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An Invasive Species as a Threat to Native Biodiversity: Larval Competition Between Native Anurans and an Invasive Treefrog

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AN INVASIVE SPECIES AS A THREAT TO NATIVE BIODIVERSITY:
LARVAL COMPETITION BETWEEN NATIVE ANURANS AND AN INVASIVE
TREEFROG

By

MCKENZIE J. WASLEY, Bachelor of Science

Presented to the Faculty of the Graduate School of

Stephen F. Austin State University

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LARVAL COMPETITION BETWEEN NATIVE ANURANS AND AN INVASIVE
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ABSTRACT

Cuban Treefrogs (*Osteopilus septentrionalis*) are established in Florida and Louisiana and have invasive potential further westward, possibly impacting native anurans at multiple life stages. In anurans, competition at the larval stage can decrease adult fitness through slower development and smaller size at metamorphosis, ultimately decreasing recruitment rates. To examine the potential impacts of *O. septentrionalis* at the larval life-history stage, I quantified activity levels and growth of their tadpoles and those of two anurans native to eastern Texas: Green Treefrogs (*Hyla cinerea*) and Gulf Coast Toads (*Incilius nebulifer*). Tadpoles of the three species were raised in con- or heterospecific treatments. In heterospecific treatments including *O. septentrionalis*, *H. cinerea* growth was lowest but *I. nebulifer* activity and growth were highest. Activity level and growth of *O. septentrionalis* were highest within the conspecific treatment whereas growth was lowest in heterospecific treatments with *H. cinerea*, a potential consequence of collective activity level within treatments. To avoid local population declines of native anurans, management of Cuban Treefrogs should focus on preventing establishment of new populations.

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INTRODUCTION

The transportation of humans and commercial goods is occurring with an ever-increasing frequency and is a leading cause of species introductions throughout the world (Elton 1958, Ruiz and Carlton 2003, Hulme 2009). Higher rates of commercial trade and human traffic result in greater frequencies of species introductions that would not likely otherwise occur if dependent upon natural dispersal events, thereby providing increased opportunities for organisms to interact with novel ecosystems (Elton 1958, Semmens et al. 2004). Anthropogenic dispersal vectors range from boats, planes, and motorized vehicles to shoes, and even the pet trade (Meshaka 1996, Davis 2009, Johnson et al. 2010). Plants are brought in for agricultural or ornamental purposes, and fish, birds, and mammals have been imported as game or for aesthetic purposes (Cox et al. 1997, Gordon and Thomas 1997, Davis 2009). Historically, the United States was one of the largest wildlife importers in the world (Cox et al. 1997). In 2005, the pet trade industry was responsible for legal importation of 210 million animals into the United States (Davis 2009). Small animals such as reptiles and amphibians often arrive in cargo containers or plant shipments (Meshaka 1996, Kraus 2003).

An organism whose human-aided introduction to a novel location or habitat is referred to as exotic, non-native, nonindigenous or introduced (Davis 2009, Adams 2016). A non-native species is considered invasive if its presence results in negative effects on an ecosystem, the economy, or human health (Davis 2009). The spread of

invasive species is the second greatest threat to biodiversity, while habitat loss associated with increasing human population remains biodiversity's greatest threat (Butterfield et al. 1997, Wilcove et al. 1998). Between 1960 and 2000, urbanized areas have more than doubled in coverage within the United States and are expected to increase from 27.4 million ha (2010) up to 66.0 million ha by the year 2060 (Heimlich and Anderson 2001, Nowak and Greenfield 2018). This is concerning especially considering altered habitats are more susceptible to invasion by non-native species than undisturbed habitats (Elton 1958, Simberloff 1997, Davis 2009).

Biodiversity is often a key indicator of quality and community stability within a given habitat (Elton 1958, Davis 2009). Invasive species have a myriad of effects on native species including but not limited to predation, loss of biodiversity and competition, all of which can negatively impact native populations (Simberloff 1997, Davis 2009). Burmese Pythons (*Python bivittatus*) in the Everglades National Forest in Florida have caused up to a 99% decline in some mammal populations by means of predation (Dorcas et al. 2012). In Guam, native vertebrate species have been driven to extinction by invasive Brown Tree Snakes (*Boiga irregularis*; Savidge 1987). In some cases, native species experience predator naiveté, as seen in varanid lizards preying on invasive Cane Toads (*Rhinella marina*) and failing to avoid consumption of the toxic species (Doody et al. 2009). An alternative perspective is prey naiveté, where native species do not recognize an invasive species as a predator (Anton et al. 2020). For example, several small fish species swim in close proximity to an invasive predator, Lion Fish (*Pterois volitans*), and

contribute to the predation efficiency of the invasive species (Anton et al. 2016). For both Cane Toads and Lion Fish, the native species make no behavioral adaptations to maximize survival, and therefore fall into an evolutionary trap leading to population-level declines (Schlaepfer et al. 2002). If given an adequate chance to recognize and respond to threats from non-native taxa, native species might adapt quickly enough to avoid falling into such trap (Schlaepfer et al. 2005).

Individuals compete for resources such as food, shelter, and mating opportunities (Bruno et al. 2005). Exploitative competition occurs when a resource is utilized by one individual before being encountered by others, whereas physical confrontations between individuals are characteristic of interference competition (Holomuzki et al. 2010). In a mesocosm experiment, Cane Toads caused a decrease in activity levels of the native anuran, *Cyclorana australis*, suggesting interference competition for space (Greenlees et al. 2007). Introduced Ring-necked Parakeets (*Psittacula krameri*) outcompeted native Starlings (*Sturnus vulgaris*) at feeding sites in Europe through direct attacks (interference competition; Le Louarn et al. 2016). In a laboratory experiment, invasive Italian Wall Lizards (*Podacris siculus*) arrived at food sites prior to native Iberian Wall Lizards (*P. virescens*), allowing the former species to exploit available food resources, gain more mass, and displace the members of the latter (Damas-Moreira et al. 2020).

Amphibians are unique among vertebrates in that many species exhibit complex life cycles or require different habitat types dependent on life stage (Wilbur 1980, Baldwin et al. 2006). These life-history stages often have different survivorship rates, and studies

suggest body condition of an adult is related to experiences in larval developmental (e.g., Wilbur and Collins 1973, Petranka 2007, Van Allen et al. 2010, Schalk et al. 2018). Factors such as density, duration of the larval period, and size at metamorphosis can have strong carry-over effects to adult fitness (Travis 1980, Newman 1988, Semlitsch et al. 1988, Chelgren et al. 2006, Yagi and Green 2018, Bredeweg et al. 2019). Invasive species have the potential to influence larval performance through competition, altering age-dependent survival probabilities (Lehtonen 2002), ultimately causing a decrease in recruitment rates costly to the population (Wyatt and Forsys 2004). Larval anurans typically increase activity level in response to the presence of competitors (Skelly 1994, Relyea 2002). Although increased activity level provides an individual with a competitive edge, the increase in activity is also associated with increased predation risk, presenting individuals with a fitness trade-off (Morin 1983, Werner and Anholt 1993, Relyea 2004, Schiwitz et al. 2020).

To maximize survival throughout the vulnerable egg and larval life stages, anurans express a wide range of morphological and behavioral traits in response to various environmental pressures (Skelly 1994, 1997, Warkentin 1995, Relyea 2002, Urban 2007, Touchon et al. 2015). The variation in phenotypic expression of a gene is referred to as phenotypic plasticity (Bradshaw 1965, Stearns 1989). Larval competition can be one driver of this variation in phenotype and can include competition for food, space and influence the chances of predation and/or cannibalism (Crump 1986, Newman 1987, Werner and McPeck 1994, Dayton and Fitzgerald 2001). For example, increased density

of Wood Frogs (*Lithobates sylvaticus*) led to an increase in intraspecific competition for food (Wilbur and Collins 1973). Larval California Red-legged Frogs (*Rana draytonii*) reduced their activity level in the presence of invasive Bullfrogs (*Lithobates catesbeianus*) to reduce competition and predation risk (Anderson and Lawler 2016). An additional study on larval competition found that two native anurans did not survive to metamorphosis as a result of predation by invasive Indian Bullfrogs (*Hoplobatrachus tigerinus*; Mohonty and Measey 2020).

Focal Species

Cuban Treefrogs (*Osteopilus septentrionalis*) are native to Cuba, the Isle of Pines, the Cayman Islands, and the Bahamas (Schwartz and Henderson 1991, Hedges 1999). Cuban Treefrogs are an invasive species and have dispersed to the Hawaiian Islands, Puerto Rico, and have been established in the Florida Keys since 1931 and on mainland Florida since 1951 (Schwartz 1952, Allen and Neill 1953, Schwartz and Henderson 1991, Meshaka 2001). Royal palm trees (*Roystonea* sp.) are an effective dispersal vector because the trees provide adequate food, shelter, and are a popular ornamental plant frequently imported to disturbed habitats (Meshaka 1994). Upon the arrival of Cuban Treefrogs in southern Florida, their dispersal was further facilitated through the ornamental plant trade, in addition to hitchhiking on vehicles (Meshaka 1994).

Cuban Treefrogs effectively exploit many types of refugia in both natural and disturbed habitats (Meshaka 1996, 2001, Hoffmann 2007, 2009, Johnson 2007). The diet

of Cuban Treefrogs includes a wide range of species, which increases their efficiency in acquiring enough energy reserves to allocate towards reproduction and growth (Meshaka 2001, Glorioso et al. 2012). In Florida, predators have not hindered the establishment of Cuban Treefrogs likely on account of the frog's large body size and foul-smelling, sticky skin secretion (Meshaka 2001, Powell et al. 2016).

Cuban Treefrogs produce a mean clutch size of 3,961 eggs (range: 1,177–16,371 eggs) and provided the appropriate conditions, will breed year-round (Meshaka 2001). Life-history traits contributing to increased larval survival of Cuban Treefrogs include a sticky coating found on the eggs during the first 15 hours post-oviposition (detering the handling of eggs by predators), and hatching typically occurring within 30 hours (Duellman and Schwartz 1958, Meshaka 1993). The mean duration of the larval period is 3–4 weeks, however, abundance of resources and warm water can facilitate metamorphosis in less than three weeks (Meshaka 2001). Males are thought to reach sexual maturity within three months, and females within 6–8 months (Meshaka 2001).

Meshaka (2001) suggested that Cuban Treefrogs are highly competitive with native species and other research has linked population declines to the arrival of the invasive species. For example, in areas where Cuban Treefrogs are established, a decreased abundance of native anurans has been observed and partially attributed to predation by Cuban Treefrogs (Meshaka 2001, Waddle et al. 2010, Rice et al. 2011, Glorioso et al. 2018). Cuban Treefrogs have been documented preying upon Green Treefrogs (*Hyla cinerea*), Squirrel Treefrogs (*Hyla squirella*), and Eastern Narrow-mouthed Toads

(*Gastrophryne carolinensis*; Meshaka 2001, Wyatt and Forsys 2004, Glorioso et al. 2012). Although their abundance is affected by the presence of Cuban Treefrogs, detection of native anuran species by researchers is not, which suggests that native species are naive to the risk of predation associated with the invasive treefrog (Waddle et al. 2010, Hoffmann et al. 2018). Additionally, acoustic competition has been observed as male Green Treefrogs call louder and with decreased call length, in the presence of Cuban Treefrogs (Tennessen et al. 2016). The mating call of Cuban Treefrogs can also potentially interfere with auditory processing of male Green Treefrogs' call (Kennedy et al. 2020). Cuban Treefrogs are also highly fecund and have generation times that span less than a year (in contrast, the most productive clutch sizes of native hylids contain 70% fewer eggs; Meshaka 2001). Male Cuban Treefrogs have been documented in amplexus with females of other anuran species, which can result in unfertilized eggs and an unsuccessful breeding attempt (Smith 2004). Predation and interfering with breeding behavior negatively affect the abundance and reproductive efficiency of adults, decreasing recruitment rates and contributing to population declines at the local level.

My study focused on the impacts of competition from larval Cuban Treefrogs on the larvae of native anurans. Amphibians display plastic responses to variation in pond environment, predation, limited food resources, and competition (Morin and Johnson 1988, Newman 1989, 1992, Skelly and Werner 1990, Kiesecker and Blaustein 1997, Relyea 2002, 2004, Richter-Boix et al. 2004). Under conditions with high densities and low food abundance or quality, larval Cuban Treefrogs consumed tadpoles of other

species including Cane Toads, Squirrel Treefrogs, and even conspecifics (Babbitt and Meshaka 2000, Smith 2005a). The presence of Cuban Treefrogs also resulted in the decreased growth and development rates of Southern Toads (*Anaxyrus terrestris*) and Green Treefrogs (Smith 2005b). A Florida study using various densities, ages, species combinations, and environmental conditions to examine the effects that larval Cuban Treefrogs have on larval Squirrel Treefrogs and Southern Toads reported that competition from the invasive anuran drives species interaction rates and community assembly (Knight et al. 2009). This study found the survival of larval Squirrel Treefrogs, regardless of environment, was lower in the presence of Cuban Treefrog larvae that had either hatched earlier or were the same age as the native treefrog. Results from this study also demonstrated that size at metamorphosis of Southern Toads were smaller in the presence of Cuban Treefrogs, consequently affecting survival and reproductive capacity post-larval stage. Compared to Squirrel Treefrogs, however, the overall negative effects of Cuban Treefrogs were weaker in Southern Toads, likely a result of the toads' short larval periods (Knight et al. 2009). Holding constant factors such as density, food availability, lack of predators, and environment, while manipulating species combinations, will allow me to investigate whether or not spatial competition among species is present.

Objective and Predictions

Glorioso et al. (2018) reported that Cuban Treefrogs are established in Orleans Parish, Louisiana. Given both the pattern of dispersal and the increased rate of habitat alteration throughout the southeastern U.S., there is concern that Cuban Treefrogs will expand their range further west to include Texas (Meshaka 1996, 2001, Johnson 2007, Blaustein et al. 2010). Cuban Treefrogs have already been reported in Houston, Dallas, and as far west as Midland (Somma et al. 2018; S. Holt and J. Cappadonna, personal communications), the occurrences of which were associated with shipments of ornamental plants. Winter temperatures in several portions of Texas can reach lows that are not suitable for Cuban Treefrogs in natural habitats, but individuals taking refuge inside buildings and on adjacent urban structures are capable of surviving periods with cooler temperatures (Simpson 2013). The impacts that existing populations of Cuban Treefrogs are known to have, at both adult and larval stages (e.g., Babbitt and Meshaka 2000, Smith 2005a,b, Waddle et al. 2010, Glorioso et al. 2012, 2018) support my motivation to examine the potential effects on native species in areas where the invasive species can, but has not yet established.

This research investigated the larval interactions between *Osteopilus septentrionalis* (OSSE) and two anuran species that occur in eastern Texas: *Hyla cinerea* (Green Treefrogs; HYCI) and *Incilius nebulifer* (Gulf Coast Toads; INNE). The purpose of my research was to identify the effects of species presence on the body size and activity level of each of the three species. *Hyla cinerea* was an appropriate study species because there

is prior evidence to suggest that *O. septentrionalis* has a negative effect on adult and larval *H. cinerea* in Florida (Meshaka 2001, Smith 2005b, Glorioso et al. 2012, Tennessen et al. 2016). *Incilius nebulifer* was selected as a study species because *I. nebulifer* and *O. septentrionalis* have several reproductive life history traits in common (e.g., high fecundity, short egg period, and rapid development suitable for ephemeral ponds; Mendelson 2005). *Osteopilus septentrionalis* overlaps with *H. cinerea* in their established range in Florida, and with *I. nebulifer* in their newly established range in Orleans Parish (Powell et al. 2016). Both native species are abundant in eastern Texas, so there is potential for all three species to interact at various life stages. *Osteopilus septentrionalis* completes metamorphosis in 21–28 days at 15–18.5 mm snout vent length (SVL; Meshaka 2001). *Hyla cinerea* larvae are typically 4.5–5.5 mm total length (TL) at hatching, grow to 49–60 mm TL, and completes metamorphosis at 12–17 mm SVL in 55–65 days (Duellman and Trueb 1994, Redmer and Brandon 2005, Altig and McDiarmid 2015). *Incilius nebulifer* tadpoles grow to 25 mm total length and complete metamorphosis in 21–28 days at 7–12 mm SVL (Mendelson 2005, Altig and McDiarmid 2015).

Based on the presumed competitive superiority of the invasive species, I predicted that activity levels of larval *H. cinerea* would be lower, and body size would be smaller, when raised with *O. septentrionalis* compared to larvae raised with conspecifics or *I. nebulifer*. Knowing that *I. nebulifer* exhibit a weak relationship between size at metamorphosis and adult size (Werner 1986), I predicted that the activity level and

growth of larval *I. nebulifer* would be similar when comparing individuals raised with conspecifics to individuals raised with heterospecifics. I predicted that the activity level of *O. septentrionalis* would not change across treatments. When all three species are raised together in the same habitat, I predicted interspecific competition would contribute to differences in growth, and that these differences would be most apparent in *H. cinerea*, given that this species has the lowest baseline activity level and longest larval stage (Altig and McDiarmid 2015, Schiwitz et al. 2020). As a result of intraspecific competition, I predict that growth in *O. septentrionalis* will be greater when raised with heterospecifics compared to being raised with conspecifics only.

MATERIALS AND METHODS

Laboratory Set-up

I conducted this research in a laboratory within the Animal Care Facility of the Miller Science Building at Stephen F. Austin State University (Nacogdoches County, Texas) at a constant ambient temperature of 27°C. This temperature falls within the upper range of larval thermal tolerances for all three species (Brattstrom 1963; Hubbs et al. 1963; Blouin 1992; Meshaka 2001, Mendelson 2005). Prior to the experiment, I deployed iButtons throughout the lab to ensure the temperature and humidity remained constant. I supplemented the room lighting with a Coralife Aqualight T5 Freshwater ColorMax Fluorescent UVB Lamp (28 W) mounted above each aquarium and programmed to a photoperiod of 14:10 h (L:D). Larvae were raised in 38 L aquaria filled with 20 L of aged tap water to establish a water depth of ~20 cm (Meshaka 2001, Smith 2005b). Although visual acuity is presumed to be weak in larval amphibians (Blaustein and O'Hara 1982), I covered the sides and back of each tank with contact paper to control for effects of visual cues from neighboring tanks. I placed artificial plants (as opposed to organic leaf litter) within each tank to serve as refugia without compromising water quality (Smith 2005b, Walston and Mullin 2007, C.M. Schalk, personal communication). Using aged tap water, I conducted water changes as needed to prevent build-up of organic material and maintain water clarity. I provided food *ad libitum* throughout the experiment in the form of Hikari Tropical Algae Wafers for Herbivorous Fish. Many terrestrial amphibians

experience an ontogenetic shift in nutrient ratio demands from nitrogen for muscles and tissues to increased phosphorus later in development for rapid ossification (Reynolds 1977, Elser et al. 1996, Altig 1999). Therefore, I supplemented diet *ad libitum* with Wardley Premium Amphibian and Reptile Sticks following Gosner stage 26 (Gosner 1960). The constant availability of food was designed to minimize the potential of food being a limiting resource within each tank and to better isolate the effects of competition for space among species (Smith 2005b).

Specimen Collection

I collected egg masses for all species between May and August 2020. Clutches of the native larvae were obtained from amplexed adults collected from Alazan Bayou Wildlife Management Area (31°29'12.6"N, 94°44'44.3"W) in Nacogdoches County, Texas. The first clutches of *Hyla cinerea* and *Incilius nebulifer* were laid on the morning of 29 May 2020 by adults collected during the night of 28 May 2020. The second clutches were laid on morning of 29 August 2020 by adults collected during the night of 28 August 2020. I placed the amplexed adults in critter carriers with aged tap water ~4 cm deep and PVC pipe or rock perches and left them in the lab overnight. The following morning, I placed adults in separate containers. I then filled the critter carriers containing the egg masses to ~12 cm with aged tap water and equipped them with an aerator and fluorescent lights until hatching. I released the adult frogs at their respective capture sites within 48 h of initial collection.

Osteopilus septentrionalis egg masses were oviposited in large plastic kiddie pools at a residential property in Gainesville, Alachua County, Florida (29°38'38.3"N 82°21'30.2"W), on the morning of 27 May 2020. I assumed that the collected egg masses were sourced from the same female. *Osteopilus septentrionalis* typically hatches within 1–2 days of oviposition, so the eggs were temporarily placed on ice for shipment in order to delay hatching (Moore 1939, Altig and McDiarmid 2015). The eggs arrived in the lab on 28 May 2020 and were transferred to a critter carrier filled with ~12 cm of aged tap water and equipped with an aerator and fluorescent lights until hatching.

Experimental Design

The day that eggs of each species became free swimming tadpoles was considered day 1 of a given trial. At that time, I transferred groups of tadpoles to 38 L aquaria. I conducted trials across seven different treatments that represented different combinations of the larvae representing each species (Table 1). I held the initial density of tadpoles per aquarium constant across all treatments regardless of how many species were represented. Conspecific treatments consisted of 30 individuals of one species and served as the control. Heterospecific treatments consisted of different combinations of the three species—15 individuals of each species in the two-species trials, and 10 individuals of each species in the three-species trials. These densities are comparable to those used in a similar experimental design by Smith (2005b).

I conducted the initial round of trials from May to June 2020, which included all treatments involving *O. septentrionalis* larvae: control (OSSE only) and different combinations involving the native species (hereinafter, treatment acronyms: OSSExHYCI, OSSExINNE, OSSExHYCIxINNE). *Osteopilus septentrionalis* hatched three days post oviposition (30 May 2020). *Hyla cinerea* hatched on 1 June, three days after oviposition and *I. nebulifer* hatched on 31 May, two days after oviposition. The control treatment *O. septentrionalis* began on 31 May, one day post-hatch (DPH), whereas all other treatments began on 1 June, with *H. cinerea* at 0 DPH and *I. nebulifer* at 1 DPH.

The single clutch of all three species produced enough tadpoles to satisfy the densities for the four trials of replication for each treatment involving *O. septentrionalis* larvae. I released tadpoles of *H. cinerea* and *I. nebulifer* not used during this initial round of trials at a water body nearest to where the amplexed adults were collected. I either euthanized tadpoles of *O. septentrionalis* by immersion in a 6% solution (7.2–7.6 pH) of MS-222 bath or allowed them to develop in a separate tank. The individuals from the separate development tank were later euthanized with MS-222 in order to produce a staged development series of *O. septentrionalis* larvae for the Vertebrate Museum at Stephen F. Austin State University (Gosner 1960, Beaupre et al. 2004).

I conducted the second round of trials in September 2020 which included the conspecific treatment for each of *H. cinerea* (HYCI) and *I. nebulifer* (INNE), as well as the heterospecific treatment involving the two native species (HYCIxINNE). Both *H.*

cinerea and *I. nebulifer* hatched three days post oviposition and began treatments on 1 September 2020 at 1 DPH. The clutches of both species produced enough tadpoles to satisfy all four trials of replication for the three treatments, and larvae not used in these trials were released where I had collected the amplexed adults. Treatments were conducted at two different points in time as the result of misidentifying an egg mass collected in the wild from unknown adults. This introduces confounding variables to the experiment in terms of interclutch variation and maternal effects that will be further explored in the discussion.

Beginning on the first day of a trial, I recorded body size measurements for five haphazardly-selected individuals representing each species within each treatment. The selection of five individuals was intended to provide representative measurements for that species within the particular treatment. I took photos and measurements (D_m) every three days, and on the day the trial ended (as defined when at least one individual within the trial reached Gosner stage 41). Hereinafter, measurement interval is denoted using the variable D_m , where the subscript denotes the trial day on which the measurements were recorded. I removed selected subjects from their tank and placed them in a temporary holding container to avoid replicate measurements of the same individual for that day (Walston and Mullin 2007). Depending on their size, I either pipetted or scooped up tadpoles with a loose-leaf tea strainer and placed them in a petri dish. I took photos by mounting an iPhone 7s Plus to an Olympus Sz30 Binocular Stereo Zoom Microscope with an Olympus TL2 Light Source. When the tadpoles became too large to fit in the

field of view of the microscope, photos were taken using a Canon EOS 50D with a Tamron 90 mm F2.8 lens. I mounted the camera on a tripod placed directly over the petri dish containing the tadpole, with supplemental light provided by the Olympus TL2 Light Source.

I calculated tail length and total length (both ± 1.0 mm) from the photos using the “Segmented Line” feature in the program ImageJ v1.52a (Rasband 2018). The line length was calculated in pixels using the “Measure” tool and, based on a ruler included in the photos, converted to mm. Total length was measured as anterior margin of oral disc to tail tip, and tail length was measured as venter to tail tip (Altig 2007). I measured blot-dry mass using a Sartorius R160P Digital Lab Analytical Balance (± 0.01 mg), following Stephens et al. (2017). Tadpoles were again transferred to a petri dish. I tared the dish prior to the addition of a tadpole and used a paper towel to blot as much excess water from around the tadpole as possible to ensure the most accurate mass reading. Some tadpoles experienced minor tail damage during transfer and the measuring processes.

On various days and at various times throughout trials, I recorded a minimum of 10 observations of activity level (% active) for each replicate aquarium, resulting in a total 40 observations per treatment (May – June 2020 and August – September 2020). Standing 30–40 cm from the tank, I counted how many individuals were moving at that particular moment in time (Skelly 1994). From this distance, diagnostic characteristics of the larvae were sufficiently discernable to correctly assign them to the respective species. For each observation, I took the average of three activity level readings recorded at 20-

min intervals. For control treatments, I considered all individuals within trials and calculated the proportion of tadpoles moving as a measure of activity level among tadpoles. Within heterospecific treatments, I calculated the proportion of moving individuals for each species.

For each replicate aquarium, the trial was ended when the first individual within that aquarium reached Gosner stage 41 (Gosner 1960, Dayton and Fitzgerald 2001). Although individuals have not completed metamorphosis at stage 41, they begin to lose significant mass and length after this stage as the tail is reabsorbed (Wilbur and Collins 1973). Following the conclusion of a trial, I euthanized all tadpoles within the treatment by immersion in a 6% solution (7.2–7.6 pH) of MS-222 bath and accessioned the specimens into the Vertebrate Museum at Stephen F. Austin State University (Beaupre et al. 2004). The methods regarding the use of vertebrate animals were reviewed and approved by the Institutional Animal Care and Use Committee at Stephen F. Austin State University.

Statistical Analyses

I conducted statistical analyses using the software JMP[®] version 14.0.0 (SAS Institute Inc. 2019). I first analyzed the data for growth and activity level using Shapiro-Wilk Goodness of Fit Test to determine if they exhibited a normal distribution. Data representing growth and activity levels violated the assumption of normality ($p < 0.001$). I analyzed growth and activity levels for a single species across all treatments using Kruskal-Wallis Nonparametric Comparisons (McDonald 2014). Overall growth and gain

in mass, total length and tail length were used as surrogates for growth to compare the performance of a single species across treatments. Overall growth was calculated as an average of values recorded each trial day on which measurements were taken (D_m). Gain in growth metrics for each species were calculated by averaging the measurements of the haphazardly selected individuals on the first trial day, and subtracting that value from the average of selected individuals on the last trial day. *Hyla cinerea* experienced the greatest variation in trial duration (D_{13} – D_{25}). In order to achieve a more accurate understanding of how *H. cinerea* responds in the presence of other species, I conducted analyses for gain in mass, total length and tail length for the first 13 days of each trial in addition to analyses of complete trial durations, D_{13} being the last day that included measurements from all treatments containing *H. cinerea*. Kruskal-Wallis comparisons were followed by a Dunn's Control post hoc tests with Bonferroni correction (Dinno 2015) Dunn tests are based on joint comparisons of rank means in comparison to a control and do not require equal sample sizes (although statistical power decreases as the number of treatment comparisons increase; Shingala 2016). Despite being a conservative test, Bonferroni corrections provide an appropriate adjustment to the p-value while preserving alpha ($\alpha = 0.05$) and protecting against family-wise Type I error when making multiple pairwise comparisons (Steel and Torrie 1980, Pedhazur 1982, Shingala 2016).

RESULTS

Hyla cinerea activity levels did not differ in the presence of the native or invasive heterospecifics ($Z \leq 0.83$, $p > 1.00$; Figure 1). In the presence of *Osteopilus septentrionalis* larvae, *Incilius nebulifer* larvae had significantly higher activity levels than when housed with larval *H. cinerea* ($Z \geq 3.91$, $p < 0.001$; Figure 2). Activity levels of *O. septentrionalis* larvae were significantly higher in the absence of either or both native species ($Z \geq 2.44$, $p < 0.04$; Figure 3).

Raised in the absence of native and invasive heterospecifics, larval *H. cinerea* had significantly higher overall growth ($Z \geq 4.26$, $p < 0.001$; Figure 4) in addition to higher gains in mass, total length, and tail length from the first trial day to the last (D_1 subtracted from D_{25} ; $Z \geq 3.00$, $p \leq 0.008$, Figure 5). Conversely, when I analyzed the first 13 days among *H. cinerea* treatments (D_1 subtracted from D_{13}), gains in growth metrics were significantly lower in the heterospecific treatment that involved *O. septentrionalis* only ($Z \geq 3.70$, $p < 0.001$; Figure 6).

Overall growth of *I. nebulifer* larvae in the control treatment was not significantly different from individuals raised with either native or invasive heterospecifics ($Z \leq 2.29$, $p \geq 0.07$; Figure 7). However, gains in mass, total length and tail length of *I. nebulifer* (D_1 subtracted from D_{21}) within the control were significantly lower in heterospecific treatments that included larval *O. septentrionalis* ($Z \geq 3.10$, $p \leq 0.06$; Figure 8).

Overall growth of *O. septentrionalis* in the control treatment was not significantly different from the heterospecific treatments that included native species ($Z \leq 2.02$, $p > 0.71$; Figure 9). In contrast, when compared to measurements from the control group, gain in mass of *O. septentrionalis* (D_1 subtracted from D_{18}) was significantly lower in the heterospecific treatments that included *H. cinerea* larvae ($Z \geq 3.12$, $p \leq 0.005$; Figure 10a). Gains in total length and tail length among *O. septentrionalis* larvae were not significantly different in the presence of the native species ($Z \leq 1.22$, $p \geq 0.67$; Figure 10b,c).

DISCUSSION

In the presence of other species that compete for a common pool of resources, many animals respond by increasing their activity levels such that the rate of resource encounter and acquisition is increased for the individual (Skelly 1997, Schiwitz et al. 2020). The success of some invasive species can be attributed to such increase in rate and efficiency of acquiring resources in comparison to native species (Dick et al. 2013, Alexander et al. 2014). With an increased activity level, however, comes an associated cost of increased probability of detection by potential predators (Morin 1983, Relyea 2002, 2004). My results did not support the prediction that *Hyla cinerea* would increase activity levels in the presence of *Osteopilus septentrionalis*, the latter species putatively being a superior competitor. Larval *H. cinerea* have an intrinsically low baseline activity level because they live in permanent ponds which, when compared to temporary ponds, have more aquatic predators (Schiwitz et al. 2020).

The negative relationship between activity level and hydroperiod is supported by a study that focused on the desert-adapted tadpoles of several anuran species (Dayton and Fitzgerald 2001). This study found that these species do not overlap in breeding habitats because the study species with the shortest larval period (*Scaphiopus couchii*) had higher activity levels and outcompeted the other species, but could not persist in ponds with longer hydroperiods because of the increased risk in predation (Dayton and Fitzgerald

2001). A separate study discovered that tadpoles of a large bodied, ephemeral species (*Pelodytes punctatus* [Common Parsley Frogs]), were reported to have a strong negative effect on the growth and survival rate of *Bufo bufo* (Common Toads) tadpoles, a permanent pond species that increased activity level in the presence of a competitor (Richter-Boix et al. 2004). Species able to persist in temporary ponds, such as *Incilius nebulifer* and *O. septentrionalis*, experience intense levels of competition because there is a limited amount of time to acquire resources and develop. As a result, the larvae typically have higher baseline activity levels and growth rates (Morin 1983, Wilbur 1987, Morin and Johnson 1988, Dayton and Fitzgerald 2001, Glorioso et al. 2018). *Osteopilus septentrionalis* have short larval periods, which corresponded to my results with high activity levels observed in the control treatment. In spite of the fact that they also experience a brief larval period, the activity levels of *I. nebulifer* larvae were highest not in the control treatment, but in the heterospecific treatments including *O. septentrionalis*. Therefore, Richter-Boix et al. (2004) suggest that, in addition to a short larval period, *I. nebulifer* could be increasing activity level in the presence of *O. septentrionalis* to compensate for the interspecific competition.

The density of individuals within an environment can influence the likelihood of *O. septentrionalis* consuming other individuals, including cannibalizing conspecifics (Crump 1986, Babbitt and Meshaka 2000, Smith 2005a). Although some studies that included *O. septentrionalis* did not examine activity levels as a proxy for competition (Babbitt and Meshaka 2000, Smith 2005a), an increase in activity levels that facilitate

escape or accelerate development is a viable explanation for minimizing the risk of being consumed by a larger bodied hetero- or conspecific. In support of this explanation, activity levels of *O. septentrionalis* and *I. nebulifer* were highest in treatments that consisted of the greatest density of highly active, large-bodied competitors; the *O. septentrionalis* control treatment and the heterospecific treatments with *O. septentrionalis* and *I. nebulifer*. This result also provided evidence for intraspecific competition in *O. septentrionalis* larvae, thus supporting one of my predictions.

An animal's competitive ability, activity level, and foraging efficiency all tend to correlate positively with individual body size and growth rate (Relyea 2002, 2004, Richter-Boix et al. 2004, Denver 2010). As such, changes in total length, tail length, and mass that occurred over the larval period were important variables to analyze in my research. The ability to acquire resources influences the timing when an anuran larva initiates metamorphosis (e.g., at what size and mass), which can have carry-over effects to subsequent life stages that impact both survival and recruitment rates (Wilbur and Collins 1973, Travis 1980, Semlitsch et al. 1988, Chelgren et al. 2006, Yagi and Green 2018, Bredeweg et al. 2019). For example, when raised with invasive *Rhinella marina*, *Limnodynastes convexiusculus* (Marbled Frogs) had an extended larval period and metamorphosed at smaller body sizes (Greenlees et al. 2013). Negative long-term effects associated with *L. convexiusculus* metamorphosing at smaller body sizes included lower survivorship and lower reproductive output, compared to larger bodied conspecifics (Greenlees et al. 2013). Similarly, under high density conditions, intra- and interspecific

competition resulted in a decrease in body size at metamorphosis and an increase in larval period for Crawfish Frogs (*Lithobates areolatus*), which had negative carry-over effects on terrestrial survival as well as size and reproductive capacity of adults (Stiles et al. 2020).

Hyla cinerea have the longest larval stage out of the three species used in my study (~9 weeks compared to 3–4 weeks in *I. nebulifer* and *O. septentrionalis*; Altig and McDiarmid 2015). The difference among the larval periods for each species resulted in variation in trial duration for *H. cinerea* (D₁-D₂₅), and could explain why *H. cinerea* larvae in the control treatment were larger and experienced the highest overall growth and gain in mass, when compared to the other treatments. However, gains in growth for all metrics of *H. cinerea* larvae housed with *O. septentrionalis* during the first 13 days (D₁-D₁₃) of trials were significantly lower when compared to subjects from the control treatment, indicating that *O. septentrionalis* could be imposing negative effects of competition on *H. cinerea* growth early in the larval period. Intense competitive pressure during the early larval life-history stages, that potentially depress activity levels, plays a role in determining the overall growth and development rates of an individual (Ficetola et al. 2010).

In addition to differences in larval stage, the variation in trial length was reflective of when I concluded the trials. In ending trials on the day that any individual, regardless of species, reached Gosner stage 41, I eliminated any opportunity to evaluate compensatory growth. This phenomenon occurs when some individuals develop faster, complete

metamorphosis, and leave the aquatic environment, resulting in a decrease in the density of individuals and a reduction in competitive interactions (Travis 1984, Hector et al. 2011). Provided with the ideal conditions, however, *O. septentrionalis* can breed year-round, allowing no opportunity for compensatory growth in slower developing species, such as *H. cinerea* (Meshaka 2001).

The result of no change in overall growth among *I. nebulifer* larvae across all treatments was expected because this species completes metamorphosis at a relatively small body size and experiences majority of its growth in the terrestrial life-stage (Werner 1986). Some anuran larvae also rely on antipredator defenses other than decreased activity level (Relyea 2004, Gunzburger and Travis 2005, Schiwitz et al. 2020). As with other bufonids, a primary defense to deter predation of *I. nebulifer* is the distastefulness of eggs and larvae (Adams et al. 2011, Üveges et al. 2019), thereby reducing the risk of predation and lending additional support for maintaining a small body size throughout the larval stage. In contrast to results for overall growth, results for gains in growth did not support my predictions seeing that gains in all growth metrics for *I. nebulifer* in the heterospecific treatments with *O. septentrionalis* were higher compared to larvae within the control treatments. Although an increase in growth for ephemeral species under intense competitive pressure might seem counterintuitive in natural conditions, food was not intended to be a limiting resource in my study. Therefore, an increase in activity level in the presence of a competitor might have allowed individuals to gain more mass, total length, or tail length. This result aligns with the association

between activity level and efficiency in resource acquisition (and associated mass gain). For example, when activity levels within a treatment were highest, such as *O. septentrionalis* housed with conspecifics or *I. nebulifer* larvae housed with *O. septentrionalis*, overall growth or gains in growth metrics were also higher when compared to all other treatments.

Among the species used in my experiments, larval *H. cinerea* have the lowest baseline activity level and *O. septentrionalis* larvae have the largest body size. Potentially, as a result of more individuals with larger body sizes exhibiting high activity levels, larvae within the *O. septentrionalis* control treatment and the heterospecific treatments containing *O. septentrionalis* and *I. nebulifer*, were acquiring food faster. In order to keep up with the foraging rate of these individuals and maintain a constant availability of food, I might have been feeding these treatments more often and at higher quantities, which could explain the higher overall growth for *O. septentrionalis* in the control treatment and the higher gains in growth metrics for *I. nebulifer* within the heterospecific treatments containing *O. septentrionalis*. Conversely, low activity level and foraging rate of *H. cinerea* would not have required the same feeding frequency or quantity to achieve satiation, meaning *O. septentrionalis* in the heterospecific treatments with *H. cinerea* might have received less food compared to *O. septentrionalis* within the control treatment. My results support this relationship, considering larval *O. septentrionalis* gained less mass in heterospecific treatments in which *H. cinerea* larvae were present. This pattern was not apparent in larvae within the *I. nebulifer* control

treatment, likely a result of this species metamorphosing at small size and undergoing majority of growth post-larval stage (Werner 1986).

The collective levels of activity and foraging rate among all individuals within treatments might have determined the frequency at which I provided food to treatments, and thus the overall quantity of food available throughout the duration of each trial. This begs the question of whether or not food was sufficiently abundant across all treatments. Although food was supplied *ad libitum*, there was no assurance of quantity or equal distribution throughout the aquarium and, therefore, no guarantee of equal access to food. Considering gains in growth metrics for *H. cinerea* larvae were lower in the presence of *O. septentrionalis* larvae, and the variation in biomass and activity level between species, it is plausible that *O. septentrionalis*, being the larger and more active species, imposed a combination of exploitative and interference competition on *H. cinerea* by taking up available feeding space as well as physically pushing out the native species from a particular food item. This competitive interaction is an unlikely explanation for the lower mass gain for *O. septentrionalis* in the heterospecific treatments including *H. cinerea*, seeing that *H. cinerea* have a much smaller body size and lower activity level than the invasive species.

Two factors that limit the interpretations available from my results are the timing of my assessment activity levels across all trials and the temporal separation of the two sets of trials representing some treatments (June vs. September 2020). I did not start recording the activity levels for individuals across all treatments at the same time of the day. This

might have introduced variation in activity level data on account of behavior patterns that change in association with circadian and circannual rhythms (Richter-Boix et al. 2007, Fraker 2008). Additional variation in activity level data might be attributable to differences in Gosner stage, as tadpoles at different ages and body sizes exhibit different activity levels in response to factors such as ontogenetic shifts in foraging behavior, locomotor efficiency, and changes in predation risk (Crump 1986, Golden et al. 2001, Denver 2010, Currie et al. 2016). Trials representing two of the treatment combinations were conducted during different times of the year, meaning that some measurements were not recorded on the same day, or the same time of day. As such, assessing the activity levels and growth of the larvae might have also been impacted by behavioral or physiological changes associated with seasonality (Laurila et al. 2001, Wu et al. 2007).

Another potential source of variation in my data is the fact that the clutches of *H. cinerea* and *I. nebulifer* used in experiments with *O. septentrionalis* were sourced from different paired adults, as compared to the clutches used in the treatments involving only native species. These clutches were also collected at different times of the year. This is cause for concern in terms of interspecific clutch variation and maternal effects and whether or not the differences between treatments were a result of competition or if confounding variables such as genetic variation and influence from females' body condition played a role. Many of the clutch characteristics, such as egg size, yolk content, and pH tolerance, are determined by the environment the female is exposed to and her body condition at the time of reproduction (Parichy and Kaplan 1992, Pierce and Wooten

1992, Bennett and Murray 2014). Maternal effects initiate a cascading effect from egg size to larval size at hatching, to size throughout development, suggesting that individual body size is influenced early on in development (Ficetola et al. 2010, Warne et al. 2013). Maternal effects play a role in adaptation, however, by signaling offspring with indirect information based on the female's environment (Kuijper and Hoyle 2015). Although they are not mutually exclusive, phenotypic capacity of individuals and their ability to adapt to cues directly from their environment are stronger and more efficient than adaptations driven by maternal effects (Kuijper and Hoyle 2015), suggesting my results might not be compromised by the confounding variables associated with using tadpoles from two different clutches. However, I cannot dismiss either the inherent genetic variation between clutches, or the fact that larval performance is related to female body condition (Morrison and Hero 2003, Dziminski and Roberts 2005, Altig and McDiarmid 2007, Warne et al. 2013).

In this research, I compared an invasive species, *O. septentrionalis* (which has been sighted but not yet reported as established in Texas) to species that occur naturally in eastern Texas, *H. cinerea* and *I. nebulifer*. Compared to mesocosm experiments (e.g., Wilbur 1987), the lab setting allowed me to use species from eastern Texas while minimizing risk of accidental release of any *O. septentrionalis* associated with my experiment. Although my research provides a baseline for understanding the potential effects of *O. septentrionalis* on native larval anurans within the confines of a lab setting, the interactions that I observed are not necessarily indicative of processes that would

occur in a natural setting (Dunham and Beaupre 1998, Lawler 1998). Food availability, tadpole density, and hydroperiod are examples of factors that vary considerably in the wild, and are drivers of competitive interactions (Wilbur 1987, Richter-Boix et al. 2007, Smith et al. 2014). I also did not consider the role larval predators might play in the establishment of *O. septentrionalis*. Predators and competitive interactions are influential factors in determining amphibian community assemblages (Skelly 1997, Dayton and Fitzgerald 2001, Arribas et al. 2018). Although predators were not a significant pressure inhibiting establishment of *O. septentrionalis* in Florida, there has been little study of novel interactions between predators and anuran larvae beyond their currently-invaded range (Meshaka and Jansen 1997, Meshaka 2001). For instance, there is evidence for the potential of predatory *Notophthalmus viridescens* (Eastern Newts) to lower the survivorship of *O. septentrionalis* within mesocosms by as much as 50% (Smith 2006). The range of *N. viridescens* overlaps with that of *O. septentrionalis* in Florida (Powell et al. 2016) and, despite decreasing survivorship of larvae, research has not linked predation by the newt to population control of *O. septentrionalis*. The range of *N. viridescens* extends along the Gulf Coast into eastern Texas (Powell et al. 2016), where predation of *O. septentrionalis* tadpoles could minimize the risk of this invasive species establishing.

Conclusion

Under natural conditions, Cuban Treefrogs will likely have negative impacts on native anuran species throughout all life-history stages. In combination with habitat loss

and climate change, competitive pressure from this species might drive local population declines or, in extreme cases, extirpation (Simberloff 2000, Davis 2009, Rödder and Weinsheimer 2009, Blaustein et al. 2010). However, populations of some native anurans in Florida are recovering in response to the removal of Cuban Treefrogs, with as many as 589 individuals being removed from a single site (Rice et al. 2011). My results reinforce the importance of management strategies that prevent the establishment of Cuban Treefrogs to eastern Texas. Checkpoints for transported produce and plants between and within states would help prevent the spread of this species, especially when shipments originate in areas known to contain established populations of Cuban Treefrogs (Keller et al. 2007, Rothlisberger et al. 2011). Developing citizen science programs and informing susceptible communities, and even communities already exposed to Cuban Treefrogs, could contribute to more effective monitoring and population control efforts (Johnson 2007, Johnson et al. 2010).

It cannot be denied that humans have some of the most impressive dispersal capabilities of any animal. With that ability, comes a rate of habitat alteration that is not likely to slow or acknowledge many ecological or ethical boundaries, and therefore further facilitating spread of nonnative species (Vitousek et al. 1997). Research on the consequences of species introductions should aim to identify preventative approaches to mitigate the impacts of invasive species such that biodiversity can be preserved at both the local and global scales.

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Table 1. Experimental design for assessing competition between larval anurans, including treatment name and number of additional species present (0, 1, 2) in each treatment type. Total number of individuals at the beginning of each trial equaled 30; 30 individuals of a single species in conspecific (0) treatments, 15 of each species in heterospecific (1) treatments, and 10 of each species in the heterospecific (2) treatments.

Treatment Name	Treatment Type
<i>Osteopilus septentrionalis</i> (OSSE)	conspecific (0)
<i>Hyla cinerea</i> (HYCI)	conspecific (0)
<i>Incilus nebulifer</i> (INNE)	conspecific (0)
<i>O. septentrionalis x H. cinerea</i> (OSSExHYCI)	heterospecific (1)
<i>O. septentrionalis x I. nebulifer</i> (OSSExINNE)	heterospecific (1)
<i>H. cinerea x I. nebulifer</i> (HYCIxINNE)	heterospecific (1)
<i>O. septentrionalis x H. cinerea x I. nebulifer</i> (OSSExHYCIxINNE)	heterospecific (2)

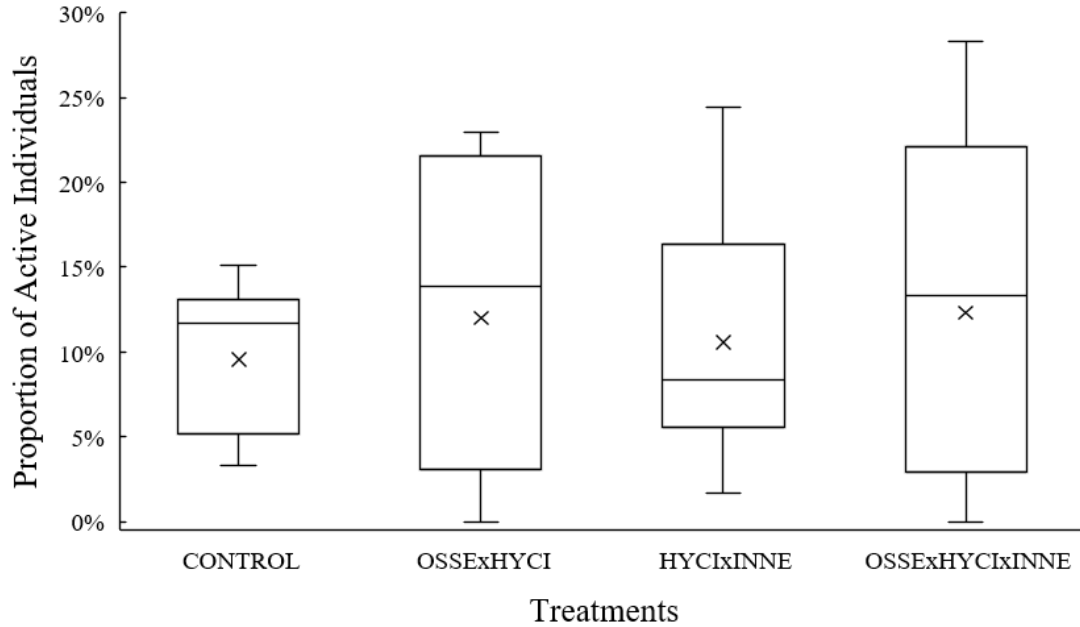


Figure 1. Activity levels of larval *Hyla cinerea* among treatments containing different combinations of individuals representing each of three species (HYCI, *Hyla cinerea*; OSSE, *Osteopilus septentrionalis*; INNE, *Incilius nebulifer*). Activity levels were calculated as the proportion of individuals of a particular species moving at a single point in time within a treatment. Activity levels of the 30 HYCI individuals in the Control treatment were not significantly different in the presence of native or invasive species.

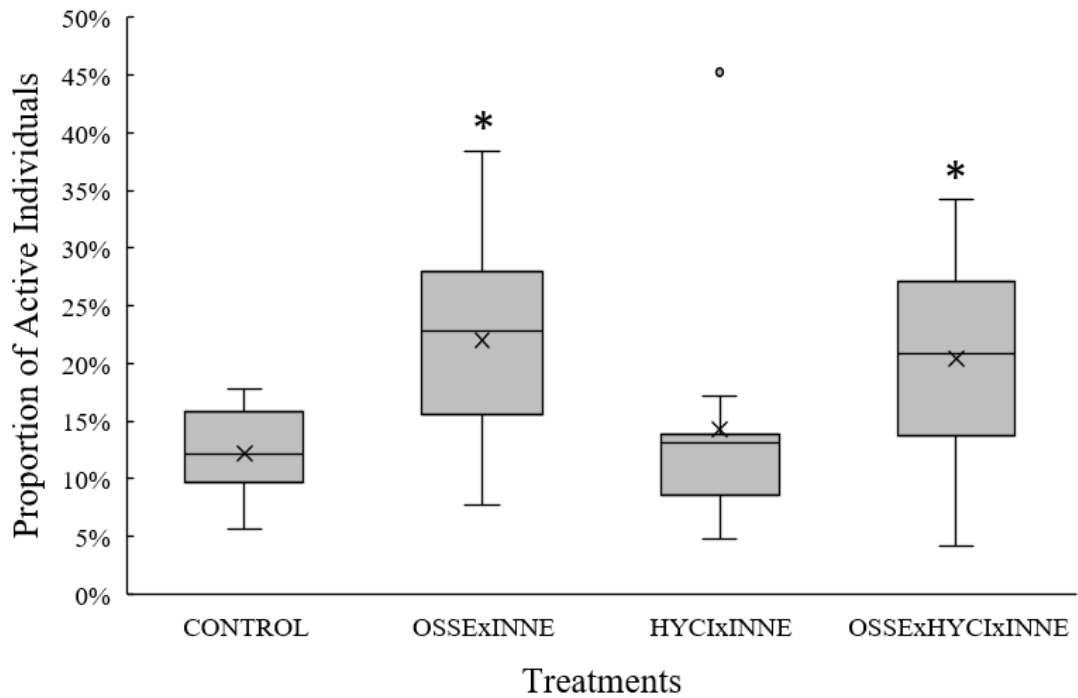


Figure 2. Activity levels of larval *Incilius nebulifer* among treatments containing different combinations of individuals representing each of three species (INNE, *Incilius nebulifer*; OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*;). Activity levels were calculated as the proportion of individuals of a particular species moving at a single point in time within a treatment. The Control treatment consisted of 30 individuals of INNE. The open circle indicates an outlier. Asterisks (*) indicate significant differences in activity level from the Control treatment (Dunn’s Nonparametric Multiple Comparisons tests).

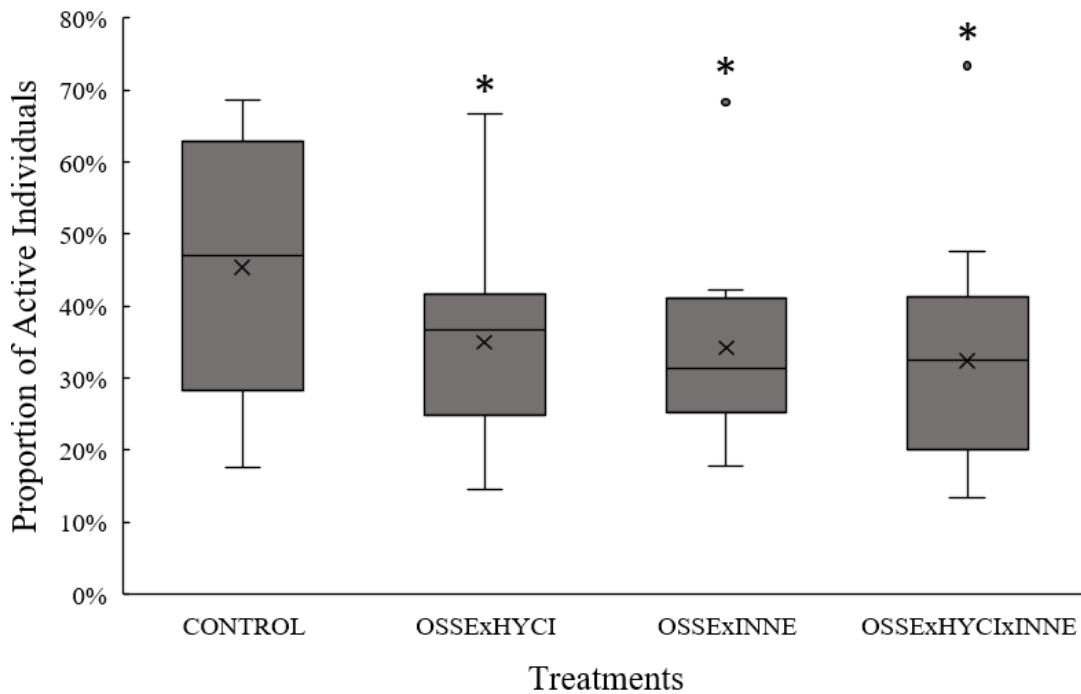


Figure 3. Activity levels of larval *Osteopilus septentrionalis* among treatments containing different combinations of individuals representing each of three species (OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*; INNE, *Incilius nebulifer*). Activity levels were calculated as the proportion of individuals of a particular species moving at a single point in time within a treatment. The Control treatment consisted of 30 individuals of OSSE. Open circles indicate outliers. Asterisks (*) indicate a significant difference in activity level from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

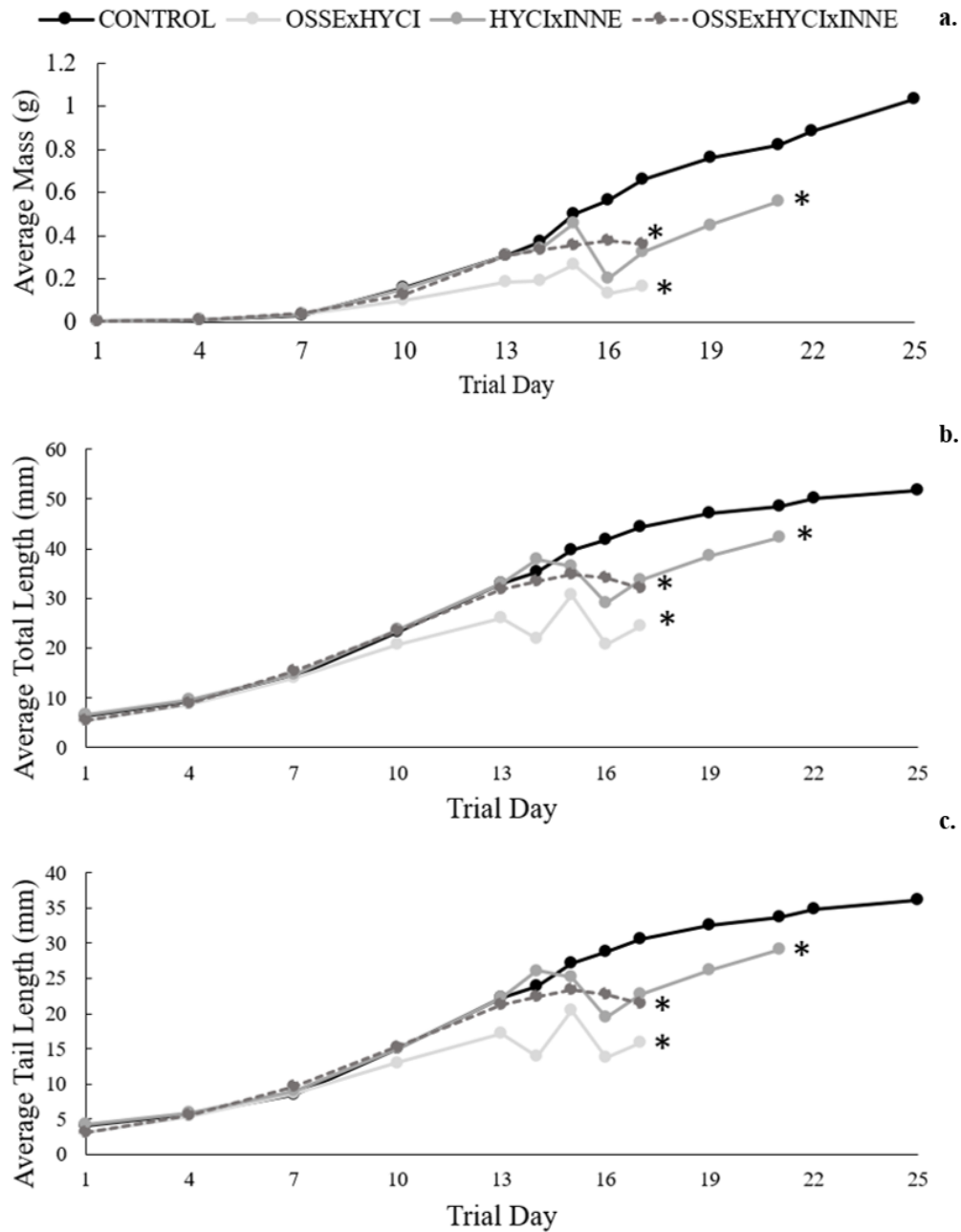


Figure 4. Average mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) each trial day (D_m) as measures of overall growth for *Hyla cinerea* among larval competition treatments (HYCI, *Hyla cinerea*; OSSE, *Osteopilus septentrionalis*; INNE, *Incilius nebulifer*). The Control treatment consisted of 30 individuals of HYCI. Asterisks (*) indicate a significant difference in overall growth from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

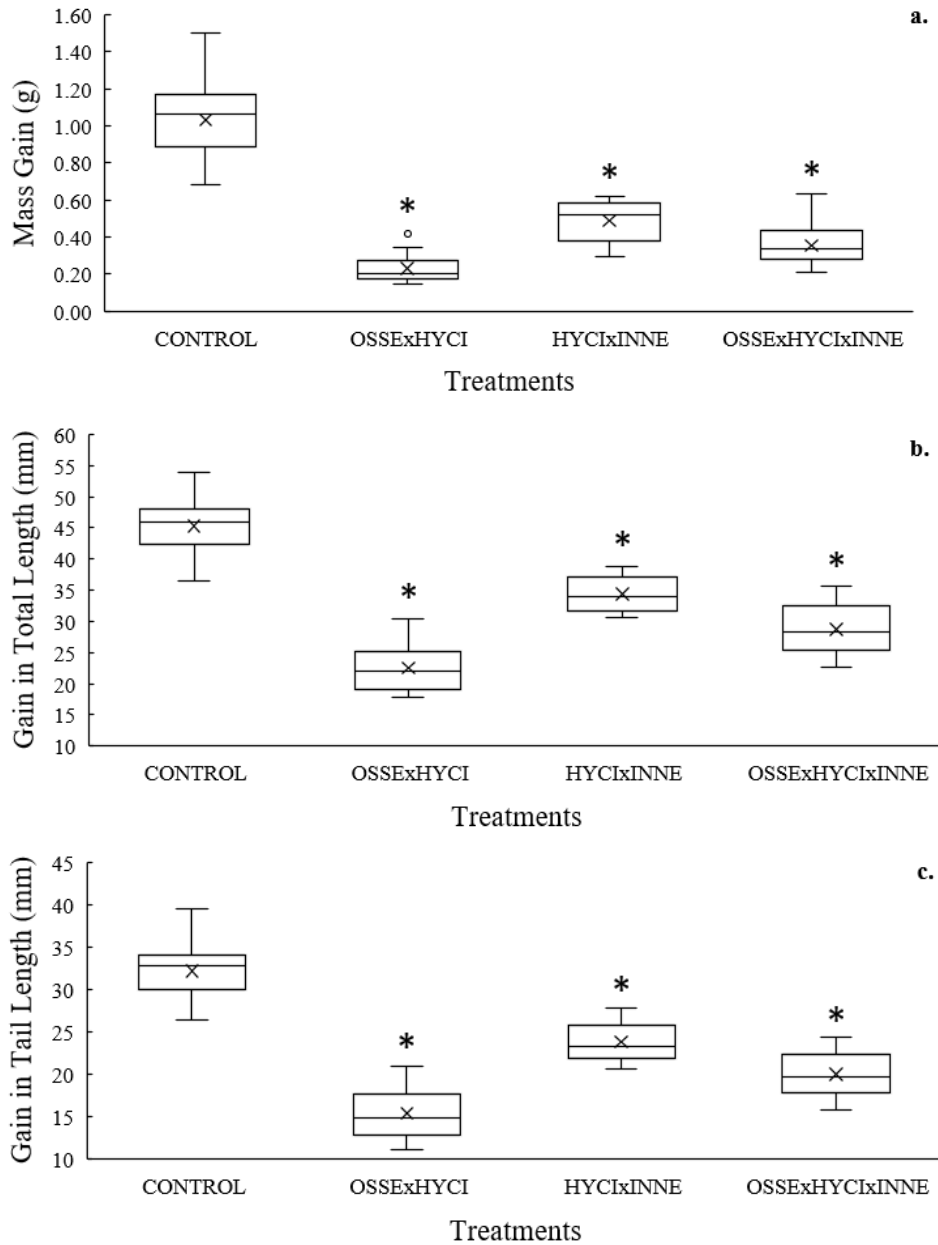


Figure 5. Gain in mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) during the full duration of trials (D_1 subtracted from D_{25}) involving *Hyla cinerea* among larval competition treatments (HYCI, *Hyla cinerea*; OSSE, *Osteopilus septentrionalis*; INNE, *Incilius nebulifer*). The Control treatment consisted of 30 individuals of HYCI. The open circle indicates an outlier. Asterisks (*) indicate gain in growth metric is significantly different from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

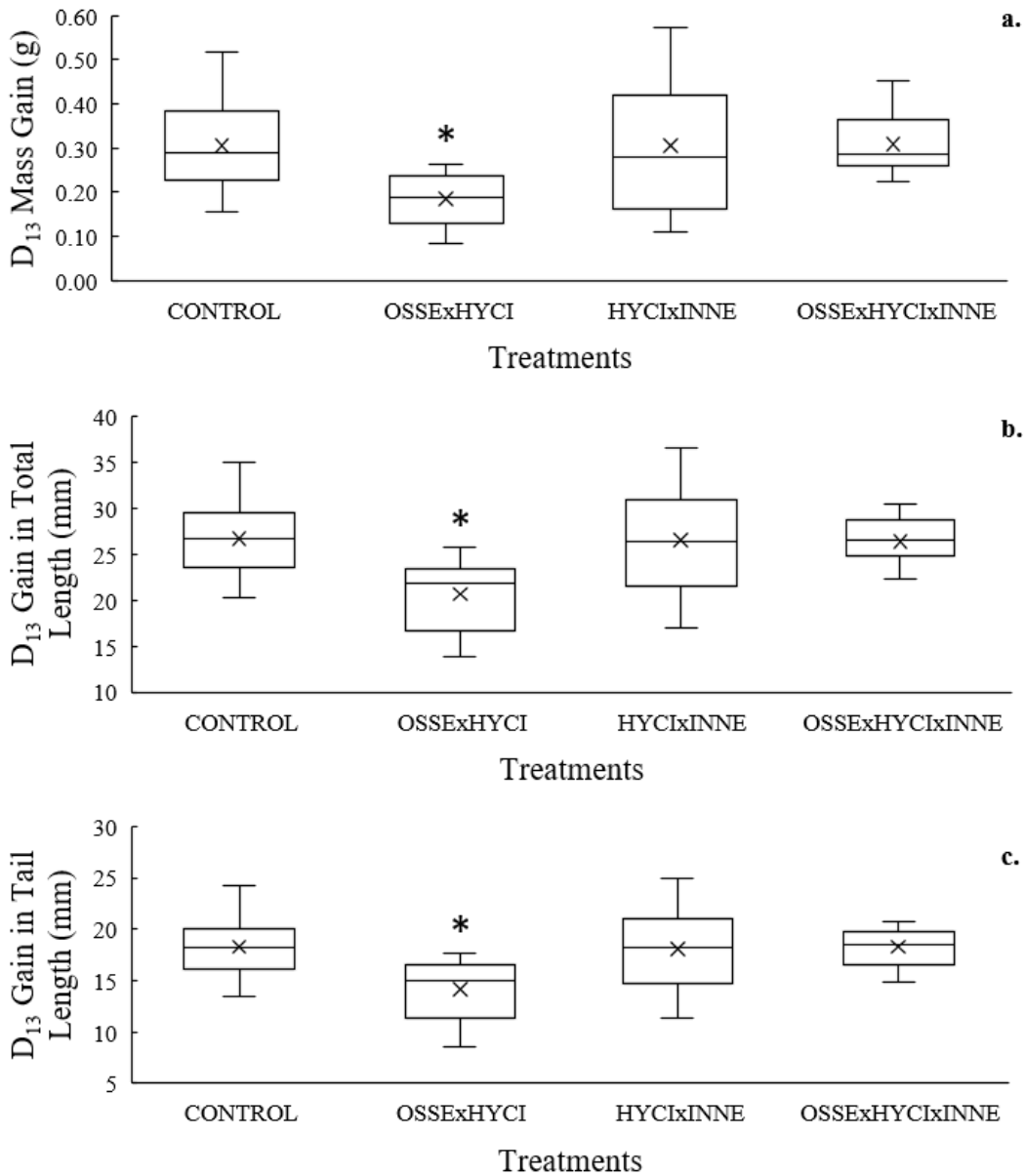


Figure 6. Gain in mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) during the initial 13 days of trials (D_1 subtracted from D_{13}) involving *Hyla cinerea* among larval competition treatments (HYCI, *Hyla cinerea*; OSSE, *Osteopilus septentrionalis*; INNE, *Incilius nebulifer*). The Control treatment consisted of 30 individuals of HYCI. Asterisks (*) indicate gain in growth metric is significantly different from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

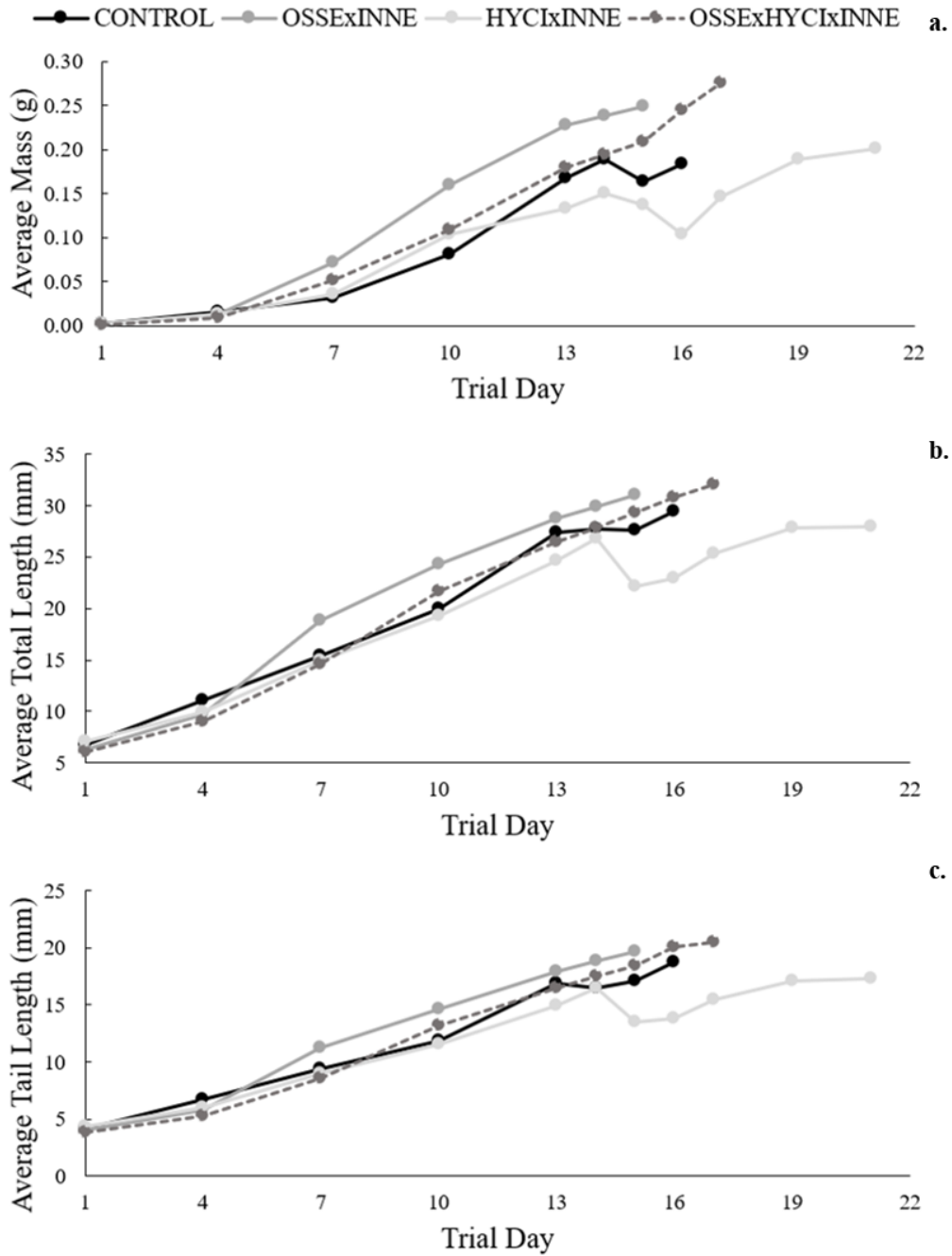


Figure 7. Average mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) each trial day (D_m) as measures of overall growth for *Incilius nebulifer* among larval competition treatments (INNE, *Incilius nebulifer*; OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*). Overall growth of the 30 INNE individuals in the Control treatment were not significantly different in the presence of native or invasive species.

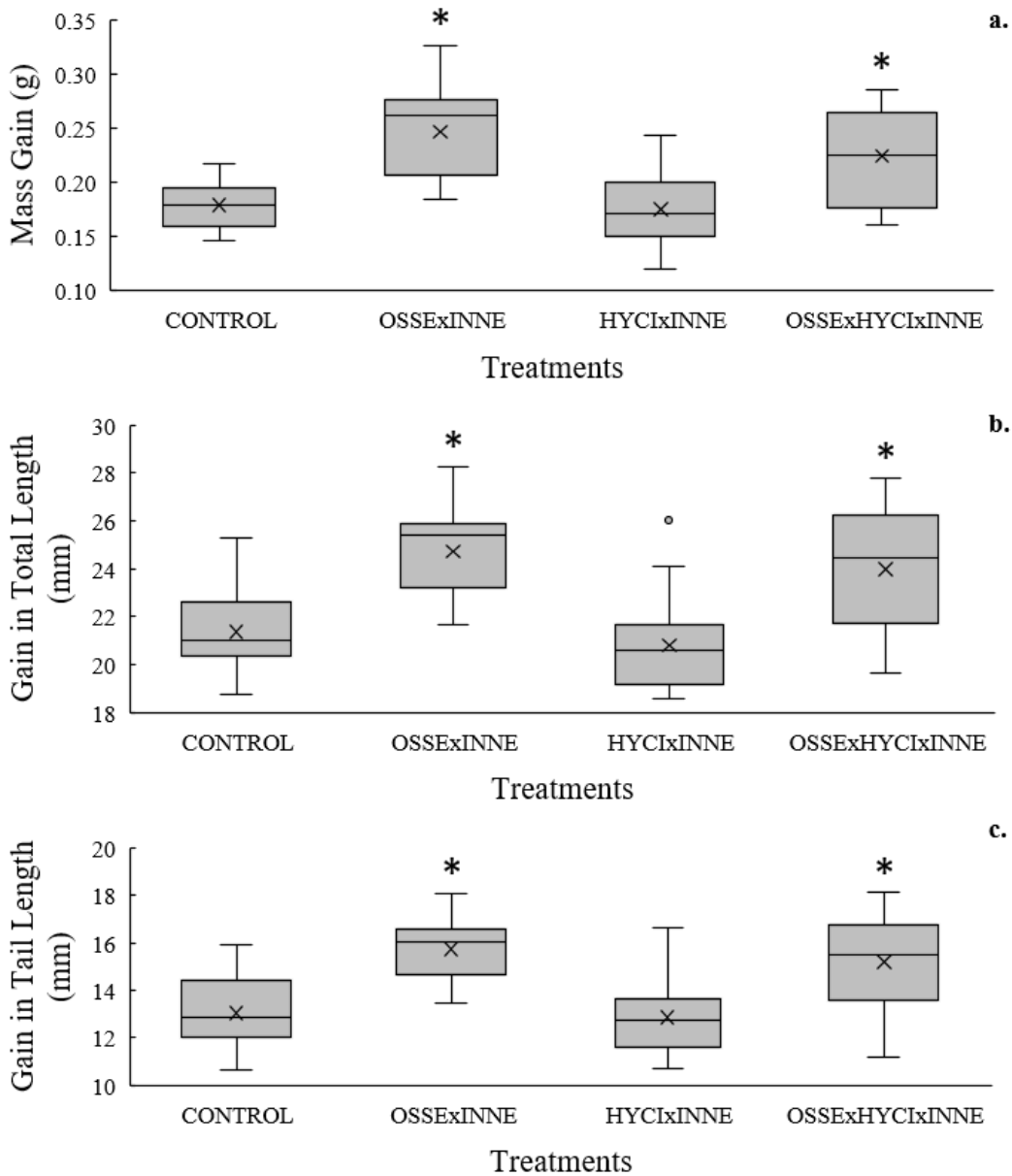


Figure 8. Gain in mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) during the full duration of trials (D_1 subtracted from D_{21}) involving *Incilius nebulifer* among larval competition treatments (INNE, *Incilius nebulifer*; OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*). The Control treatment consisted of 30 individuals of INNE. The open circle indicates an outlier. Asterisks (*) indicate gain in growth metric is significantly different from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

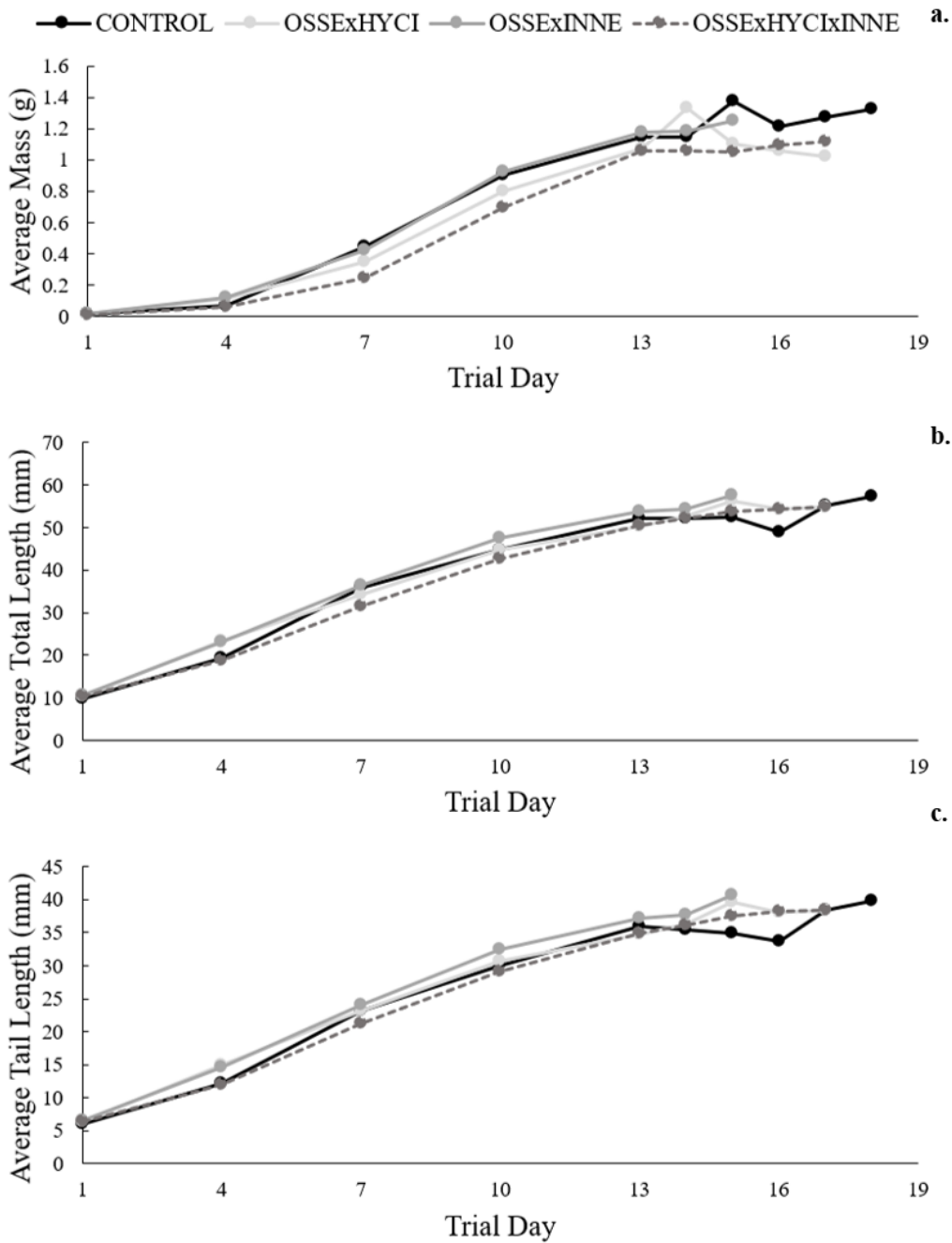


Figure 9. Average mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) each trial day (D_m) as measures of overall growth for *Osteopilus septentrionalis* among larval competition treatments (OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*; INNE, *Incilius nebulifer*). Overall growth of the 30 OSSE individuals in the Control treatment were not significantly different in the presence of native species.

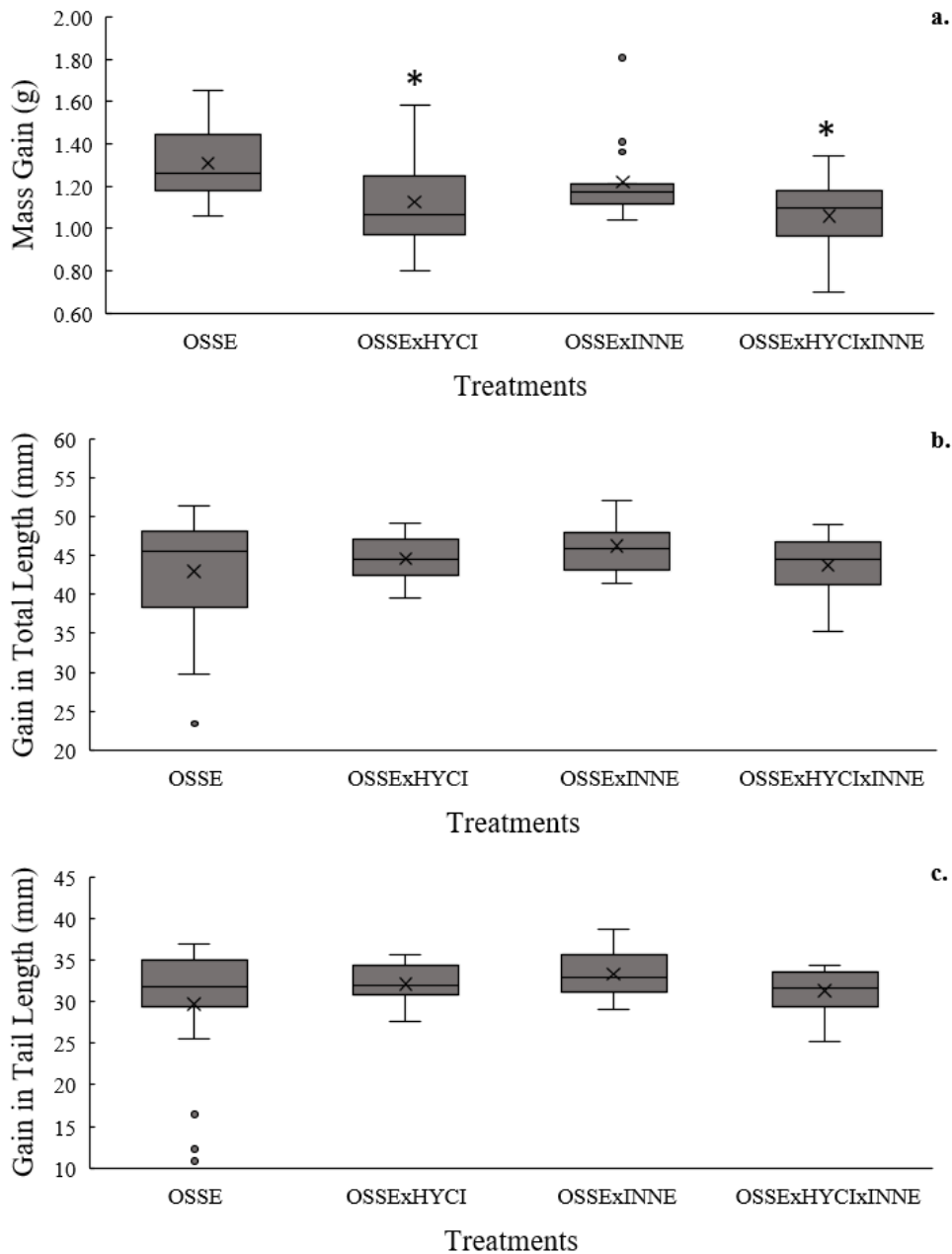


Figure 10. Gain in mass (g; **a**), total length (mm; **b**), and tail length (mm; **c**) during the full duration of trials (D_1 subtracted from D_{18}) of *Osteopilus septentrionalis* among larval competition treatments (OSSE, *Osteopilus septentrionalis*; HYCI, *Hyla cinerea*; INNE, *Incilius nebulifer*). The Control treatment consisted of 30 individuals of OSSE. Open circles indicate outliers. Asterisks (*) indicate gain in growth metric is significantly different from the Control treatment (Dunn's Nonparametric Multiple Comparisons tests).

VITA

After graduating from Galen High School, Reno, Nevada in 2012, McKenzie J. Wasley attended the University of Nevada, Reno in pursuit of a major in Wildlife Ecology and Conservation. She received the degree of Bachelor of Science from the University of Nevada, Reno in December 2016. McKenzie spent the following year employed as a lab assistant for researchers at the University of Nevada, Reno who focused on ecology and evolutionary biology. In August of 2018, she enrolled in the Graduate School of Stephen F. Austin State University at Nacogdoches, Texas, and received the degree of Master of Science in December 2020.

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This thesis was typed by McKenzie J. Wasley