *P. acutus* than in treatments with *P. clarkii* when crayfish densities are low (Figure 5-A). There were significant differences in survivorship and mass of anurans ($F_{2,6} = 6.38$, $p = 0.0327$ and $F_{2,6} = 23.59$, $p = 0.0014$, respectively) but not for larval period ($F_{2,6} = 0.76$, $p = 0.5074$, Plate 11, Figure 6) among treatments with two crayfish. Specifically, at high density, survivorship was highest in the single-species *P. clarkii* treatment, and was lower in both the single-species *P. acutus* treatment and when *P. acutus* was combined with *P. clarkii* (Figure 6). All three high-density treatments differed in the mass of metamorphosed toads, with the heaviest toads emerging from the multi-species treatments and the lightest from the single-species *P. clarkii* treatment (Figure 6).

Although increasing the density of *P. acutus* did not cause observed mortality to differ from expected mortality (given independent effects of individual *P. acutus*, $p = 0.283$), there was a strong tendency for risk reduction in the higher density of *P. clarkii* ($p = 0.052$, Plate 12, Figure 7).

Discussion

Contrary to what was expected, *Procambarus acutus* appears to have more of an effect on tadpole populations than *P. clarkii* under the conditions tested. In these circumstances, in which a non-native is transplanted only slightly out of its native range and among species similar to those in its native environment, its impact may not be as detrimental as if it were transplanted into a vastly different area. Additionally, it is likely that the anti-predatory responses of *Bufo fowleri* tadpoles were effective against the non-native *P. clarkii* as crayfish are a predator that occur commonly in habitats that *B. fowleri* frequents.

As in previous studies, I found that crayfish presence generally had an effect on tadpole populations (Gherardi, Renai et al. 2001; Renai and Gherardi 2004; Gherardi and Barbaresi 2007). Across treatments containing a crayfish predator, only an average of 24% of the tadpoles survived to metamorphosis, whereas 81% of the tadpoles in the controls survived (Figure 1). For the *Procambarus clarkii* in the single-species treatments, density appeared to have little effect on the response variables, although overall trends could be seen that mirrored the slightly more obvious trends in the single-species *P. acutus* treatments (Figures 3 and 4). This suggests that density of the crayfish may be eliciting functionally similar responses from the tadpoles, but to a lesser degree in *P. clarkii* than *P. acutus*. This may be indicative of lower intraspecific interference (competition) in *P. acutus* because they were able to kill more tadpoles than *P. clarkii*. Differential tadpole response between the two crayfish species was also found at low predator density; there was a strong trend for reduction in survivorship by *P. acutus* compared to *P. clarkii* (Figure 5).

The effects of crayfish on tadpoles are probably not only predatory. Crayfish are omnivorous, and will consume the same detrital and algal resources as tadpoles, making them competitors for resources as well as predators (Holdich and Lowery 1988). In addition to consuming detritus, crayfish also are important shredders of leaf litter in aquatic habitats (Usio 2000; Montemarano, Kershner et al. 2007). This action and the production of feces high in nitrogen may increase the bioavailability of nutrients locked inside dead leaves, increasing local nutrient levels and possibly increasing primary productivity (Covich, Palmer et al. 1999). It was found in a previous study that crayfish presence was associated with higher biomass of periphyton in late summer but that this trend was not maintained over time (Dorn and Wojdak 2004). This phenomenon could be present in the experiment reported for this thesis as the experiment only lasted one season and periphyton production may have increased enough to enhance anuran metamorph body mass. The possible increase in periphyton due to crayfish nutrient enhancement may have mitigated some of the competitive effects of crayfish on tadpoles. This phenomenon may increase the body mass of tadpoles (due to higher resources) relative to other predators. For instance, a non-crayfish predator that reduces survival of tadpoles by a similar amount but does not add nutrients to the water might have less of a positive effect on mass at metamorphosis. In this way, crayfish may actually be indirectly beneficial to the remaining larvae that it does not kill.

Interestingly, even though the single-species high density *P. acutus* treatments showed similar survivorship to the mixed-species treatments, *Bufo* mass at metamorphosis was significantly higher in the mixed-species treatments (Figure 6). This may be explained by the differential effects of each crayfish species. If the *P. acutus* in

the mixed-species treatment is reducing populations to levels similar to the all *P. acutus* treatments and the *P. clarkii* is adding nutrients to the water through increased substrate breakdown, it follows that the emerging metamorphs would have greater algal resources divided among fewer individuals, giving survivorship similar to the high-density *P. acutus* treatments but with greater masses.

Mass at metamorphosis has been long-considered an indicator of future fitness in anurans (Wilbur and Collins 1973; Goater, Semlitsch et al. 1993). Although increased mass has been linked with higher sprint speeds and endurance in *Bufo* metamorphs, it has also been found that metabolic rates also increase, presumably because of a higher energy requirement for maintenance at larger sizes (Beck and Congdon 2000). This trade-off may enhance performance in young anurans, increasing predator-avoidance capabilities; but at the same time may decrease future fitness by requiring more energy to be shunted to maintenance rather than reproductive output (Beck and Congdon 2000). In this case, mass of metamorphs was higher in crayfish treatments than controls, and increased as predator density increased (Figures 1 and 2). The increase in mass at metamorphosis in these treatments is likely due to a release from competition mediated by the consumption of larvae by crayfish (Van Buskirk and Yurewicz 1998).

Presence of crayfish also affected the larval period of *B. fowleri* (Figure 1). Larval period of anurans has been found previously to increase or decrease in unpredictable ways with differences in predators and larval density [reviewed in (Relyea 2007)]. In the case of this study, as crayfish predator density increases, survivorship and larval period decreases and mass of metamorphs increases (Figure 2). A combination of predator-mediated metamorphic induction and thinning of competitors likely leads to earlier larger

metamorphs. This pattern of emerging earlier when lethal predators are present may be uncommon as it only occurred in 32% of the studies reviewed by Relyea. In only one of the eleven lethal predator studies reviewed did a member of the genus *Bufo* emerge earlier and larger in treatments with lethal predators than in treatments with no predators. None of the studies utilizing *Bufo* included a crayfish predator (Relyea 2007).

Predators may induce larvae to emerge more quickly so as to escape predation, and at the same time may reduce competition (through thinning of competitors) enough so that the resources left are more plentiful and perhaps of better quality, allowing for faster growth. Rapid development (short larval period) has been linked to increased sprint speed and endurance in *Bufo* metamorphs and may confer an advantage in dispersal and predator avoidance once they have left the pond (Beck and Congdon 2000). In this study, a marked reduction in larval period is only clear when presence and absence of crayfish predators is considered; density and identity of the crayfish had no significant effect (Figures 2-5).

When compared to the calculated expected prey mortality, there was an overall trend across treatments for risk-reduction when two crayfish predators were present (Figure 7). Because *P. clarkii* are more active than *P. acutus* it is possible that the movement of the predators frightened the tadpoles into staying clear of them, increasing the level of risk reduction in the conspecific *P. clarkii* treatment. (Acquistapace, Daniels et al. 2004). Acquistapace et al. noted that *P. clarkii* responded more actively to food and heterospecific cues than did *P. acutus* (Acquistapace, Daniels et al. 2004). Higher activity levels may help to explain the similar consumption rates in the single-species *P. acutus* and the treatments with both crayfish species present (Figure 7). If the *P. clarkii* are

responding more actively to food cues (tadpoles) and heterospecifics (*P. acutus*), the increased activity may chase the prey from the *P. clarkii* and toward the less active (but now more successful) *P. acutus*.

Procambarus acutus may be capable of out-competing *P. clarkii* in some heterospecific settings (Eversole, Mazlum et al. 1999; Eversole, Mazlum et al. 2004; Mazlum and Eversole 2005; Mazlum and Eversole 2008); nevertheless, there are areas that likely do not have a large enough population of *P. acutus* to do so. *P. clarkii* is capable of year-round recruitment in warmer climates (Holdich and Lowery 1988) and as such may be able to rapidly increase its population in a short amount of time in the absence of competitors. In this way, and despite its tendency toward risk-reduction at higher densities (Figure 7), it is possible that *P. clarkii* could become a pest as it has in areas far outside of its home range.

In this study only adult crayfish were used. However, it has been found previously (in crayfish species other than the ones in this study) that juvenile crayfish are more likely to feed on animal prey than the adults of the species while adult crayfish were found to have a higher plant and detritus-based gut content (Holdich and Lowery 1988). Although there was a significant impact on larval anurans by adult crayfish in this study, there may be different impacts *in vivo*. For instance, the per capita depredation of anurans may increase if the juvenile crayfish are more voracious predators than adult crayfish. However, it is also possible that the presence of adult crayfish may influence juveniles in some way so as to consume fewer anuran larvae. Additionally, because adult *P. clarkii* can be quite large, it is possible that the bait industry (which is the likely source of many *P. clarkii* introductions) would sell a higher proportion of juvenile crayfish to suit a gape-

limited fish predator. This influx of highly predatory juvenile crayfish may further reduce anuran and macroinvertebrate populations in areas where bait buckets are likely emptied.

I found differences in the effects of *Procambarus acutus* and *P. clarkii* on *Bufo fowleri*. However, contrary to my original hypotheses, *P. acutus* seems to have a greater lethal effect than *P. clarkii*. Even at low density, *P. clarkii* reduced survival by 44% over predator-free controls, whereas *P. acutus* reduced survival by 54% over predator free controls. Although *P. clarkii* seems to have less of an effect at a higher density than *P. acutus*, *P. clarkii* is capable of surviving at very high densities whereas *P. acutus* is not known to exhibit this invasive behavior in nature. The high density of individuals that *P. clarkii* are capable of producing, both in and out of aquaculture, are evidence of its ability to survive and persist in less than optimal conditions (Holdich and Lowery 1988). High concentrations of crayfish predators, such as those that may occur in populations of *P. clarkii*, may have unknown repercussions for the aquatic communities of North Carolina.

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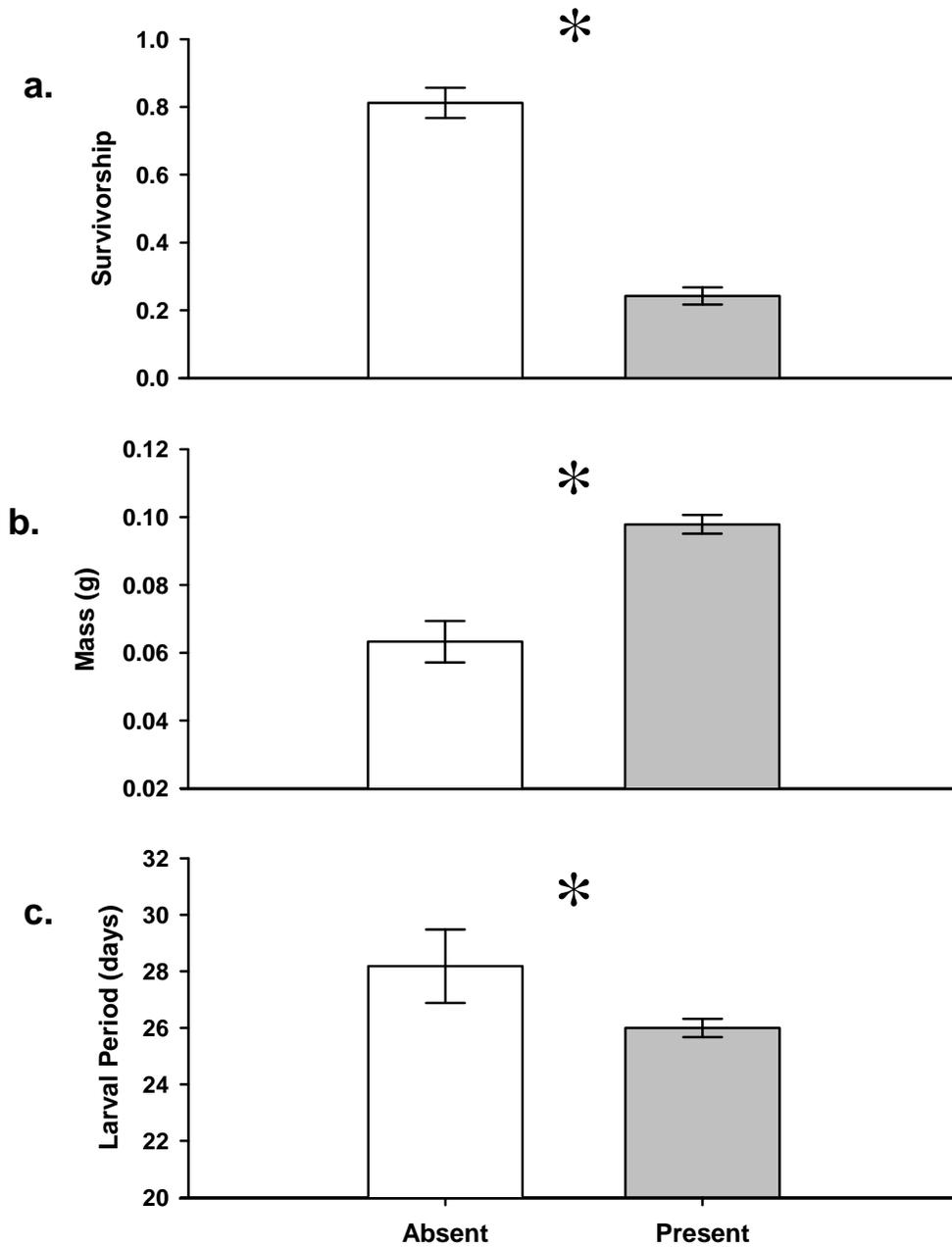


Figure 1: Average effect of crayfish presence on a) survival, b) mass at metamorphosis and c) larval period in *Bufo fowleri*. Asterisk (*) above bars indicate significant differences. One positive and negative standard error is indicated.

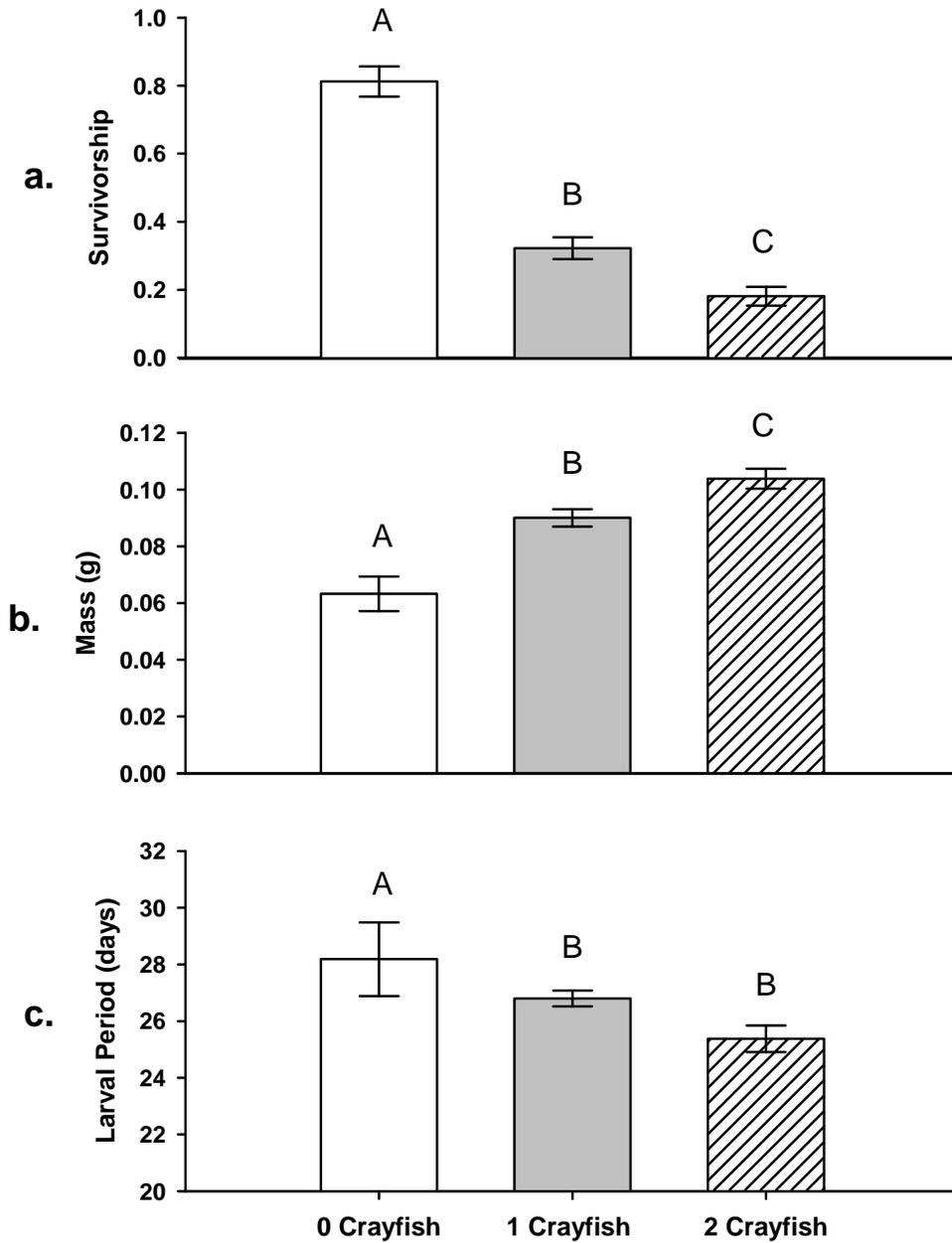


Figure 2: Average differences in a) survival, b) mass at metamorphosis, and c) larval period mediated by crayfish density. Letters above bars indicate significant differences as calculated by a post-hoc REGW. One positive and negative standard error is indicated.

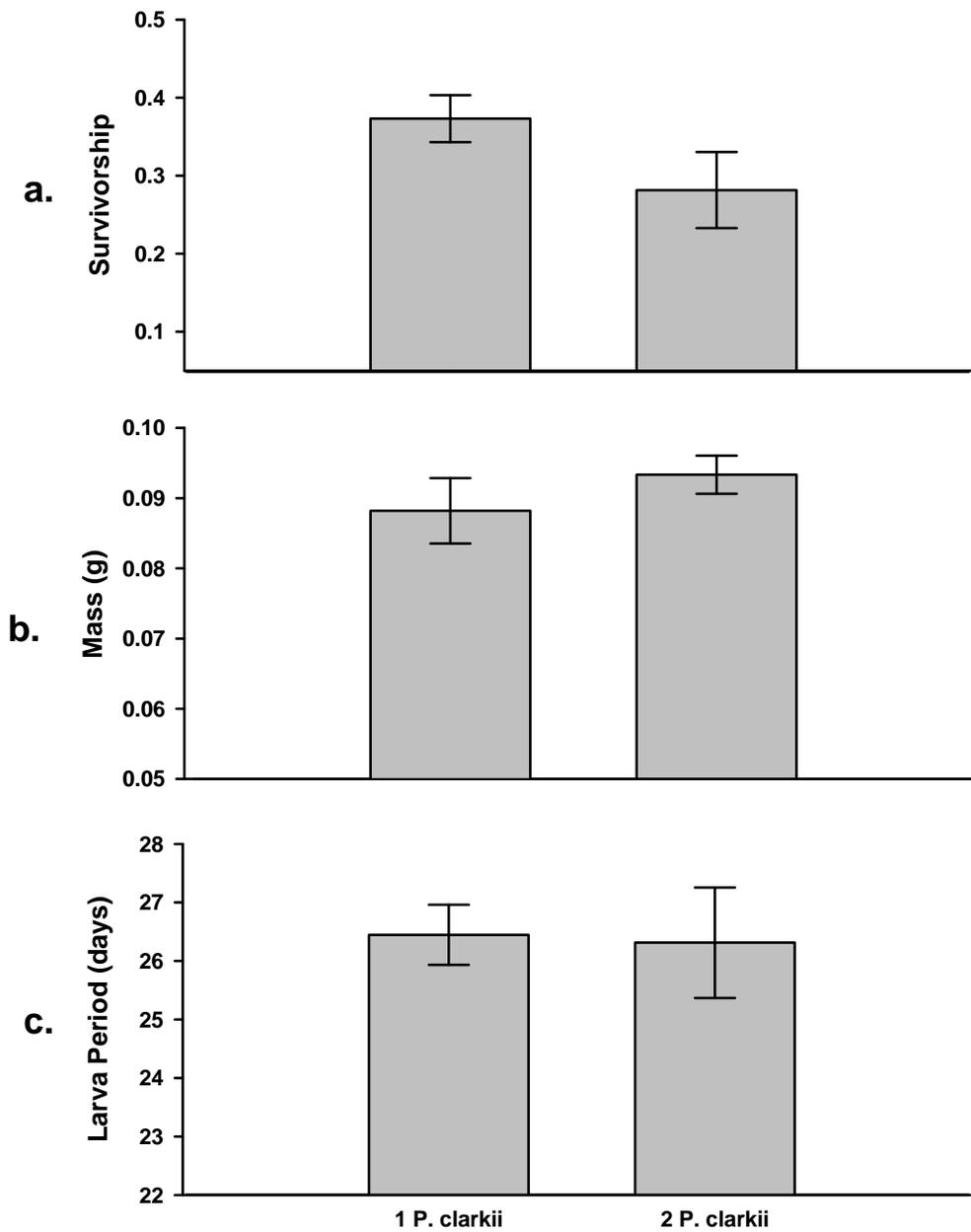


Figure 3: Average differences in a) survival, b) mass at metamorphosis and c) larval period as mediated by *Procambarus clarkii* at low and high densities. One positive and negative standard error is indicated.

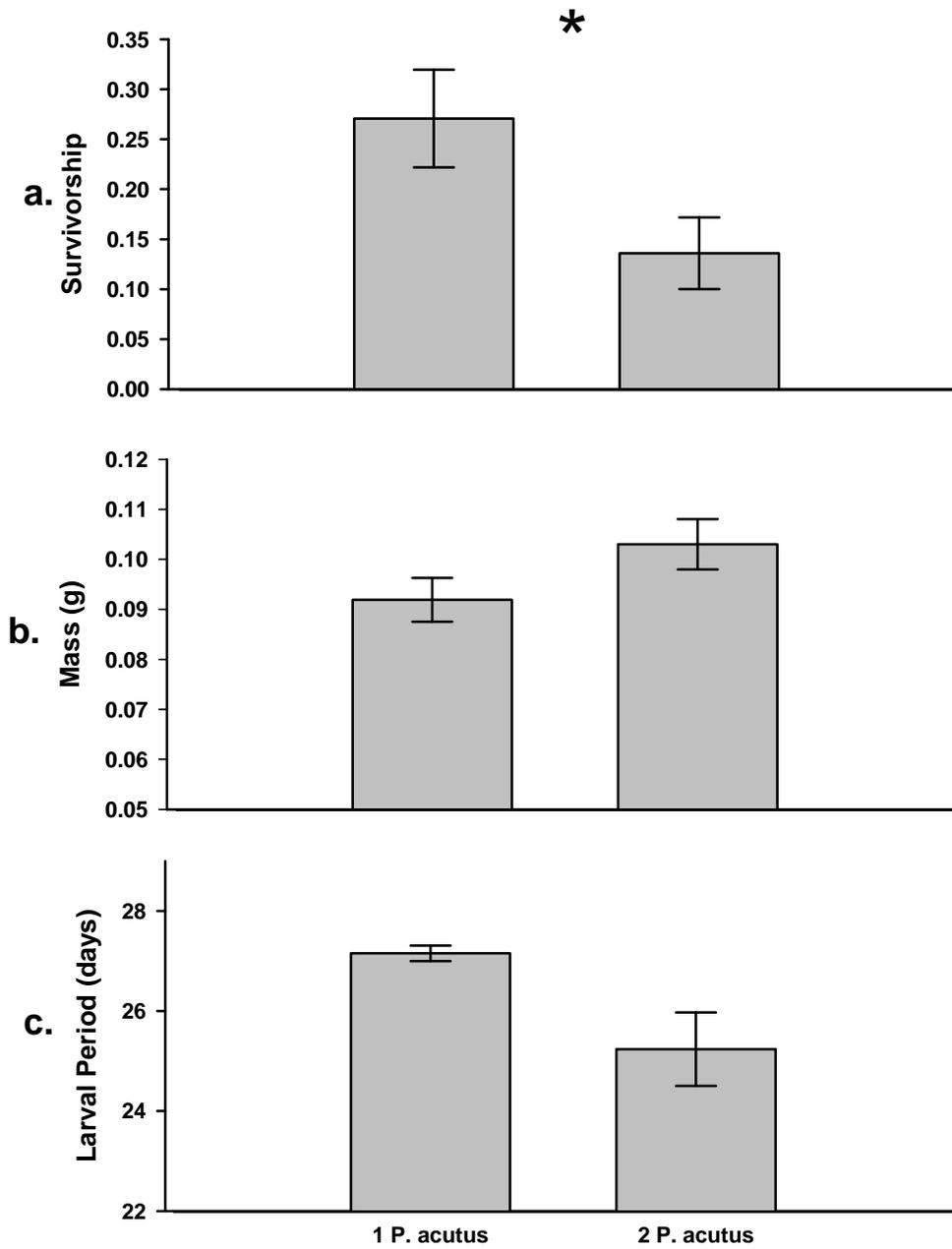


Figure 4: Average differences in response variables as mediated by *Procambarus acutus* at low and high density. Asterisks (*) above bars indicate significant differences. One positive and negative standard error is indicated.

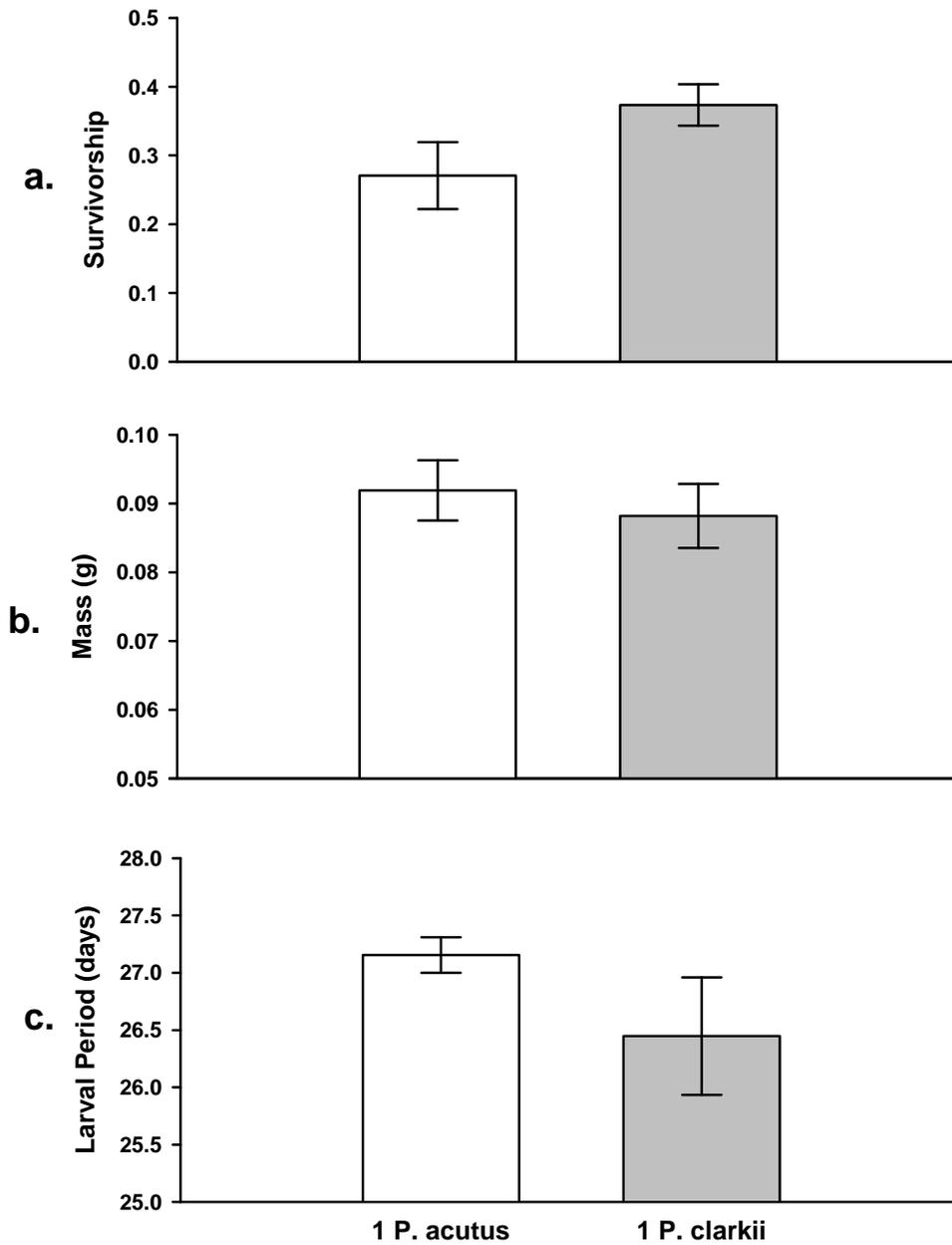


Figure 5: Average differences in a) survival, b) mass at metamorphosis and c) larval period when crayfish were at low density. One positive and negative standard error is indicated.

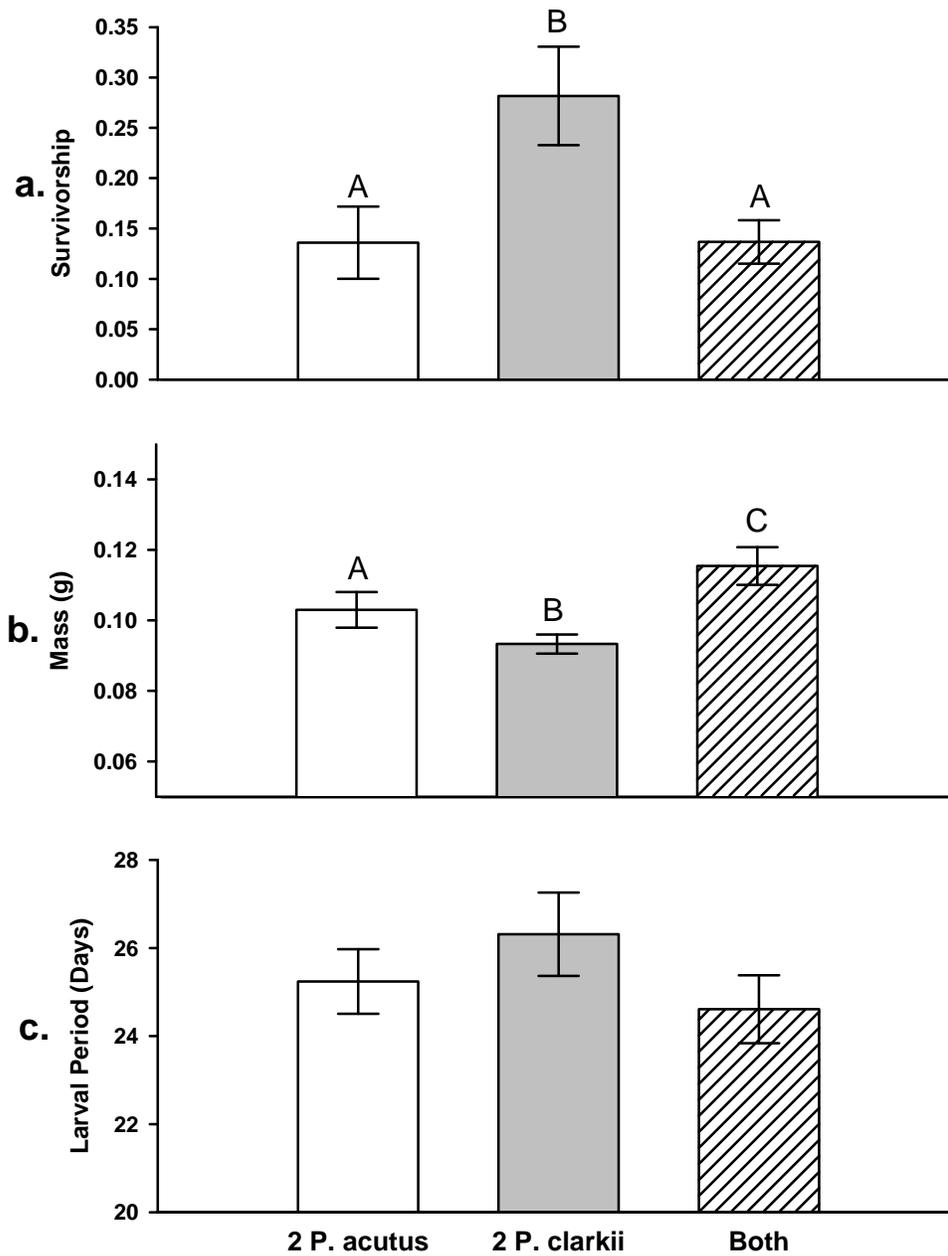


Figure 6: Average differences in a) survival, b) mass at metamorphosis and c) larval period in *Bufo fowleri* by crayfish species at high density. The “Both” category indicates that one of each species was present. Letters above bars indicate significant differences according to a post hoc REGW. One positive and negative standard error is indicated.

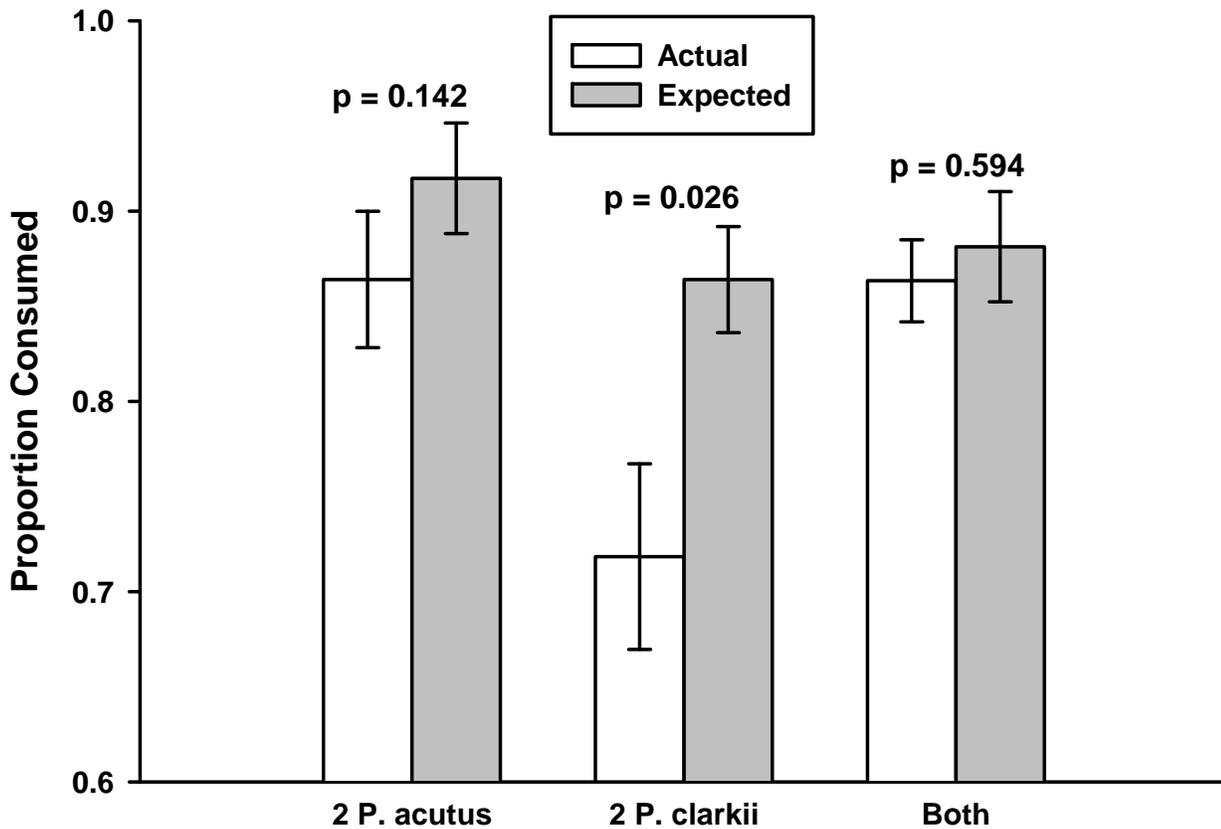


Figure 7: Actual and expected proportions of *Bufo fowleri* consumed. The “Both” category indicates the treatments contained one *P. acutus* and one *P. clarkii*. Expected consumption was calculated using Soluk and Collins’ multiplicative risk model (1988). Values above the bars indicate p-values associated with a one-tailed paired-t test. One positive and negative standard error is indicated.

CHAPTER 3: Assessment and Implications of this Study

In this study, it was found that *Procambarus acutus*, a North Carolina native, was a more successful predator than the invasive *Procambarus clarkii* at high density.

Although this was unexpected, it was not surprising as the native has a natural history with the prey item and *P. acutus* and *P. clarkii* are congeners. Additionally, because *P. clarkii* is known to be more aggressive than *P. acutus*, agonistic interactions in the high density *P. clarkii* treatments may have reduced predatory efficiency. Therefore the fact that the two crayfish have comparable effects on prey at low density (as I have shown) is also not surprising because of the lack of possible competitive interference at low density.

Although this study found that native species of crayfish are better predators, it still informs invasive species biology. In many instances, invasive species are found in areas that have similar types of organisms but of different species. Such is the case for invasive ant species, earthworms, vines, grasses, etc. in the U.S (APHIS 2009; Global Invasive Species Database 2010). However, release from predation, natural enemies such as pathogens, and introduction into an area that may be climatologically advantageous may help to give invasives the upper hand against natives (Wonham 2006). The two crayfish species in this study are congeners, have overlapping ranges and are known to co-occur in the same body of water in the native range of *P. clarkii* (Huner, Barr et al. 1984). It is therefore possible that any advantage the *P. clarkii* might have in the invaded range would be similar to the advantages it has in its native range. In this way, the ecological processes that keep the two in coexistence in the native range of *P. clarkii* may be similar in the invaded range in Eastern North Carolina.

In this study, there were no differences between the two crayfish species in any of the response variables (survival, mass at metamorphosis, larval period) at low density of crayfish. In the case of these two crayfish, it is possible that the similarity between the two is enough that the effects of crayfish identity will not matter at low or normal density. This may suggest to biologists that when a transplanted species is placed in an environment in which many species are similar (or the same) as in its home range, its effects on the community may also be similar. Like many areas in the native range of *P. clarkii*, Eastern North Carolina is riddled with floodplains, cypress swamps and other habitats suitable for crayfish. Because some of the same species are located in both areas (both flora and fauna) it is conceivable that *P. clarkii* simply fit in the same niche that it occupies in its native range. However, any predator at high density is certain to have negative effects on prey species, and *P. clarkii* is known to reach very high densities in invaded areas (personal observation).

The effects of “short range” introductions of crayfish are not extensively studied, but information does exist for *Orconectes rusticus*. This species forces other crayfish from hiding places, making them more susceptible to predators than if the invasive were not present (Global Invasive Species Database 2010). In addition, *O. rusticus* has a higher metabolism and therefore consumes more macrophytes than native crayfish. Macrophytes are a food source that is limited in the northern lakes where *O. rusticus* has invaded (Global Invasive Species Database 2010). In this case, when an introduced species has significantly different traits than natives, a short-range introduction can be detrimental. However, if short-range introductions are analogous to range expansions, such as those seen naturally in bird species, then the outcome may not always be damaging. For

instance, in the U.S. the range expansion of the great-tailed grackle (*Quiscaus mexicanus*) has been relatively benign to other species, although it is a bit of an agricultural pest (Wehtje 2003). The expansion of the western bluebird (*Sialia mexicana*), on the other hand, has begun to displace the less-aggressive mountain bluebird (*Sialia currucoides*) as the western bluebird expands its range eastward (Duckworth and Badyaev 2007). The different aggression levels of the grackle and the western bluebird toward sympatric species are likely what make one range expansion benign and the other detrimental.

Introduced species, by virtue of being inserted into a new environment, will undoubtedly have interactions with local species other than immediate annihilation. These exchanges with native flora and fauna may shape community characteristics that are not directly associated with the interacting species. For instance, in Hawaii, 80% of the habitat invaded by pigs has been degraded to vegetation-free bare ground (Pimentel, Zuniga et al. 2005). This direct exchange between the pigs and the vegetation causes a disturbance that, along with fecal distribution of seeds by the pigs, contributes to the spread of the strawberry guava (*Psidium cattleianum*), another invasive species that is pushing out native Hawaiian plants (Van Driesche and Van Driesche 2000). The interaction of the pigs with native plants both directly changes the landscape (through plant removal) and indirectly alters the species composition in the area by facilitating invasive plant species.

Although native and non-native species interactions are inevitable, relatively few studies have examined the impacts of native and invasive predators together. The interactions of invasive species with native species and the effect of both together on native prey populations may have different impacts than invasive or native species

separately. Although in this study there were no differences in impact on larval anurans between *Procambarus acutus* and *P. clarkii* at low density, at high density I found that the two species together induced a higher mass at metamorphosis than either species alone. It is therefore possible that when *P. acutus* and *P. clarkii* occur at a 1:1 ratio, surviving tadpoles will be indirectly benefited by the interaction of the two crayfish considering there was no difference in mortality between the mixed-species and high density *P. acutus* treatments. Although this may be good news for the tadpoles, and maybe a partial name-clearing for the invasive *P. clarkii*, it is unlikely that a 1:1 ratio of these species at the density examined would continue over time.

When studying invasions of species that are omnivorous, predation, detritivory and herbivory may combine to have a more pronounced effect on native species than any single trophic interaction alone. The total effects of a species in an environment may be very different from the predatory effects. For instance, *Procambarus clarkii* is known to destroy macrophytes, which are substrates for epiphytes such as diatoms. Tadpoles consume diatoms as an important part of their diet (Kupferberg 1997). In this way, crayfish could affect both the direct survival of tadpoles through predation, and also affect the quality and quantity of food available to these organisms.

In the simple habitat created for this experiment, only the effects of crayfish species in a homogenous environment are considered. In nature, and although they co-occur in some areas, these two species may inhabit different types of wetlands (Huner, Barr et al. 1984; Holdich and Lowery 1988). For instance, *Procambarus clarkii* will live in dirtier, more anoxic habitats with lower pH than *P. acutus* (Huner, Barr et al. 1984 231) and *P. acutus* is more frequently associated with flowing and pristine freshwater

habitats than *P. clarkii* (Holdich and Lowery 1988). Because there is no shortage of wetlands in eastern North Carolina, native crayfish may inhabit the wetlands that suit it best and that it inhabited first, while the introduced species may be relegated to the less-preferred habitat. This hypothesis has not been tested, but a reciprocal transplant experiment with caged crayfish of both species is a simple way to assess performance in different types of wetlands. In addition to individual performance, it is possible that competition between crayfish is context-dependent; the better predator may vary with environmental conditions. Environmentally-dependent performance should be considered when looking at species that can live in more than one type of habitat. An organism that is very detrimental in a cypress swamp may simply coexist with natives in a temporary pond or a stream.

In the example of *Procambarus clarkii* in Eastern North Carolina, seasonal changes in the density and activity of this crayfish may affect native populations of amphibians differently than other types of predators. The density of crayfish (and the size ratios of adults and juveniles) in freshwater systems will change with season and rainfall (Huner, Barr et al. 1984; Holdich and Lowery 1988). Crayfish in temporary ponds will move from their burrows into the open water of ponds as the ponds fill with rain. This rain can be the same trigger for many frog species to begin breeding. In a spring rain event, ponds will fill, frogs will breed and crayfish will emerge from their burrows all at once. The fluctuation of all of these factors could have unpredictable effects on a pond community, especially if the crayfish is an invasive species with no evolutionary history with the area species. Although this is not the case with crayfish in Eastern North

Carolina (many temporary ponds in this area contain crayfish), differences between native and introduced crayfish may be more pronounced in other areas.

When a species is beneficial to humans, it is more likely to be moved to new areas (Wonham 2006). Some of the most successful invasions have been mediated by humans moving species around for our own benefit (Williamson and Fitter 1996; Pimentel, Zuniga et al. 2005). When considering the introduction of new species into an area for agriculture, aquaculture, forestry, or recreation, we must consider ways to prevent that new species from escaping and colonizing. This is especially true when the species of interest is a habitat generalist and can proliferate without human intercession. Prevention of species escape is also critical when the species is a known invasive in other areas.

There are several aquaculture facilities that utilize *Procambarus clarki* in Eastern North Carolina and no preventative measures are taken to insure that the crayfish do not escape the aquaculture ponds (Steve Gabel, NC Cooperative Extension, personal communication). However, the likely pathway for introduction of the population used in this study is fishermen's bait buckets (personal sleuthing). In both cases, prevention of escape by an introduced species could be fairly simply accomplished by using native crayfish for bait and building retaining fences around aquaculture ponds. Fortunately, *P. clarkii* has not yet spread across the entirety of Eastern North Carolina and does not yet have the devastating effects here that it has elsewhere.

That invasive species are a leading cause of reduction of global biodiversity is hotly debated in some scientific arenas (Brown and Sax 2004). However, there are known mechanisms by which invasive species can reduce biodiversity and they include introduction of new pathogens and diseases, crowding out of species through competition

for space or food, direct predation of species, hybridization with local species, and altering the environment to make it unsuitable for natives (Wonham 2006). Even when an introduced species does not directly damage the other species in the new environment, it still adds to the global homogenization of species and may alter the path of evolution for the species it comes in contact with. Additionally, it is possible that the introduction of new species may change the environment in subtle ways that may make native species more susceptible to extinction without the direct killing of any species. We can never truly understand the full impact of invasive species because we will never stop changing the rules of the game. We continuously move species; we constantly disturb habitats. Even if no direct change is apparent, the world's climate is changing. These factors, along with others, will alter the lives of species in ways that may change their interactions with each other.

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APPENDIX A: Animal Use Protocol Approval



Animal Care and Use Committee

East Carolina University

212 Ed Warren Life Sciences Building

Greenville, NC 27834

252-744-2436 office • 252-744-2355 fax

June 16, 2008

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "The Differing Effects of the Red Swamp Crayfish and White River Crayfish on Larval Treefrogs," (AUP #D221) was reviewed by this institution's Animal Care and Use Committee on June 16, 2008. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

A handwritten signature in black ink that reads "Robert G. Carroll, Ph.D.".

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure

APPENDIX B: PLATES

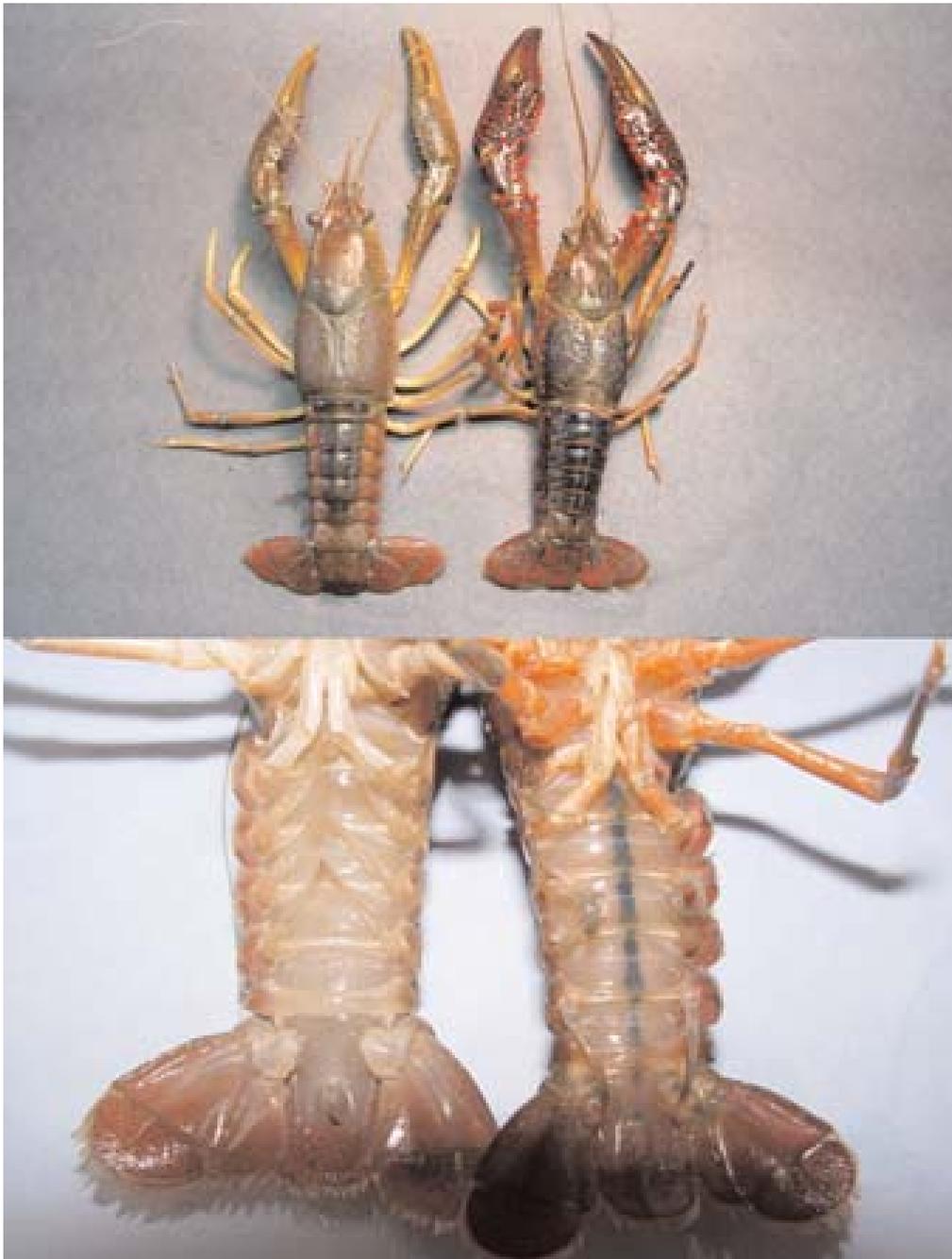
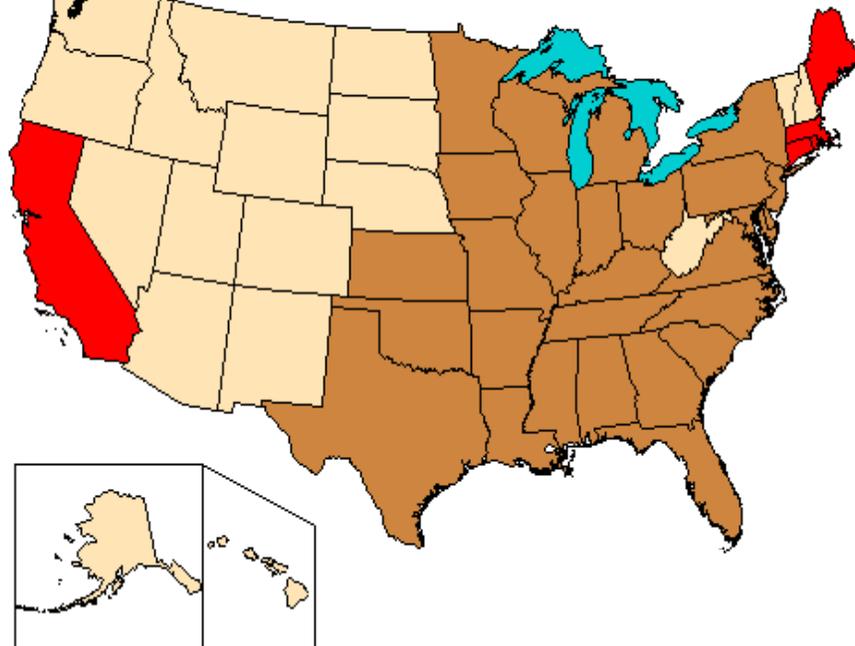


Plate 1: *Procambarus acutus* and *Procambarus clarkii* (left and right, respectively)

Procambarus acutus
White River crawfish



Procambarus clarkii
red swamp crawfish

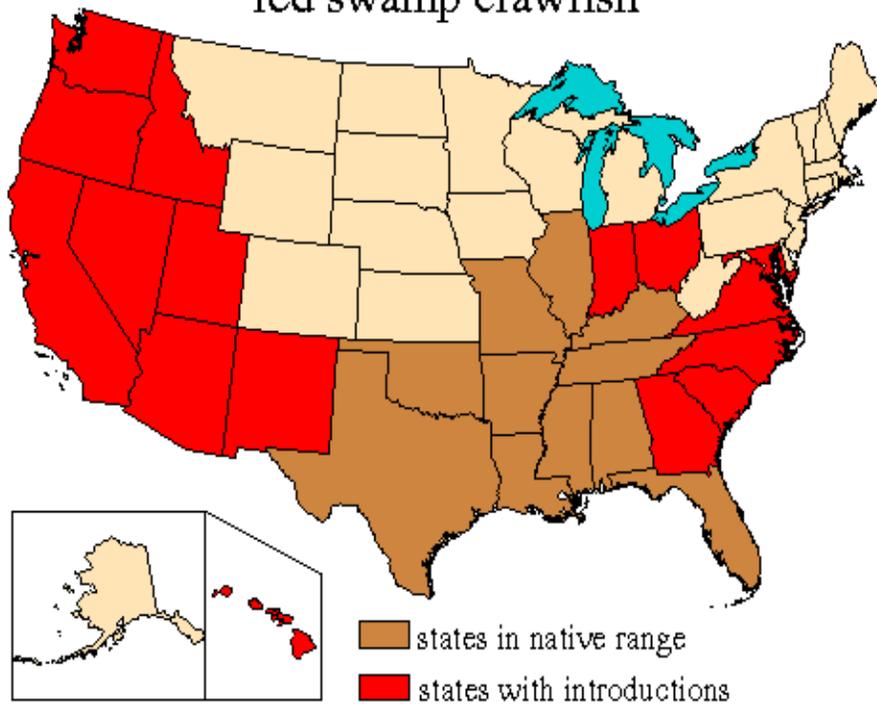


Plate 2: States included in the range of *Procambarus acutus* and *P. clarkii*. Map courtesy of the United States Geological Survey (USGS)



Plate 3: *Bufo fowleri* adults and larvae. Larvae drawing courtesy of the USGS.

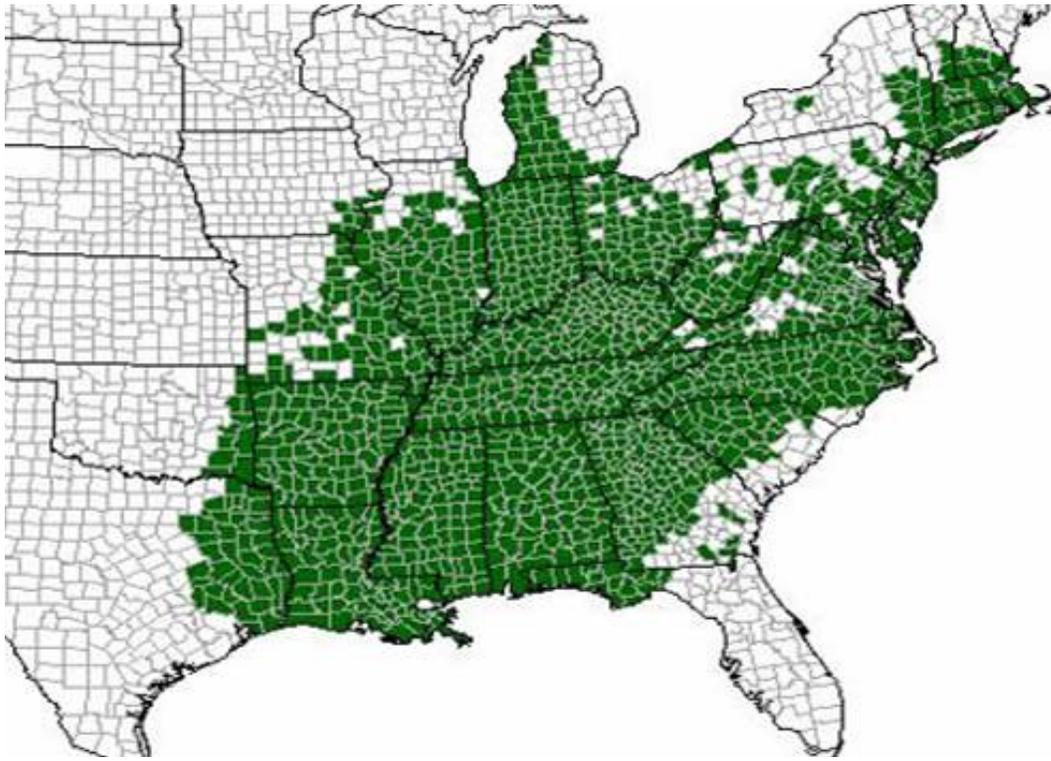


Plate 4: Range map of *Bufo fowleri*. Map courtesy of the USGS.

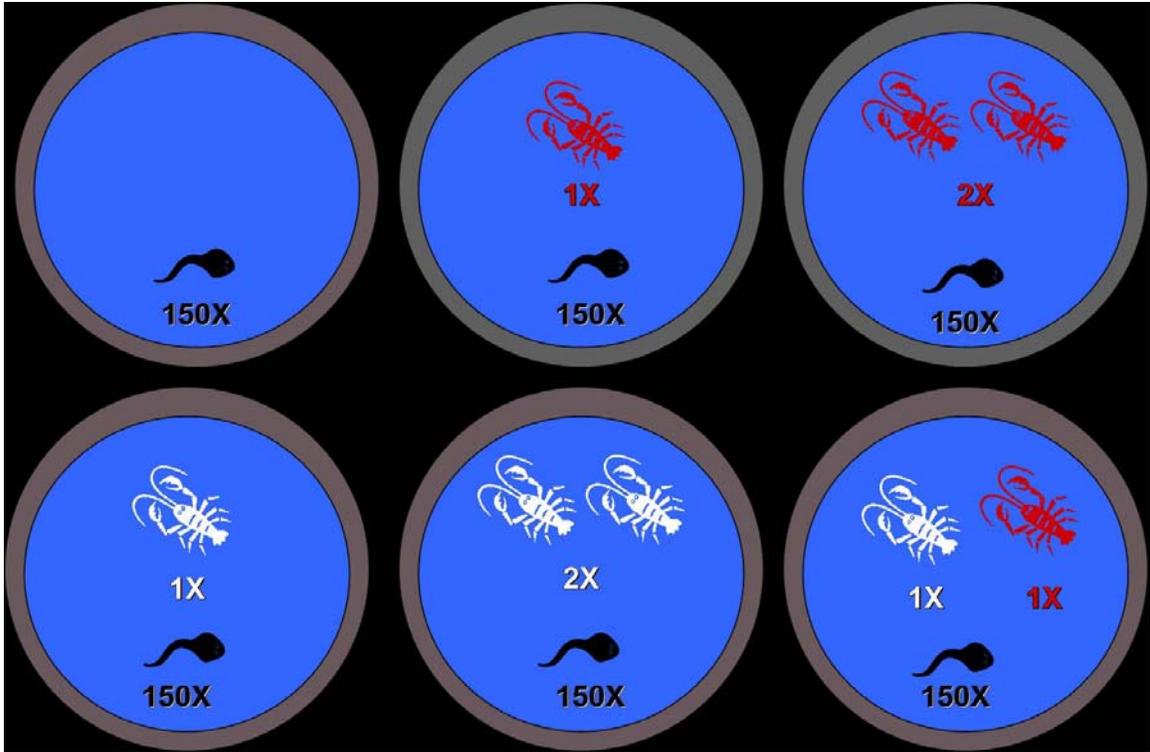


Plate 5: Pictorial representation of treatments. Red crayfish represent *Procambarus clarkii*, white crayfish represent *P. acutus*, and black figures represent tadpoles. Numbers of individuals of each species included are indicated.



Plate 6: Photograph of experimental mesocosms (smaller tubs, foreground). West Research Campus, East Carolina University.

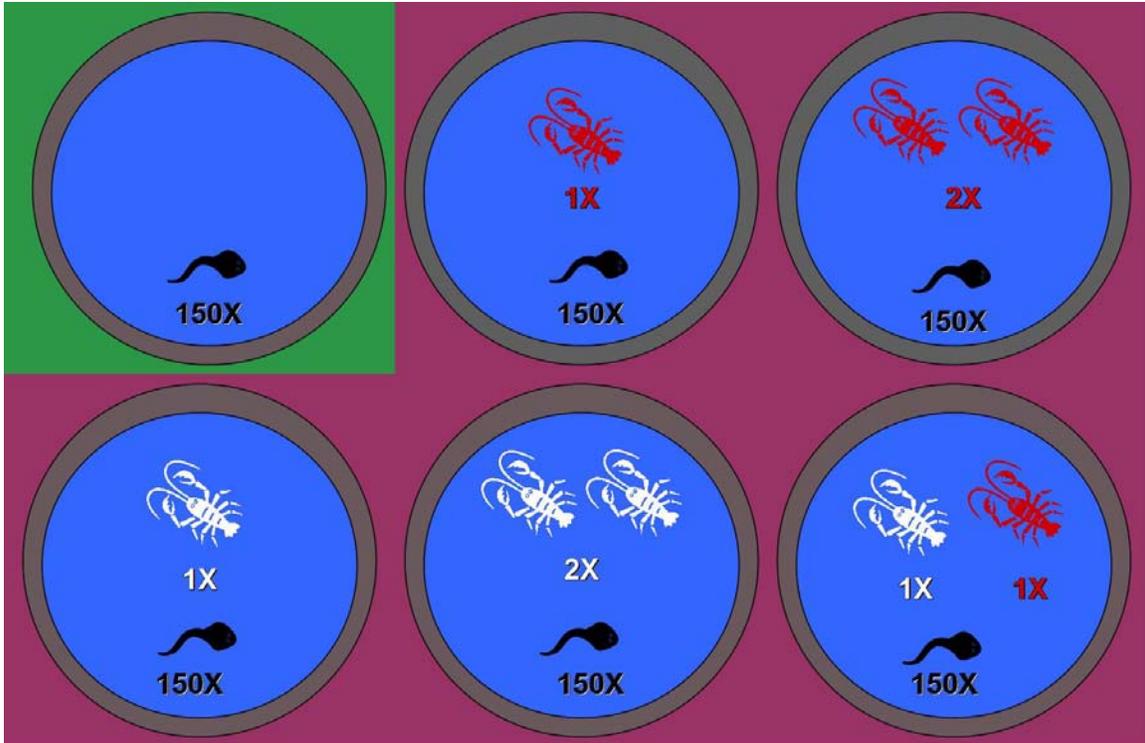


Plate 7: Pictorial representation of the treatments used to calculate the effect of crayfish presence. Treatments with the same color background were combined to enhance statistical power.

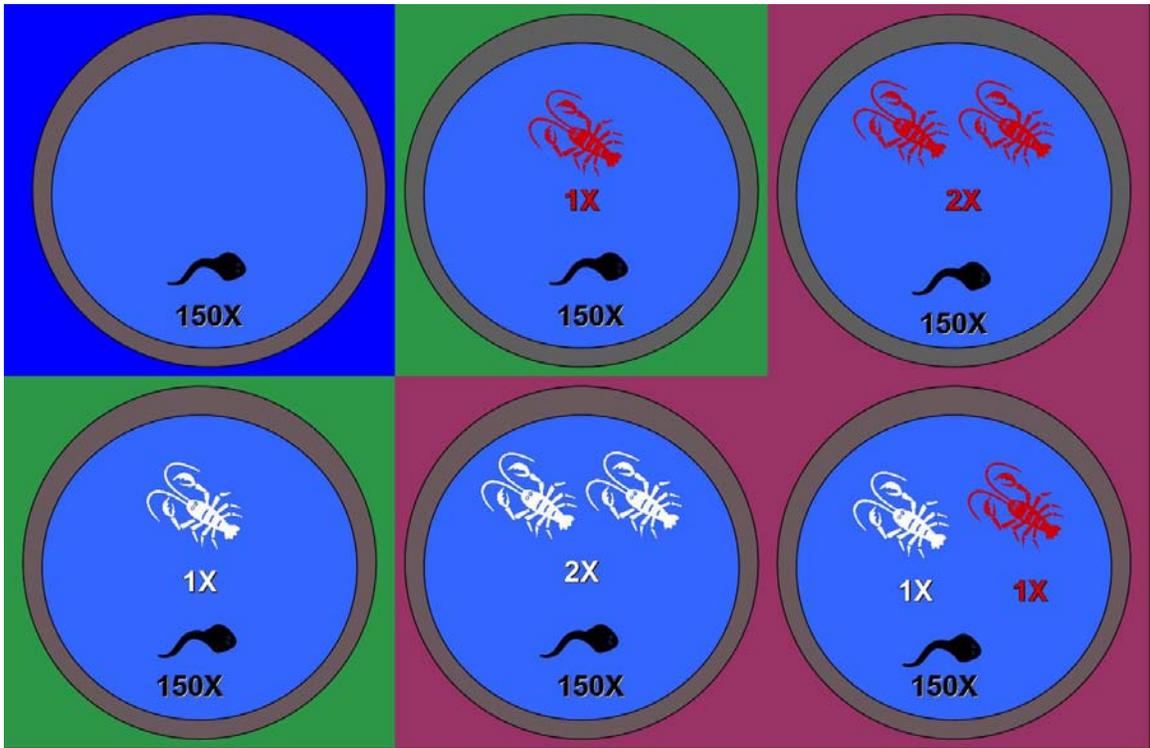


Plate 8: Pictorial representation of the treatments used to calculate the effect of crayfish density. Treatments with the same color background were combined to enhance statistical power.

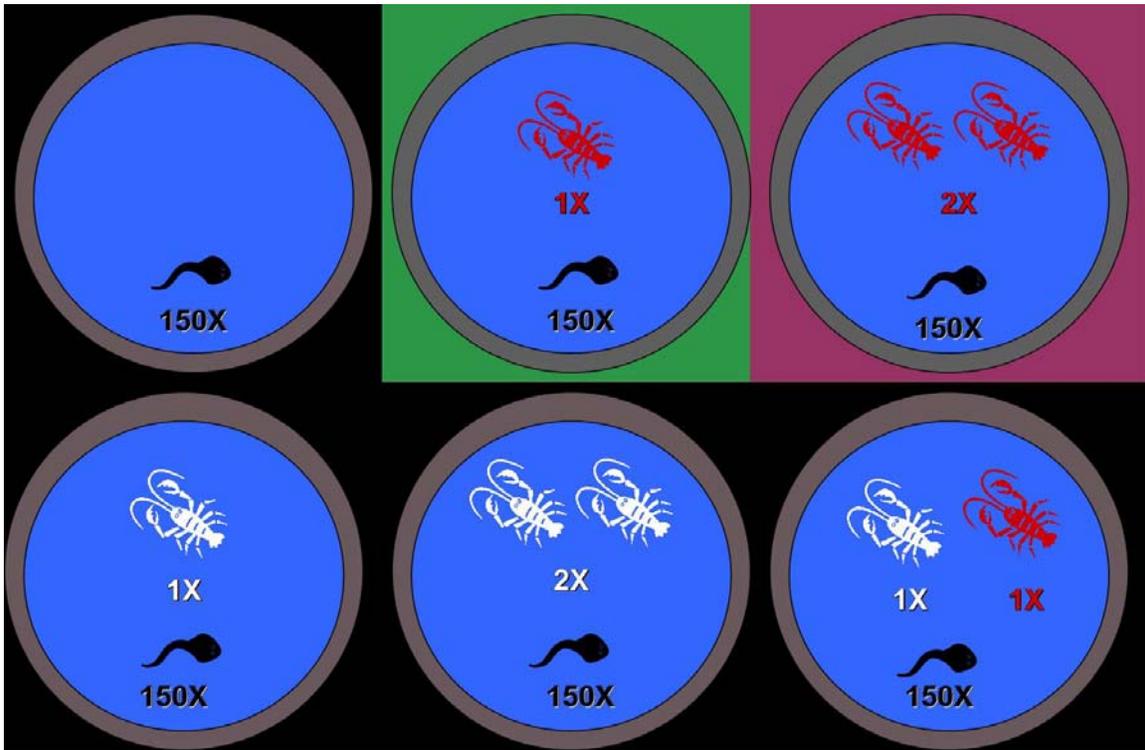


Plate 9: Pictorial representation of the treatments used to calculate the effect of *Procambarus clarkii* density. Treatments with a black background were not used in this calculation.

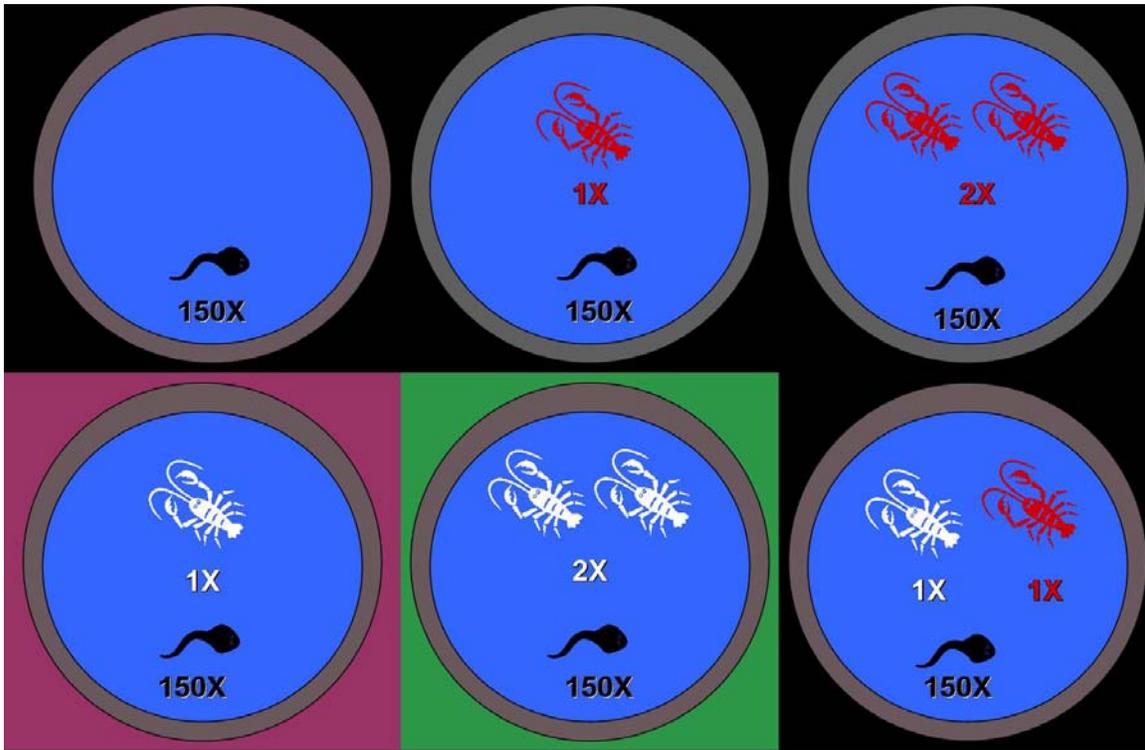


Plate 10: Pictorial representation of the treatments used to calculate the effect of *Procambarus acutus* density. Treatments with a black background were not used in this calculation.

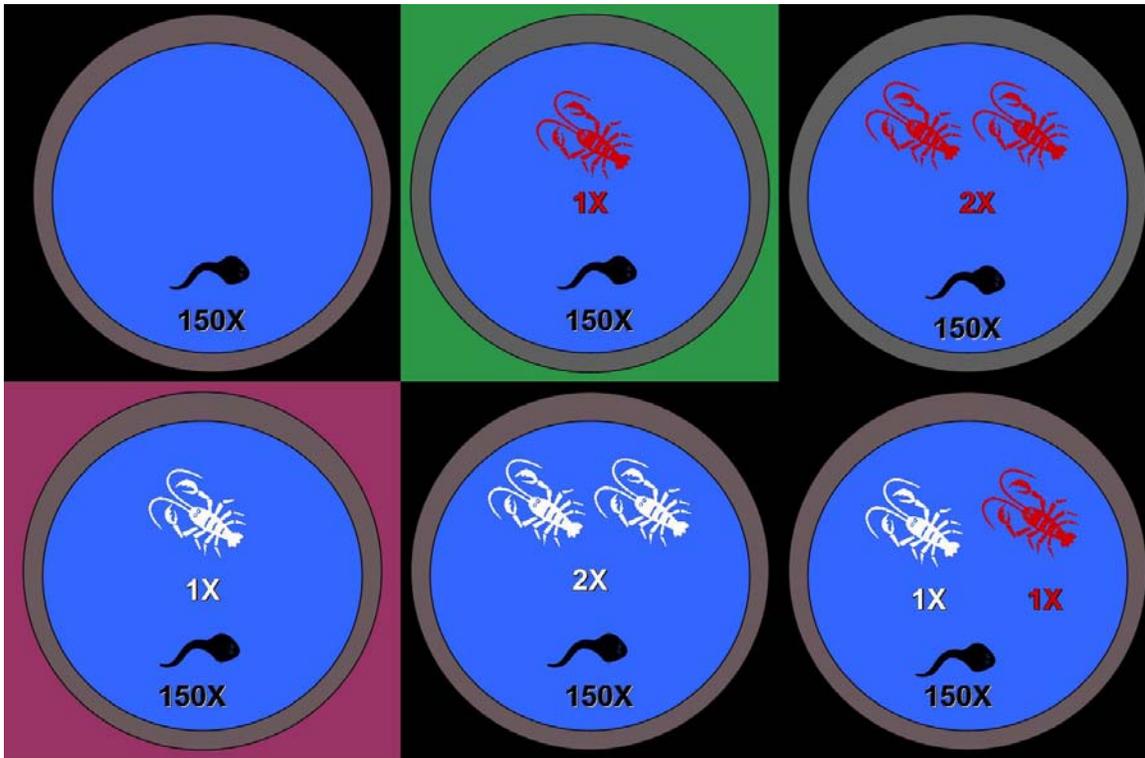


Plate 11: Pictorial representation of the treatments used to calculate the different effects of *Procambarus acutus* and *P. clarkii* at low density. Treatments with a black background were not used in this calculation.

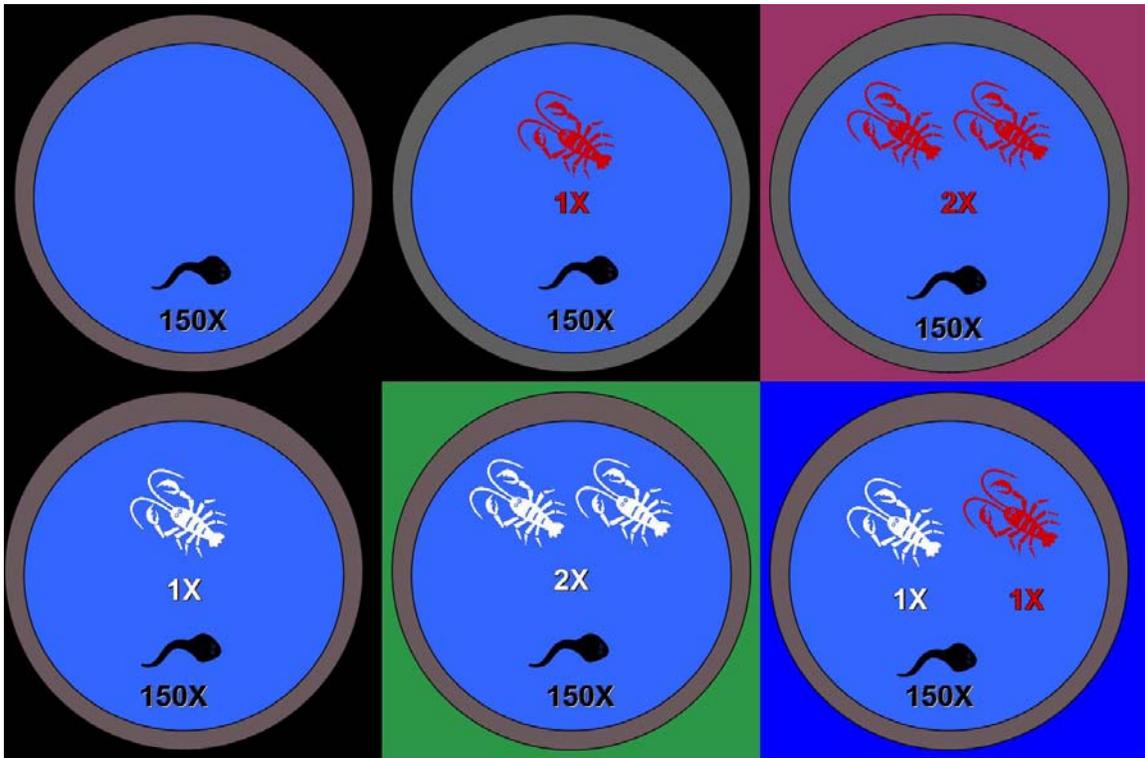


Plate 12: Pictorial representation of the treatments used to calculate the different effects of *Procambarus acutus* and *P. clarkii* separately and together. Treatments with a black background were not used in this calculation.

