Herpetological Conservation and Biology 13(1):167–174.
Submitted: 18 August 2017; Accepted: 14 February 2018; Published 30 April 2018.

The Salamander Daisy: A Novel Captive Rearing Method For Cannibalistic Salamander Larvae

Brian J. Tornabene[1,2,3] and Jason T. Hoverman[1]

[1]Department of Forestry and Natural Resources, Purdue University, 195 Marsteller Street, West Lafayette, Indiana 47907, USA
[2]Present address: Department of Wildlife Biology, University of Montana, 32 Campus Drive, Missoula, Montana 59802, USA
[3]Corresponding author, email: brian.tornabene@umontana.edu

***Abstract*.—Salamanders are often reared in captivity to support reintroduction efforts and laboratory experiments. However, larval salamanders can experience intense competition and cannibalism in captive environments that can influence survival, morphological traits, and developmental rates. Collectively, these outcomes can reduce the effectiveness and success of conservation and research efforts. Herein, we present a novel captive rearing method for cannibalistic salamanders, the salamander daisy. Our method houses salamanders in individual chambers floating in shared water, compared to traditional methods that house salamanders either communally or in individual containers. Additionally, we compared differences in survival and body size after rearing 21 Tiger Salamanders (*Ambystoma tigrinum*) using a traditional method compared to salamander daisy method. Survival of *A. tigrinum* reared using the salamander daisy method was 2.5 times higher (95%) than using the traditional method (38%). Therefore, more animals could be available for experiments or reintroductions using our novel method. We observed no difference in mean snout-vent length (SVL) or mass between methods. However, we found that variance in SVL and mass in the traditional method was > 31 times higher than when using the salamander daisy method. Our method is simple to construct, minimizes cannibalism, and makes animal husbandry more tractable. By using our method, we hope that investigators can produce more animals that have limited variation and are standardized in morphology. Moreover, this might maximize the efficiency and success of future experiments and reintroductions of imperiled salamanders.**

Key Words.—amphibian; Caudata, community ecology; disease ecology; ecotoxicology; management; translocation; Urodela

Introduction

More than 60% of salamander species worldwide are listed as threatened or near-threatened (International Union for the Conservation of Nature [IUCN] 2017) and require direct conservation actions. To aid in species recovery, the Amphibian Conservation Summit listed captive rearing and translocation as requiring development and implementation in the Amphibian Conservation Action Plan (Gascon et al. 2007). Captive rearing of salamanders for translocations and reintroductions occurs worldwide for a variety of species. For example, captive rearing and translocation programs are active for threatened Frosted Flatwoods Salamanders (*Ambystoma cingulatum*; O'Donnell et al. 2017) and endangered Yellow Spotted Mountain Newts (*Neurergus microspilotus*; Sharifi and Vaissi 2014; Vaissi and Sharifi 2016). Captive rearing to support reintroductions has also been proposed for Tiger Salamanders (*A. tigrinum*) in British Columbia (Southern Interior Reptile and Amphibian Recovery Team 2008), California Tiger Salamanders (*A. californiense*) in California, and Sonora Tiger Salamanders (*A. tigrinum stebbinsi*) in Arizona (U.S. Fish and Wildlife Service 2002, 2017). However, success of translocation projects may require captive rearing of large numbers of amphibians because the most successful projects generally release > 1,000 animals (Germano and Bishop 2009). Moreover, only 42% of published translocation efforts for amphibians are deemed successful, and this number may be overestimated because of publication bias (Germano and Bishop 2009). Salamanders are also currently used in largescale experiments (e.g., disease ecology and ecotoxicology) to investigate the influence of hypothesized stressors (e.g., Forson and Storfer 2006; Hoverman et al. 2011; Kerby et al. 2011). Given that conservation and research efforts require thousands of animals to be successful, there is a need to develop inexpensive and effective salamander husbandry techniques.

Cannibalism is a major challenge associated with husbandry of salamander larvae in natural and laboratory environments (Collins and Cheek 1983; Crump 1983; Semlitsch and Reichling 1989; Vaissi and Sharifi 2016).

Table 1. Descriptions and associated prices (unit and total price, USD$) for materials used to construct small and large salamander daisy rearing systems (prices as of July 2017). The small setup provides materials to house up to 35 salamanders and the large setup provides materials to house up to 1,029 salamanders. Most items were purchased from a local home improvement store, but shock cord and polyethylene jars were purchased from online vendors.

		Small		Large	
Detailed description	Unit price (USD$)	Quantity	Total (USD$)	Quantity	Total (USD$)
Oval tank, 189 L	90.06	1	90.06	–	–
Stock tank, 1136 L	210.05	–	–	7	1,470.35
White polyethylene jar, 946 ml	0.99	35	34.65	1029	1,018.71
R5 insulation sheathing, 2.54 × 61 × 51 cm	6.24	1	6.24	2	12.48
Shock cord, 0.32 × 762 cm	6.99	2	13.98	36	251.64
Fiberglass screening, 91 × 213 cm	5.99	1	5.99	3	17.97
Small insect fiberglass screening, 91 × 122 cm	15.06	1	15.06	6	90.36
Clear, waterproof silicone sealant, 290 ml	5.32	2	10.64	40	212.80
Hole saw, 28 mm	8.37	1	8.37	1	8.37
		Total:	184.99	Total:	3,082.68

Larval salamanders feed on eggs, limbs, and tails of conspecifics, and can consume entire smaller larvae of their own species (Wells 2007; Vaissi and Sharifi 2016). Cannibalistic larval salamanders prey on each other even in environments with high food densities and low animal densities (Vaissi and Sharifi 2016). Moreover, cannibalism can lead to larger body mass and faster growth rates (Claessen et al. 2000; Wakano 2004), and quicker time to metamorphosis (Semlitsch 1985). However, cannibalism can also inhibit growth and development (Newman 1987; Scott 1990), and cannibals may acquire parasites from conspecifics that also influence growth and surival (Pfennig et al. 1998). Despite these contrary conclusions, it is clear that cannibalism and competition influence natural populations and individuals reared in captivity.

Current methods for captive rearing of amphibians can be burdensome and wasteful when rearing cannibalistic salamanders. Communal rearing is a common and traditional method to rearing amphibian larvae. With this method, egg masses are collected, hatched, and held indoors or outdoors in large communal containers (e.g., 200–1000 L), and fed *ad libitum*. Communal rearing works well for anuran larvae (i.e., tadpoles), which generally do not prey on each other, and is cost effective (Bloxam and Tonge 1995). However, salamander larvae housed together are highly competitive for food resources and prey on conspecifics, thereby reducing the number of animals produced and increasing variability in morphology (Collins and Cheek 1983; Vaissi and Sharifi 2016). Variation in density of conspecific salamander larvae, and food resources, can also influence cannibalism rates (Crump 1983; Semlitsch and Reichling 1989). Because of competition and cannibalism in communal rearing containers, wide variation in morphology can occur because a cannibal is typically larger than its victim (Elgar and Bernard 1992; Claessen et al. 2000; Persson et al. 2000). An alternate method is to raise larvae singly in small containers (e.g., about 500 mL; Forson and Storfer 2006; Brunner et al. 2007; Kerby and Storfer 2009; Kerby et al. 2011), but regular feeding and water changes for hundreds of individual containers can be onerous. This is a tedious process that ties-up personnel and increases costs.

Herein, we describe the salamander daisy, a device we developed to rear cannibalistic salamander larvae in captivity. We also experimentally tested the efficacy of the salamander daisy and hypothesized that survivorship and morphology are affected by aquaculture methods. We predicted that survivorship would be higher and morphology more consistent with the salamander daisy compared to traditional aquaculture methods. Finally, we sought to develop this method to increase the success of future research and conservation efforts that focus on these species.

Materials and Methods

Construction of salamander daisies.—Each salamander daisy consisted of seven 1-L polyethylene jars, arranged with six jars in a circle surrounding a central jar (seven total jars in the shape of a daisy), tied together with two 1-m lengths of bungee cord around the outside of the jars (Fig. 1; Table 1). Before assembling each daisy, we drilled a 2.8-cm hole in the bottom of each jar and covered the hole with a circle of fiberglass screen (about 7 cm in diameter). We fixed fiber glass screen to the bottom of each jar with silicone caulk and allowed it to cure for > 2 d. We placed a smaller fiber glass screen (about 5 cm in diameter) that had tighter weave (e.g., small in-

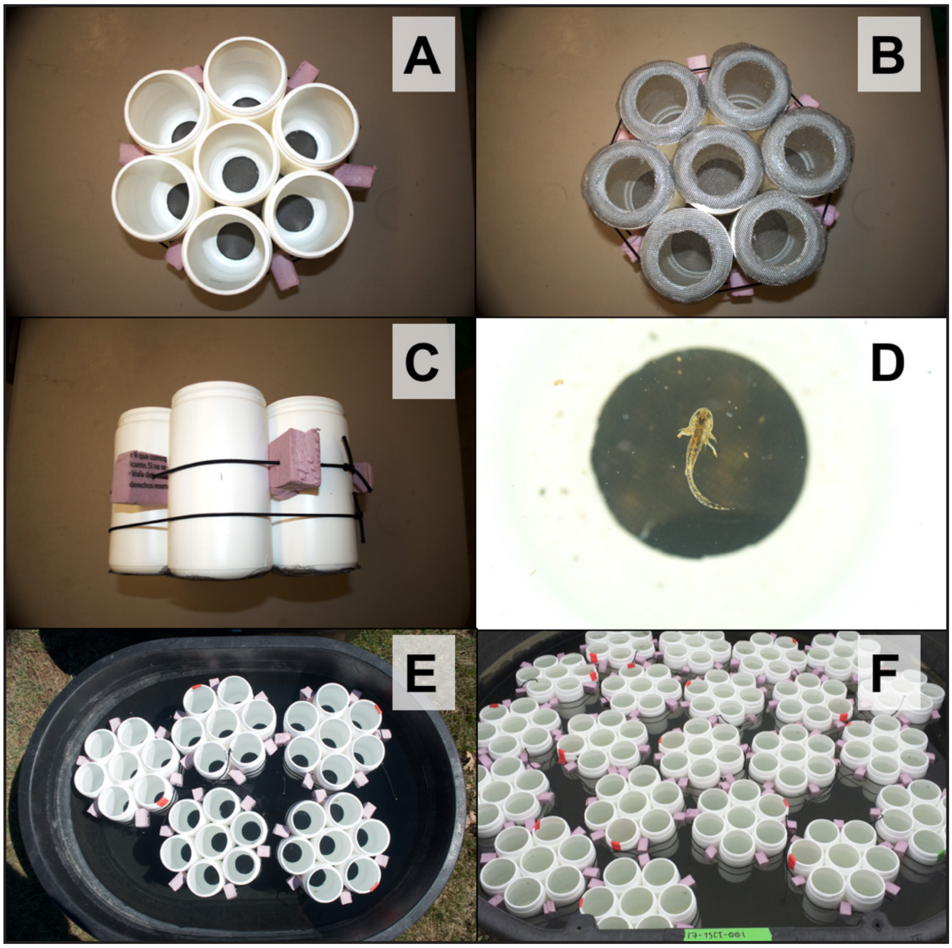

Figure 1. The salamander daisy rearing method as viewed from the top (A), bottom (B), and side (C). Note that a salamander daisy can house seven salamanders, each in a separate chamber. Screens are attached with silicone caulk to the bottom of each chamber in B, and bungee cord straps and insulation sheathing floats are visible in C. (D) depicts a small larval Tiger Salamander, *Ambystoma tigrinum*, in a chamber. (E) A small rearing system using an oval stock tank (about 200 L) and five salamander daisies (chambers for 35 salamanders). (F) large rearing system using a larger stock tank (about 1,200 L) and 21 salamander daisies (chambers for 147 salamanders). (Photographed by Brian J. Tornabene).

sect screen) inside each jar, at the bottom, to minimize the escape of smaller prey items. We strung one bungee cord through six 2.5-cm squares of insulation sheathing to float each daisy in water. A square of insulation sheathing rested where each of the six jars met. We tied bungee cords as tightly as possible around the jars, without bending them, to ensure the daisy would keep its shape. We floated each salamander daisy in the water column and added more squares of insulation sheathing underneath the tightened bungee cord, if necessary, to standardize the water line of each jar at the neck.

Five salamander daisies (35 total jars and animals) fit inside a 200 L stock tank and 21 salamander daisies (147 total animals) fit inside a 1,200 L stock tank (Fig. 1 E and F). We covered stock tanks with square (for 200 L stock tanks) or circular (for 1,200 L stock tanks) lids fashioned with a polyvinyl chloride (PVC) or polyethylene pipe frame, respectively. We filled frames with sand and stapled 70% shade cloth to the frames. A large square of 70% shade cloth and a bungee cord could also be used to cover stock tanks. Except when feeding and checking mortality, we covered stock tanks with lids at all times to prevent the colonization of invertebrate predators (e.g., dragonflies, *Anax* spp.) and breeding amphibians (e.g., Gray Tree Frogs, *Hyla versicolor*). The cost of one small salamander daisy setup to raise 35 salamanders, in five daisies with seven jars each, is < \$200 USD (including stock tank and all supplies; Table 1). The total cost of one large salamander daisy setup to raise 1,029 salamanders, in seven stock tanks with 21 daisies each (with seven jars per daisy), is about \$3,000 USD. However, new stock tanks are the most expensive items (about \$90 and \$210 USD for small and large stock tanks, respectively) and might already be available (in a variety of different shapes and sizes) or bought in bulk to reduce the price per unit.

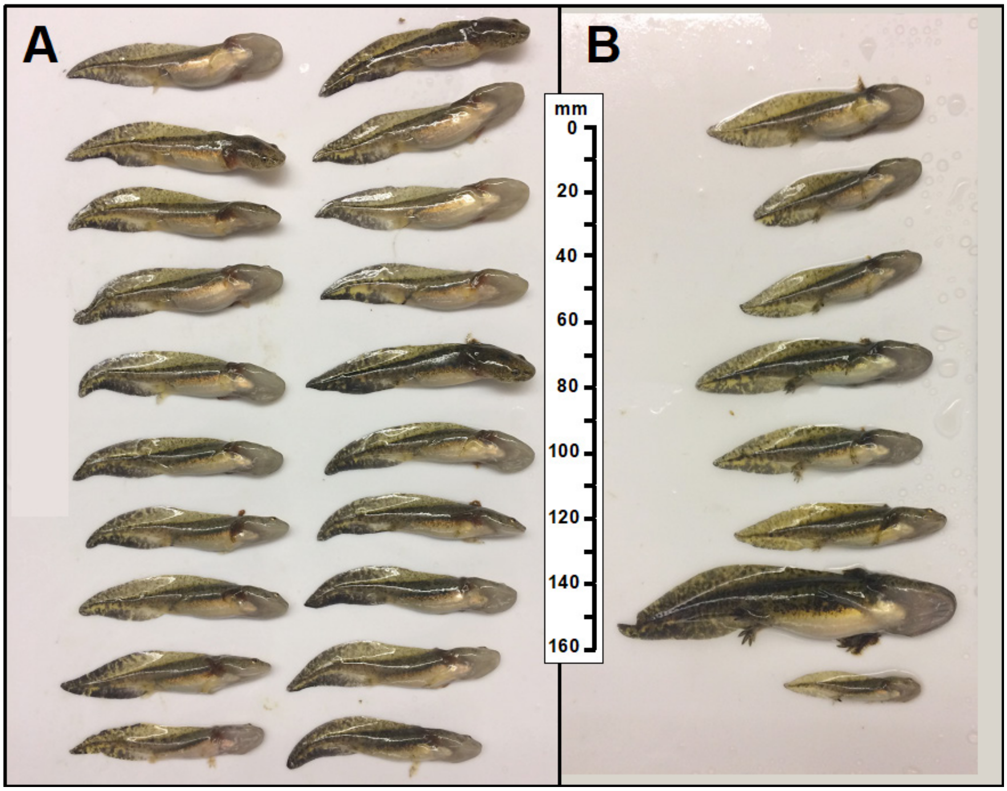

Figure 2. Larval salamanders reared for 50 d from about Harrison stage 37 to Harrison stage 46 with the salamander daisy (n = 20, survival = 95%; A) and traditional, communal captive rearing methods (n = 8, survival = 38%; B). We began both treatments with 21 larvae. Images A and B are to scale and share the central scale bar. (Photographed by Brian J. Tornabene).

Comparing traditional and novel rearing methods.—We compared the differences in survival and morphology after rearing cannibalistic salamander larvae with a traditional communal method compared to our salamander daisy method. We housed salamander eggs, and then small larvae, communally until they hatched and began to develop. The small gape sizes of salamanders during early larval stages deters cannibalism. We collected three partial egg masses of Tiger Salamanders, *A. tigrinum*, from ephemeral wetlands at the Purdue Wildlife Area (PWA) in West Lafayette, Indiana, USA, on 9 March 2017. We placed egg masses in a single outdoor 200 L stock tank filled with about 120 L of well water. We filled the stock tank with water 2 d prior to adding egg masses to allow water to equilibrate to ambient temperature. After hatching, we fed salamanders *ad libitum* (generally every other day) with concentrated zooplankton collected from permanent wetlands at PWA. We maintained very high food density in the tank during the early stage of rearing to reduce the development of cannibalistic behaviors (Vaissi and Sharifi 2016).

The larvae reached Harrison stage 37 or 38 by 5 April 2017 (Harrison 1969), and we then randomly assigned them (n = 52) to either the traditional method (n = 21), salamander daisy setup (n = 21), or a group to monitor 24-h handling mortality (n = 10). We chose this stage because larvae were large enough to handle without causing injury, but before they became noticeably cannibalistic or aggressive (e.g., tail and limb nipping) with conspecifics. All larvae were combined, and allowed to mix freely in a single container, before randomly assigning them to treatment groups; we waited 5 min between transferring individuals out of the combined container to allow salamanders to move around and mix within the container. We used a random number generator (with numbers 1–3) to assign larvae to each treatment. We used a 500 µm, nylon hand net to transfer larvae to the appropriate treatment regime. We covered all stock tanks with lids, which we removed when either feeding the larvae, or evaluating their health and mortality. We housed the 24-h handling mortality group indoors in 15-L plastic aquaria filled with 10 L of aged water at 21° C with a 12:12 photoperiod for 24 h. After 24 h, we counted living larvae, euthanized them with MS-222 (tricaine methanesulfonate), measured their snout-vent length (SVL) and mass, and accessioned larvae into teaching collections.

We reared larvae in the remaining two treatment tanks (communal and salamander daisy methods) for 50 d (Harrison stage 46, about halfway to metamorphosis), and checked their health and mortality daily. We

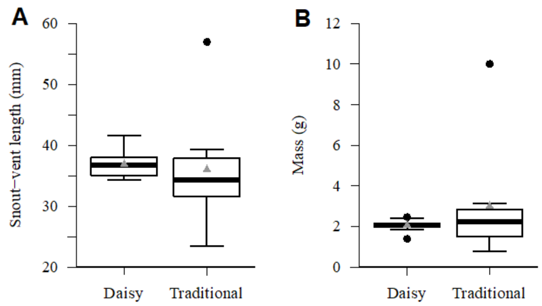

Figure 3. Snout-vent (mm; A) and mass (g; B) of surviving salamanders reared using a communal (traditional; n = 8) or salamander daisy (daisy; n = 20) captive-rearing method. For boxplots, the bottom and top whiskers describe 1.5 times the interquartile range, the bottom of the box describes the first quartile, the bolded horizontal line describes the median, the grey triangle represents the mean, the top of the box describes the third quartile, and data beyond the whiskers are outliers represented as points.

fed salamanders with concentrated zooplankton or tadpoles every other day to ensure an adequate food supply. We administered 120 mL of homogenized, dense zooplankton to each jar in the salamander daisy treatment. However, we haphazardly scattered 120 mL of concentrated zooplankton per salamander remaining in the tank to the traditional approach stock tank. When the gape size of all larvae were large enough to consume tadpoles, we switched the food of the larvae from zooplankton to tadpoles of the American Toad (*Anaxyrus americanus*). We collected tadpoles from fish aquaculture ponds at the Purdue Aquaculture Research Laboratory in West Lafayette, Indiana, USA. Each salamander was fed about three *A. americanus* tadpoles per feeding, generally every other day. We introduced tadpoles directly to each jar in the daisy treatment, and haphazardly scattered tadpoles around the stock tank for the traditional approach. Salamanders and introduced tadpoles moved around and mixed freely within the stock tank in the traditional treatment. We conducted partial (about 50%) water changes every 7 d by siphoning off grey water, with a 1.3-cm diameter hose covered with screen at the end, and slowly added aged (for 2 d), clean water. We removed filamentous algae and salamander waste from both treatments as necessary using a turkey baster. After 50 d (on 25 May 2017), we removed all salamanders from both treatments, euthanized them with MS-222, counted individuals per treatment, measured SVL and mass of each salamander, and accessioned larvae into teaching collections.

Statistical analyses.—We compared morphology (mass and SVL) and survival between traditional and salamander daisy treatments at the end of the experiment. We monitored mortality and designated it as natural (animal deceased in tank) or cannibalism (animal disappeared) throughout the experiment. We counted the number of remaining salamanders in each method after 50 d and divided by 21 (the original number stocked in tanks) to calculate survival. We examined the normality of all variables with histograms and Shapiro-Wilks tests (Zar 1999). We log-transformed mass and SVL measurements to meet assumptions of normality for statistical tests. We used *F*-tests of equality of variances to investigate differences in variance in mass and SVL between salamanders raised in the two methods (Zar 1999). To compare mass and SVL between our two methods, we used Welch's *t*-test because it is robust to differences in sample size and variance between groups (Ruxton 2006). We conducted all analyses in Program R v3.4.1 and statistical significance level for all analyses was $\alpha = 0.05$ (R Core Team 2016).

Results

Comparing traditional and novel rearing methods.—Survival was higher and variation was lower for larval *A. tigrinum* reared for 50 d in the salamander daisy treatment compared to traditional communal method. Survival in our 24-h handling mortality group was 100% (n = 10). During the first week of the experiment, one salamander from each treatment died naturally (i.e., not cannibalized). At the conclusion of the experiment, 12 larvae in the traditional treatment had been cannibalized; whereas, no larvae in the salamander daisy treatment were cannibalized. Survival was 2.5 times higher in the salamander daisy treatment (95%, n = 20) than in the traditional treatment (38%, n = 8). Mean mass and SVL of *A. tigrinum* assumed a normal distribution following log transformation ($W > 0.93$ and $P > 0.483$). Mean mass and SVL of *A. tigrinum* raised using the salamander daisy method did not differ from those raised in the traditional method ($t = -0.41$ and 0.57, df = 7.1 and 7.2, $P = 0.694$ and 0.584, respectively; Fig. 3). However, variance in SVL and mass were 31 and 212 times higher, respectively, for salamanders reared in the traditional method ($\sigma^2 = 8.55$ and 93.56) than those reared in the salamander daisy method ($\sigma^2 = 0.040$ and 3.77; $F_{7,19} = 0.04$ and 0.005, and $P < 0.001$). Snout-vent length of the largest larva reared in the traditional treatment was 1.8 times longer than the SVL of the smallest larva reared in that treatment. However, SVL of the largest larva reared in the salamander daisy treatment was only 1.2 times longer than the SVL of the smallest larva reared in that treatment.

Discussion

The success of captive rearing and reintroduction efforts for threatened and endangered salamanders is dependent on our ability to generate large numbers of individuals (Germano and Bishop 2009). Unfortunately, husbandry requirements are unknown for most species of salamanders (Pough 2007). Species interactions, such as competition and cannibalism, during the larval stages can be barriers to success when using traditional communal rearing techniques. Thus, we developed the salamander daisy, a novel method that integrates individual holding chambers within a larger body of water. Our technique raised more salamanders with less variability than the traditional communal method. Moreover, the salamander daisy method required less regular maintenance than using individual disconnected containers per animal under laboratory conditions.

Our salamander daisy method eliminated both competition and cannibalism in larval salamanders. Density of larvae per tank and contact rates influence cannibalism in salamanders (Michimae and Wakahara 2001; Wildy et al. 2001). Moreover, competition among larvae for food can lead to cannibalism (Vaissi and Sharifi 2016). By rearing larvae in individual containers within the daisies, we prevented contact between individuals and controlled resource levels provided to each individual. Ultimately, use of salamander daisies eliminated cannibalism and resulted in more standardized larvae compared to the traditional communal rearing method. An alternative method is to rear salamander larvae under laboratory conditions in individual containers (e.g., about 500 mL; Forson and Storfer 2006; Brunner et al. 2007; Kerby and Storfer 2009; Kerby et al. 2011). However, when rearing thousands of individuals, this method is labor, time, and space intensive. Additionally, rearing salamanders to metamorphosis would require containers > 1 L, which might not be feasible because of space limitations. Our approach allowed us to house salamanders in a large, stable, communal body of water. Using a larger volume of water can reduce the frequency and number of water changes during the husbandry period. Furthermore, individuals reared in outdoor salamander daisies are exposed to natural conditions (e.g., variable photoperiod, temperature, and precipitation), which influence growth and development (Wells 2007), compared to individuals reared in the laboratory under standardized photoperiod and temperature.

We suspect that the one larva from the traditional rearing method, which became significantly larger than cohorts, was cannibalizing other larvae and was responsible for most of the mortality we detected (i.e., giants and dwarves; *sensu* Claessen et al. 2000). Salamanders raised in the traditional method could be sorted regularly by size to reduce this effect. However, this increases handling stress, effort, and the number of containers necessary for rearing numerous sized-sorted groups. With the salamander daisy method, animals can remain in chambers until they outgrow it (about 100 mm SVL). Following this, salamanders can be held in groups again because cannibalism tends to cease because of large body sizes and gape size limitations among individuals (Elgar and Bernard 1992; Persson et al. 2000). However, in our experiment, no salamanders outgrew their jars in the salamander daisy method within 50 d.

Importantly, the daisy method also can be used to take numerous salamanders to metamorphosis. For a large salamander daisy setup, we used twice as many large stock tanks than we had salamanders in, and prepared duplicate stock tanks with aged water. Therefore, if necessary, we could easily lift salamander daisies (that contained salamanders) from stock tanks with grey water to stock tanks with clean and aged water. This eliminated the need to siphon grey water out of tanks with salamanders, age water in a separate location, and pump or siphon clean and aged water back in. To expedite feeding, we used turkey basters or 5 mL transfer pipettes to feed zooplankton and small anuran tadpoles (e.g., *A. americanus*) to each jar occupied by a salamander. Because small anuran tadpoles were difficult to obtain in large quantities, we also fed Black Worms (*Lumbriculus variegatus*) to larval salamanders in larger captive-rearing operations. We started feeding *ad libitum* with concentrated zooplankton, transitioned to cut then whole *L. variegatus*, then supplemented with anuran tadpoles as salamanders grew. We also used turkey basters to remove salamander waste and filamentous algae. Removing waste helped to reduce the frequency of water changes per stock tank, and algae could impede the movement of salamanders within their housing. When salamanders outgrew jars in the daisies, they were removed and placed communally in 200 L stock tanks (starting density of 20 salamanders). Using this method, we observed that large larval salamanders did not exhibit cannibalistic behaviors (or nip at limbs or tails). We progressively lowered the water level in the stock tanks, then tilted the stock tank on its side to expose a terrestrial location to elicit metamorphosis. When salamanders finished metamorphosis (i.e., absorption of gills), we housed them in moist sphagnum moss and fed them with crickets consistent with terrestrial husbandry methods (Pough 2007).

We were able to eliminate mortality associated with cannibalism during captive rearing using our salamander daisy method. Moreover, our method was easy to assemble, manage, and clean. This information enhances our ability to effectively raise, increase survival, and standardize the growth and development of salamanders raised in captivity. We hope our method can increase the effectiveness and success rate of captive rearing, laboratory experiments, and reintroductions of larvae or adults of imperiled salamander species.

Acknowledgments.—We collected all *Ambystoma tigrinum* under Indiana Department of Natural Resources permit #17-051 issued to Brian J. Tornabene. We reared and euthanized all *A. tigrinum* under the auspices of Purdue University Institutional Animal Care and Use Committee protocol #1601001355. We thank Purdue University for support. We thank Sarah Abercrombie, Michael Chislock, and Zachary Compton for helpful discussions of design and for help with animal husbandry. We also thank Erin Kenison and members of the Hoverman Lab for their constructive comments and revisions of this manuscript.

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Brian J. Tornabene is a Ph.D. student in the Department of Wildlife Biology at the University of Montana in Missoula, Montana, USA. He obtained his Bachelor of Science from Baldwin Wallace University in Berea, Ohio, USA (2008), and his Master's from Montana State University, Bozeman, Montana, USA (2014). Brian is broadly interested in the natural history, behavior, and population and community ecology of herpetofauna. He is also interested in ecotoxicology, physiology, and disease ecology to determine the effects of anthropogenic stressors on individuals and ecosystems. Currently, he is investigating the influence of brine contamination from oil and gas development on amphibian assemblages in the Prairie Pothole Region of Montana and North Dakota, USA. (Photographed by Scott See).

Jason T. Hoverman is an Associate Professor of Vertebrate Ecology in the Department of Forestry and Natural Resources at Purdue University in West Lafayette, Indiana, USA. He obtained his Bachelor of Science (2000) and Ph.D. (2007) from the University of Pittsburgh, Pennsylvania, USA. His research seeks to understand the separate and combined effects of natural and anthropogenic stressors at multiple scales of ecological organization (i.e., individuals to ecosystems). To address his research interests, he uses freshwater aquatic systems (e.g., ponds, wetlands, and lakes) and their associated taxa (e.g., tadpoles, snails, insects, fish, and parasites). Within the framework of environmental stressors, he integrates research on community ecology, ecotoxicology, and disease ecology. (Photographed by Brent Drinkut).