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Rachel Ann Settle

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**QUANTITATIVE BEHAVIORAL ANALYSES OF OZARK HELLBENDER
REPRODUCTION AND PATERNAL CARE**

A Masters Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree
Master of Science, Biology

By

Rachel Ann Settle

May 2017

QUANTITATIVE BEHAVIORAL ANALYSES OF OZARK HELLBENDER REPRODUCTION AND PATERNAL CARE

Biology

Missouri State University, May 2017

Master of Science

Rachel Ann Settle

ABSTRACT

The Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) is a federally endangered aquatic salamander. While some anecdotal observations have been reported, no quantitative assessments of reproductive behaviors have been made. I quantified video-recordings of two breeding events at the Saint Louis Zoo. Hellbenders were housed in an indoor stream outfitted with 4 cameras. General activity and agonistic behaviors increased through the first oviposition, peaked during inter-oviposition, and declined abruptly following the second oviposition. Following oviposition, males guard their eggs until hatching. I also quantified behavior of a guarding male from video footage collected by MDC from a nest in the North Fork of the White River. I observed tail-fanning of eggs, rocking, foraging at the nest, nest occupancy, egg cannibalism, and behavior of fishes/crayfish at the nest. There were high frequencies of tail-fanning and rocking, behaviors which increase aeration. The male rarely left the nest unguarded and spent over half of the time at the nest exposed at the nest entrance. Potential egg predators observed included centrarchid, cyprinid, ictalurid, and percoid fishes, with centrarchids being the most common and exhibiting the most interest in the nest. The frequency of foraging by the male was low ($n = 8$ strikes), with a 37% success rate; all successful strikes were to small cyprinids. Understanding spawning and nest-guarding behaviors can be used to inform management decisions and captive breeding programs.

KEYWORDS: reproductive behavior, oviposition, kinematic analysis, paternal care, *Cryptobranchus alleganiensis bishopi*

This abstract is approved as to form and content

Dr. Alicia Mathis
Chairperson, Advisory Committee
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INTRODUCTION

According to the International Union for Conservation of Nature's (IUCN) Red List criteria, nearly one-third of amphibian species are threatened worldwide (Stuart et al. 2004). Additionally, amphibians (427) surpass both birds (179) and mammals (184) for total number of critically endangered species (Stuart et al. 2004). Stuart et al.'s (2004) global overview amphibian Red List species, the most recent published summary, is now over 10 years old, and additional species have been added (<http://www.iucnredlist.org/initiatives/amphibians>). A broad study of amphibians available in the USGS database reported, in the United States alone, there was an estimated 3.7% annual decline in total amphibian occupancy in aquatic and terrestrial habitats over a ten-year span, and an 11.6% decline in red-listed species (Adams et al. 2013).

Global and local amphibian declines should be of concern, not only because of an interest in preserving biodiversity, but because of the ecological role of amphibians in maintaining ecosystem structure via food webs (Hocking and Babbitt 2014). From an applied standpoint, amphibians also serve as indicators of environmental health (Cooke 1981; Welsh and Ollivier 1998; Pollet and Bendell-Young 2000). One characteristic that makes amphibians particularly sensitive environmental indicators is the semipermeable nature of their skin, which allows amphibians to rely on cutaneous respiration as a source of oxygen intake (Noble 1925; Bernstein 1953; Whitford and Hutchison 1965; Guimond 1970; Feder and Burggren 1985). Although most amphibians also have access to pulmonary respiration, the majority of their respiration is through the skin (Wells 2007).

Hellbenders (*Cryptobranchus alleganiensis*) are permanently aquatic salamanders that can fill the role of both predator and prey in the ecosystem (Nickerson and Mays 1973). Because hellbenders are heavily reliant on cutaneous respiration as their main source of gas exchange (Bruner 1914; Guimond and Hutchison 1973), they are sensitive to environmental changes, such as stream pollution, and thus are a good indicator of water quality. Currently, two subspecies are recognized, although both are paraphyletic (Crowhurst et al. 2011; Tonione et al. 2011). In many parts of their ranges, populations of both subspecies have experienced declines (Wheeler et al. 2003; Mayasich et al. 2003; Foster et al. 2009; Burgmeier et al 2011). The Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) is federally endangered (USFWS 2011) and the Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is currently petitioned to be listed as threatened or endangered under the Endangered Species Act (USFWS <https://ecos.fws.gov/ecp0/profile/speciesProfile?sPCODE=D043>).

In Missouri, both hellbender subspecies are classified as state endangered (MDC 2016). As part of a strategy to combat the decline of Missouri's hellbenders, captive rearing efforts were initiated at the Saint Louis Zoo's (SLZ) Ron Goellner Center for Hellbender Conservation (RGCHC) and the Missouri Department of Conservation's (MDC) Shepherd of the Hills Fish Hatchery in Branson, Missouri. Both programs have successfully hatched eggs collected from naturally-occurring nests and reared larvae for release in the wild (Crowhurst et al. 2011; Bodinof et al. 2012; Briggler 2016a; Briggler 2016b).

Captive breeding of hellbenders proved to be more difficult. In nature, they congregate in large aggregations during the breeding season (Smith 1907; Nickerson and

Mays 1973; Humphries 2007). Male hellbenders exhibit site-specific selection of nests under large, flat rocks, within bedrock crevices, and other structures with the open end oriented in the downstream direction (Smith 1907; Bishop 1941; Nickerson and Mays 1973; Peterson 1988; Pflingsten and Downs 1989). The female selects a male and enters his nest site either freely or by coercion, deposits her eggs within the nest, and then the male utilizes external fertilization by using his tail to direct milt over her eggs in a rhythmic, fanning motion (Nickerson and Mays 1973; Pflingsten and Downs 1989). Females may lay several hundreds of eggs in one oviposition event, which may take several days to complete (Smith 1907; Bishop 1941; Nickerson and Mays 1973; Pflingsten and Downs 1989). After oviposition ceases, the female will leave the nest willingly or the male may force her out of the nest while he begins guarding the eggs during their development (Nickerson and Mays 1973; Petranka 1998). In 2011, a conservation milestone was reached, when the RGCHC, in collaboration with MDC, reported the first successful breeding events for Ozark hellbenders in captivity (Ettling et al. 2013). The SLZ has continued to successfully breed Ozark hellbenders each year since the initial success (Ettling et al. 2013; Briggler 2016a; Briggler 2016b).

The success of the captive breeding program at RGCHC appears to be largely attributable to use of artificial breeding streams that closely mimic conditions in the natural environment, including temperature, photoperiod, precipitation, water quality parameters, and prey availability (Ettling et al. 2013). Adjusting the ionic composition (total dissolved solids) and the introduction of artificial nest boxes were likely the major contributing factors to the success of fertilized clutches (Ettling et al. 2013). At the time of the first successful breeding events, the artificial stream was outfitted with a four-

camera surveillance system that recorded hellbender activity around the clock. This video recorded a detailed view of hellbender reproductive behavior in captivity that could provide insights for future captive breeding efforts for this species. In this study, I provide analysis of the video recordings of the behavior of the hellbenders during the two successful sequential breeding events of 2012, culminating in kinematic diagrams of sequences of behavior that occur before, during, and between the successful reproductive events. Although there have been numerous anecdotal descriptions of reproductive events in the wild (Smith 1907; Huheey and Stupka 1967; Floyd and Unger 2016), there has not been a systematic ethological analysis of the steps involved in courtship and mating.

Successful fertilization of eggs is only one step in the recruitment event. Once the eggs are laid, males sit at the front of the nest, guarding the eggs until hatching (e.g., Bishop 1941). For many vertebrates, including salamanders, parental care is predominantly by the female (Wells 2007). In contrast, all extant salamander species within the family Cryptobranchidae (*Andrias japonicas*, *Andrias davidianus*, *Cryptobranchus alleganiensis*) engage in paternal care of their offspring (Smith 1907; Tago 1927; Nussbaum 1985; Cogger 1999).

Males stay with the eggs until hatching (Browne et al. 2014), but field observations of paternal care by hellbenders are limited primarily to observations that males protect the eggs from predators and provide increased oxygen flow by rocking movements of their bodies (Smith 1907; Bishop 1941). More detailed information is available for paternal care in another cryptobranchid, the Japanese giant salamander, *Andrias japonicas*. Okada et al. (2015) quantified paternal care behavior of two *A.*

japonicus, in a naturally-occurring nest, and observed several additional behaviors including tail-fanning and agitation of the eggs and consumption of dead or fungal-infected eggs.

The camera arrangement in the artificial streams did not allow for detailed observations of the male at the nest box. However, I was also provided access to video footage collected from a naturally-occurring Ozark hellbender nest during the 2008 breeding season by a biologist with MDC (Jeff Briggler, personal communication). The nest was located in the North Fork of the White River and was guarded by a single male hellbender with a fertilized clutch of eggs. The data quantified from this video represent the first systematic behavioral data collected from a naturally-occurring hellbender nest. The specific goals of the study were to determine: (1) the specific nest-related behaviors that occurred at the nest and the frequency with which they occurred, (2) whether the hellbender foraged from the nest entrance, and, if so, the identity of the prey, (3) the percentage of time that the male spent at the nest versus away from the nest, (4) the percentage of time that the male spent at the nest entrance versus inside the nest, and (5) the identity of fish species (potential prey or egg predators) that were visible near the nest and their behavior with respect to the nest.

METHODS

Part I—Reproduction in an Artificial Stream at the Saint Louis Zoo

Indoor Artificial Stream. The Ron Goellner Center for Hellbender Conservation (RGCHC) is home to an indoor artificial stream (9.7 m × 1.7 m × 0.6 m; Figure 1) containing 5 male and 3 female adult Ozark hellbenders serving as broodstock. The hellbenders were collected from the North Fork of the White River (NFWR; Ozark County, Missouri; Table 1). As described in Ettlting et al. (2013), water flows through the stream in a circular direction at 227 L/min, with an average depth of 0.3 m. The stream is a closed recirculating system consisting of filters (e.g., biological, sand, etc.) and ultraviolet sterilization. Reconstituted reverse osmosis water was used for water changes. Year-round photoperiods, water temperature, water quality and precipitation events were selected to mimic values that occurred in natural habitats in the river of origin. Each day, a chiller was used to manually adjust temperatures to match data recorded by data loggers in the river of origin; annual temperatures ranged from 4.4°C to 22.2°C. Total dissolved solids were also kept similar to natural river water at 175–300 mg/L because related characteristics, such as salinity and osmolality, can influence sperm motility in some aquatic species (Alavi and Cosson 2006; Bonislawska et al. 2015). Data for other measures of water quality (pH, nitrates, nitrites, ammonia, phosphates, dissolved oxygen) are provided by Ettlting et al. (2013). A manual sprinkler system plus adjustment of water levels was used to mimic natural precipitation, and photoperiods were adjusted daily via an automatic timer. The floor of the artificial stream was covered with river gravel (10.2—15.2 cm), and a variety of large (approximately 0.2—0.7 m) sandstone and moss-

covered rocks were scattered over the gravel. Crayfish (*Orconectes* spp., *Procambarus* spp.), darters (*Etheostoma* spp.), sculpins (*Cottus bairdi*, *Cottus carolinae*), and shiners (*Notropis* spp.) were collected from various streams near the St. Louis area and introduced into the stream as a source of natural forage. See Ettlting et al. (2013) for additional details on the artificial stream and hellbender maintenance.

Artificial nest boxes (n = 6) were positioned in the stream (Figure 1) with the open end of the boxes facing in the direction of the flow. As described in Briggler and Ackerson (2012), nest boxes were constructed with a chicken-wire base frame covered with hardware cloth and a concrete/sand mixture. Nest boxes were a modified “boot” design, with an entrance tunnel (“leg” of the boot; $\sim 27 \times 7.3 \times 10$ cm) connected to a nesting chamber (“foot” of the boot, $\sim 39 \times 31$ cm). An opening with a removable lid was made on the surface of the nesting chamber so that eggs deposited inside the chamber could be monitored periodically with minimal disturbance.

A four-camera infrared video recording system positioned directly above the stream monitored the hellbenders between 20:00—08:00 hr daily. Video recordings were archived to computer hard drives at the SLZ.

Behavioral Sequence Data Collection and Analysis. Our analysis is based on video data collected from 21—26 September 2012, during which time two oviposition events occurred (Ettlting et al. 2013). I quantified the behavior of the hellbenders on the day before the first oviposition event to illustrate “Pre-oviposition behavior”. I only quantified Pre-oviposition behavior on this one day so that the time frame would be the same as for the other events; qualitative, the behavior on this day was similar to the behavior on the preceding three days. Oviposition Event A occurred on 22 September

2012 and Oviposition Event B occurred on 24 September 2012. The day between the two oviposition events (23 September 2012) was quantified as “Inter-oviposition” behavior. Post-oviposition behavior was quantified for two days following the last oviposition event. Each day’s video was viewed in its entirety using Milestone XProtect® Smart Client 2013 R2 – Player v. 8.1b. I modified an ethogram that had been developed by C. Schuette (personal communication) based on her initial observations of hellbenders in the enclosure of the SLZ (Table 2). Behaviors were categorized as “agonistic”, “solitary/locomotory”, “sexual”, or “social.” I recorded every occurrence of any of the defined behaviors, the location of the behavior (camera number, nest box number, etc.), and, when possible, the sex of individual. An individual’s sex was identified based on physical features unique to that individual, and these features were not always visible on the video.

I defined a behavioral sequence as beginning when one or multiple individuals performed any of the defined behaviors (Table 2) and ending when the hellbender(s) was/were inactive for a period of 5 min or when the individual(s) entered a next box or other cover object (i.e. natural rock). I calculated transitional probabilities (the probability that one behavioral pattern follows another) through the use of transition matrices (Martin and Bateson 2007). The columns and rows of the matrix consist of all behavior patterns, and the numbers in a cell are the percentage of times that the first behavioral pattern (columns) is followed by the second behavioral pattern (rows). I illustrated the transition probabilities using kinematic graphs (flow diagrams) (Lehner 1979). Separate transitional matrices and kinematic diagrams were made for the periods

of Pre-oviposition, Oviposition Event A, Inter-oviposition, Oviposition Event B, and Post-oviposition.

As an example, consider the following scenario: Hellbender 1 (1) Walked out of a nest box onto a rock. He (2) Approached and (3) Bit Hellbender 2, while Hellbender 2 (4) Fled. In this example, the behavioral sequence would be recorded as, “(1) Walk, (2) Approach, (3) Bite, (4) Flee.” If Hellbender 1 (1) Walked, rested for 5 or more min, (1) Walked, (2) Approached Hellbender 2, (3) Bit Hellbender 2 and caused Hellbender 2 to (4) Flee, then two separate sequences would be recorded. The first sequence would be identified as a single “Walk” with no subsequent behaviors to follow. The second sequence would be recorded as, “(1) Walk, (2) Approach, (3) Bite, (4) Flee.”

Part II—Paternal Care of a Natural Nest in the North Fork of the White River

Nest Characteristics. The nest was located within a bedrock crevice in the North Fork of the White River, and oriented perpendicular to stream flow (Figure 2A, 2B). To protect the nest site from potential disturbance, specific location information will not be disclosed. The nest contained a fertilized clutch of eggs (estimate: 2—5 d old) guarded by a single male (mass = 310 g, total length = 40 cm, snout-vent length = 26.5 cm). An infrared underwater video camera (Aqua-Vu, 2007; approximately 28 cm from nest entrance) was modified to connect to a VCR and a monitor on land. Once the camera angle was adjusted to the best viewing angle, the monitor was removed. To decrease the possibility of vandalism or other disturbance, the VCR recorder was locked in a camouflaged metal container hidden several meters up the bank, and the cord was buried in gravel. A field team from the Missouri Department of Conservation changed tapes and

batteries, generally every 1–2 days, with occasional longer gaps.

Video Collection. Video data were collected between 8 October and 15 November of the 2008 breeding season. Videos were recorded using 24-, 40-, or 64-hr recording modes (Table 3). Total length of the tapes (Table 3) varied depending on recording mode and the battery life of the camera. Variation of recording modes was due to practical considerations of when a researcher would be available to replace the spent tape and recharged battery. Faster recording modes allowed for collection of the most data per videotape but resulted in lower quality images. Conversely, the slowest mode (24-hr) recorded the highest quality images but the least amount of data per videotape. Visibility on some of the tapes was also limited due to insufficient light or obstruction of the lens by floating debris; the percentage of viewable footage per tape ranged from 0 to virtually 100% ($\bar{x} \pm SD = 53.6\% \pm 30.98$; Table 3).

Data Collection. Variables recorded in the study were: occurrence of specific behaviors (strikes at prey, tail-fanning, rocking), location of male (at nest versus away; at nest entrance versus inside nest), and observations of fishes (could be either potential egg predators or hellbender prey) near nest entrance.

For each “strike at prey”, I recorded: (1) the type of potential prey item (crayfish or fish, with species identified where possible), and (2) whether the strike was successful. Striking events were totaled at the end of each video.

The duration of the hellbender tail-fanning and rocking was measured for each video. Tail-fanning was defined as the hellbender moving his tail in a rhythmic, lateral motion over fertilized eggs (Browne et al. 2014, Okada et al. 2014). Rocking was defined as a side to side motion of the hellbender’s body when he was otherwise stationary within

the nest (Okada et al. 2014). For tail-fanning and rocking behaviors, the start time, end time, and duration of each bout was recorded. For each tape, the percent time spent in each behavior was calculated as the time spent in each behavior divided by the viewable footage of that tape.

Percent time spent in nest occupancy was calculated as the time spent away from the nest divided by the total observable footage for each tape. The measured duration away from the nest began when the hellbender was observed to swim completely out of the frame and ended when he re-entered the frame.

Nest entrance behavior was defined as proportion of nest occupancy in which the hellbender was visibly present at the entrance of the nest (Figure 3-A). For each video, cumulative time in nest entrance behavior was divided by the total duration spent at the nest to calculate the proportion of time spent guarding at the nest's entrance.

Each fish that entered the frame was identified to family, and species was recorded when possible. The orientation of each fish with respect to the nest was categorized as toward the nest (T), away from the nest (A), or lateral to the nest (L) (Figure 3-B). Orientation is a possible indicator of interest in the nest, with orientation toward the nest indicating interest in the nest, orientation away from the nest indicating low/no interest, and orientation lateral to the nest being considered as neutral (neither interested nor uninterested). To minimize the possibility of continually recording the presence of the same individual, once a fish was identified, I did not record the presence of any other fish of the same species for 2 min. This time period was chosen because individuals did not appear to remain around the nest for longer than 2 min (personal observations). When a fish oriented in more than one direction during the 2 min window

only the initial direction was used for the statistical analysis.

Data Analyses. The occurrence of tail-fanning, rocking, and nest guarding behaviors were variable over time, and data are depicted graphically to illustrate patterns. Percent time spent at the nest entrance appeared to decline linearly, and so these data were analyzed with a Spearman's Correlation Rank Test (RStudio v. 0.98.1091).

Four families of fishes were identified (Centrarchidae, Cyprinidae, Percidae, or Ictaluridae). Due to low expected values in some cells within the transitional matrices (see Appendix A), fishes were categorized as either Centrarchids or Non-Centrarchids for analysis of orientations. A chi-square test (Minitab v. 16.1.0) was used to determine whether the frequency of orientations (toward, lateral, away) differed between the two fish taxa.

Table 1. Broodstock hellbender collection information.

| Collection Date | Sex | Snout-Vent Length (cm) | Mass (g) |
|-----------------|-----|------------------------|----------|
| 2004 | F | 32.0 | 713 |
| 2004 | M | 25.5 | 444 |
| 2004 | M | 34.0 | 596 |
| 2005 | F | 33.0 | 907 |
| 2005 | F | 36.0 | 995 |
| 2007 | M | 30.0 | 569 |
| 2007 | M | 33.0 | 815 |
| 2011 | M | 29.5 | 552 |

Table 2. Ethogram of behaviors recorded during video observations.

| Behavior | Description |
|---------------------|--|
| Agonistic | |
| Bite | One hellbender bites or snaps at another |
| Charge | A hellbender swims toward another at a noticeably increased swimming speed |
| Chase | One hellbender follows another |
| Flee | An individual quickly swims away from another (flight) |
| Solitary/Locomotory | |
| Surface | Hellbender contacts surface with any part of body |
| Swim | Wave-like movements of the tail propel the body forward, and limbs are not in contact with the substrate |
| Walk | Hellbender moves forward while limbs are in contact with substrate |
| Sexual | |
| Oviposition | Female deposits eggs |
| Circle | Hellbender swims in tight circle near another who may or may not perform circling at the same time |
| Tail Swish | Male swishes tail laterally while stationary |
| Social | |
| Approach | One hellbender moves to within 0.5 m of another without changing swimming speed |
| Nose-to-nose | Individuals touch or nearly touch their noses while stationary |

Table 3. Characteristics of the 14 videotapes that were recorded. The date range refers to the dates the tapes were inserted and removed. Length includes the total length of each recorded video. Recording mode specifies the recording setting for each video. The viewable footage refers to the recorded time that included unobstructed footage from which the data were collected.

| Video number | Date Range (2008) | Length h:min:sec | Recording Mode | Viewable Footage h:min:sec |
|---------------------|--------------------------|-------------------------|-----------------------|-----------------------------------|
| 1 | 8 Oct—9 Oct | 5:20:13 | 24-hr | 5:14:19 |
| 2 | 9 Oct—10 Oct | 8:11:39 | 24-hr | 8:11:21 |
| 3 | 10 Oct—12 Oct | 8:10:16 | 40-hr | 8:05:14 |
| 4 | 12 Oct—13 Oct | 7:28:39 | 24-hr | 3:43:49 |
| 5 | 13 Oct—14 Oct | 6:23:12 | 40-hr | 3:49:30 |
| 6 | 17 Oct—20 Oct | 7:51:35 | 64-hr | 0:00:00 |
| 7 | 20 Oct—3 Nov | 2:26:39 | 40-hr | 1:22:54 |
| 8 | 3 Nov—4 Nov | 3:51:14 | 40-hr | 1:13:20 |
| 9 | 4 Nov—6 Nov | 2:40:40 | 40-hr | 1:09:09 |
| 10 | 6 Nov—8 Nov | 5:37:39 | 64-hr | 4:37:59 |
| 11 | 8 Nov—11 Nov | 3:45:55 | 64-hr | 1:27:22 |
| 12 | 11 Nov—13 Nov | 5:40:59 | 64-hr | 1:11:05 |
| 13 | 13 Nov—14 Nov | 2:11:39 | 64-hr | 0:45:30 |
| 14 | 15 Nov—16 Nov | 2:18:37 | 64-hr | 0:49:15 |

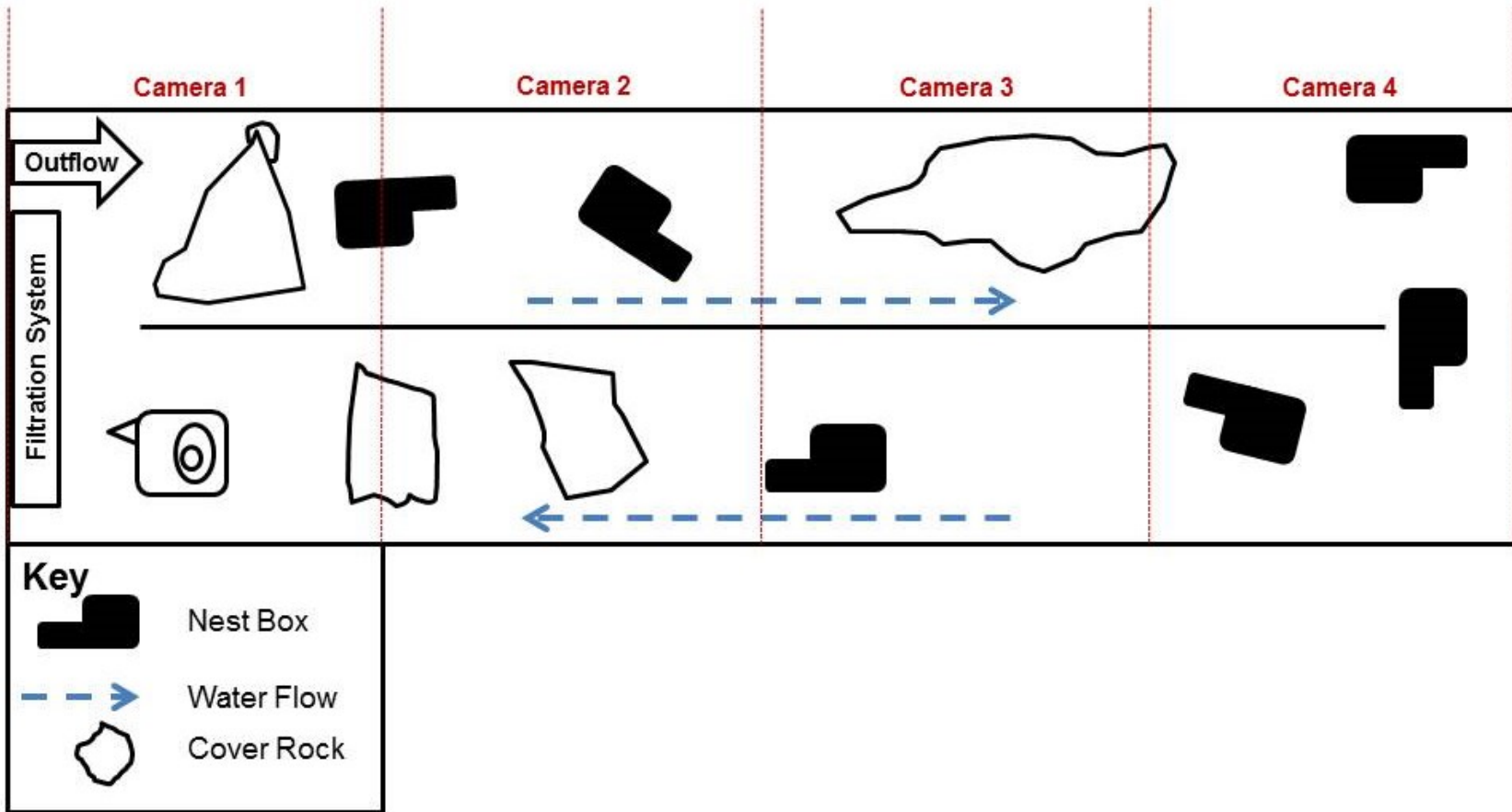


Figure 1. Diagram of the artificial indoor stream, including nest box and cover rock locations, at the Saint Louis Zoo Herpetarium,

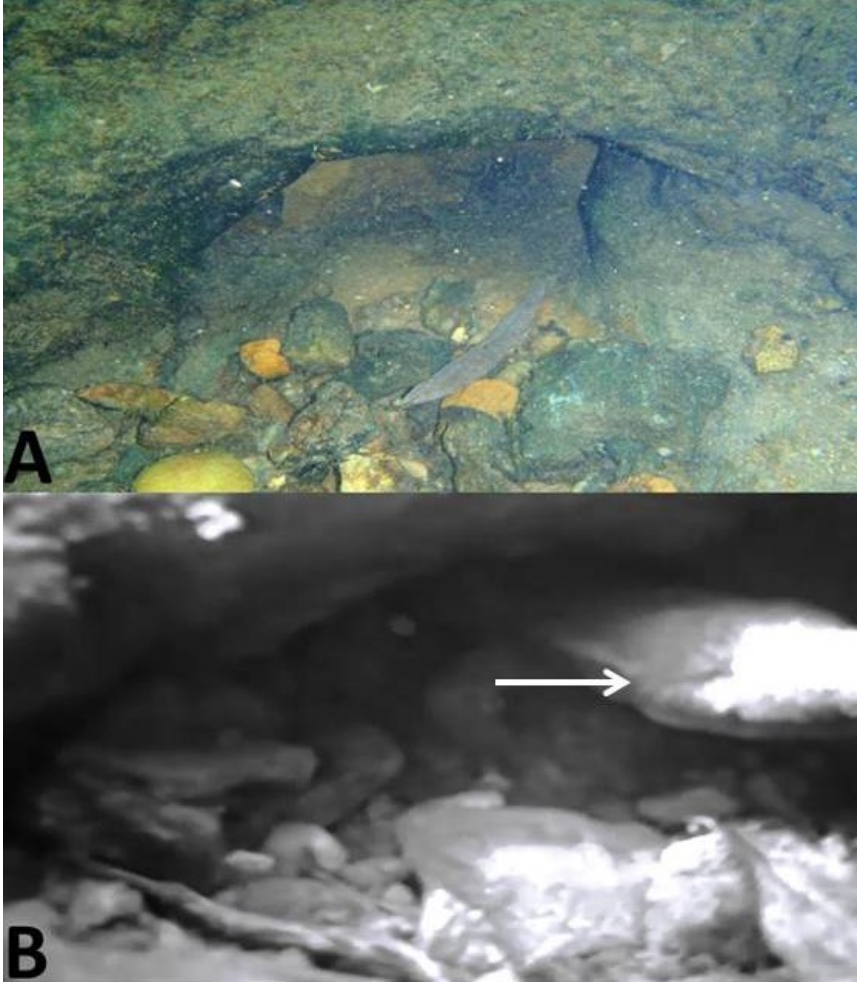


Figure 2. A: View of the entrance of the nest recorded in the study; photo by Jeff Briggler. B: Video screenshot of the nest's entrance with the hellbender's head (arrow) protruding from the nest.

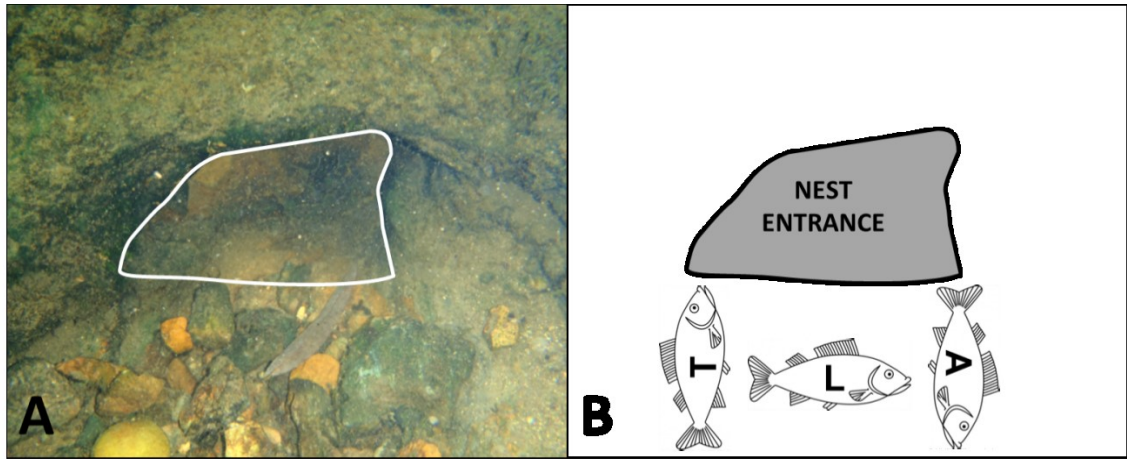


Figure 3. A: The entrance to the nest, as outlined in white, was 18 cm wide and approximately 8 cm tall. B: Arrows indicate possible orientations of fish to the nest as either Toward (T), Lateral (L), or Away (A) from the nest entrance.

RESULTS

Part I—Reproduction in an Artificial Stream at the Saint Louis Zoo

The transitional matrices used to construct kinematic diagrams are located in Appendix A.

On the day before oviposition, most behaviors were Solitary/locomotory (Figure 4). Almost all (97%) of these locomotory movements were categorized as Walking and the rest were Swimming movements (Figure 4). Only one agonistic sequence was recorded during the Pre-oviposition period. This sequence was initiated by a hellbender Approaching another hellbender and Biting it, resulting in the bitten hellbender Fleeing. No sexual behaviors (Tail swish, Circle) were observed during the quantified Pre-oviposition period (21 September 2012) or during the videos for 18—20 September 2012.

During Oviposition Event A (22 September 2012), the overall level of activity and the diversity of behavior increased substantially to include all behavioral categories (agonistic, solitary/locomotory, reproductive and social). Solitary/Locomotory behavior continued to be the most frequently performed behaviors, but, in comparison to the previous night, the frequency of Walking decreased by about 50%, from 103 to 54 instances, and Swimming behavior increased by a factor of 8 (from 3 to 25 instances). Agonistic behavior also increased in frequency, with Biting occurring 5 times, Fleeing occurring 3 times, and the first occurrences of Chasing. Surfacing behavior was also observed for the first time during this event (4 times).

Behavioral sequences were highly variable (Figure 5), although a few patterns are apparent. Surfacing events only followed the high-activity behaviors of Swimming and

Chasing. Although Bites sometimes (20%) led to Circling behavior, Circling led to only lower intensity activities, including more Circling (20%), Swimming (40%) or Walking (40). Flight resulted from either Bites or Chases. Approach led to the most intense interactions of Biting (38%), Nose-to-nose (25%) and Oviposition (25%).

The two oviposition behaviors during Oviposition Event A occurred as follows. After two females Approached a nest box, one female Bit the other, and the bitten hellbender Fled away from the nest box while being chased. The female that initiated the bite then slowly entered the nest box (~ 2:00 hr). She stayed inside the nest box for approximately 120 minutes and exited without any indication of coercion by the male. After approximately 90 minutes, the second female Approached and entered the nest box (~ 6:00 hr) and stayed inside the nest box until the video stopped recording (8:00 hr). The male did not leave the nest box after oviposition occurred.

During the Inter-oviposition period (23 September 2012), the highest frequencies of locomotory behaviors during the entire data collection period were recorded and more interactions between hellbenders were observed, including 30 sequences that were initiated by approaches (Figure 6). Walking initiated behavioral sequences 330 times, while Swimming initiated sequences 110 times (Figure 6). Agonistic behaviors also occurred at the highest frequencies during the Inter-oviposition period, with Biting occurring 20 times, Fleeing occurring 23 times, Charging occurring twice, and Chasing occurring 12 times. Charging always led to Walking, and Biting always led to Fleeing behavior. Tail swishing and Circling were the only sexual behaviors to occur, and both occurred at low frequencies. Tail swishing led to walking 100% of the time.

Oviposition Event B occurred on 24 September 2012. Although locomotory behaviors were not as frequent as during the Inter-oviposition period, Walking, Swimming, and Surfacing were still prevalent at a higher rate than in Oviposition Event A (Figure 7). Agonistic sequences occurred, but at a lower frequency than the previous day and similar to that during Oviposition Event A. Behavioral transition sequences were variable, with some similar patterns as observed during Oviposition Event A. With one exception, Surfacing events only followed the high-activity behaviors of Fleeing and Chasing. Flight continued to result from only Bites or Chases. The most intense interactions of Biting and Oviposition followed from Approach behavior. In general, sexual behavioral transitions were less complex than those occurring in Oviposition Event A; neither Circling nor Nose-to-nose behaviors were observed during this Oviposition event.

The oviposition activity during Oviposition Event B was less complex than Oviposition Event A. The remaining non-spent female approached a separate nest box that was occupied by a different male than the male that fertilized both clutches in Oviposition Event A. After Approach, the sequence of behavior by the female was: Walk, Walk, Walk, Swim, Surface, Walk, Swim, Swim, Walk, Walk, Walk, Approach, Walk, Approach, Oviposition (6:00 hr). The female stayed within the nest box for approximately 65 min and slowly exited after Oviposition. The male remained within the nest box, and so I could not observe his behavior.

The Post-oviposition period began on 25 September 2012 and ended on 26 September 2012. This period was characterized by an abrupt decrease in frequency of all behaviors, with only Solitary/Locomotory behaviors exhibited (Figures 8, 9). On the first

day Post-oviposition, Walking was the only behavior exhibited, and it occurred only four times (Figure 8). On the second day Post-oviposition, 14 instances of Walking and one of Swimming occurred (Figure 9).

Part II—Paternal Care of a Natural Nest on the North Fork of the White River

Hellbender Behaviors. A total of eight strikes at prey were observed overall (Table 4). The success rate of the hellbender foraging at the nest was 37.5%, with three successful strikes at fishes of the family Cyprinidae. The unsuccessful bites were directed toward crayfish (4) and small cyprinid fishes (1). Most bites ($n = 6$) occurred at the beginning of the season (first three videos), and there were no bites at prey observed between the third and tenth videos. Additionally, two bites were made at unidentifiable prey items, and the hellbender consumed seven of his eggs within the view of the camera.

Overall, tail-fanning was generally more frequent than rocking (Figure 10). Tail-fanning occupied at least 40% of the time observed at the nest entrance on 5/13 tapes whereas rocking peaked at about 20%. Both behaviors were highly variable, with some of the variability likely due to relatively short periods of viewable footage available on some tapes. When limiting the analysis to tapes with only at least 1.5 hr of viewable footage (Figure 11), it appears that rocking behavior peaked during the first week and then declined to a consistently low rate for the rest of the period, whereas tail-fanning reached a peak of approximately 60% on tapes 3—5 before also declining to a low level.

Nest Occupancy. Overall, the male occupied the nest for approximately 98.4% of the observable footage. Over the span of 38 days, the hellbender was recorded leaving

the nest 11 times, with the nest left unguarded for an average of 270 s (SD = 294.85, Range = 61—1111 s) per trip.

Nest Entrance Behavior. At the beginning of the recording period, the hellbender spent approximately 90% of the video exposed at the front of the nest (Figure 12), but this percentage declined to about 20—40% by the end of the study. There was a significant negative correlation between the percent of time spent in nest entrance behavior and date ($r_s = -0.780$, $P < 0.003$, Figure 12). There also was a significant positive correlation between the number of fish observations per video and the amount of time the hellbender spent at the entrance in each video ($r_s = 0.709$, $P < 0.007$, Figure 13). Overall, the hellbender spent 55% of his time at the nest guarding at the nest entrance.

Fish Observations. The total number of fishes observed in all of the videos was 56 (Table 5). Of the 56 observations, 68% of the observations were of the family Centrarchidae. Identified centrarchid species included *Lepomis megalottis*, *Micropterus* sp., *Pomoxis nigromaculatus*, and *Lepomis macrochirus*. The next most common family was Cyprinidae (23%). Because of their small size and the low image-quality of the videos, species of cyprinids were difficult to identify. In several of the observations, the species appeared to closely resemble striped shiners (*Luxilus chrysocephalus*) or perhaps hybrids of striped shiners and other shiner species (Pflieger 1997). Fishes in the families Ictaluridae and Percidae were relatively infrequent in the observations. The two observations of ictalurids were catfish (likely bullhead catfish, *Ameiurus* sp., based on MDC fish census data; Appendix B). Observed percids were two log perch (*Percina caprodes*) and one darter (*Etheostoma* sp.).

Fishes were oriented Toward the nest in 37 of the 56 observations, with the remainder being almost evenly split between Lateral and Away orientations (Table 5). Centrarchids were significantly more likely to be oriented Toward the nest than non-centrarchids ($\chi^2_{2, 56} = 13.136, P = 0.001$; Table 6). One centrarchid was observed to consume a single egg that had slowly drifted from the nest near the end of Video 4. Because the video recordings ended before the eggs hatched, there is a possibility that more fish consumed eggs after the videos ended.

Table 4. Strikes at prey by a nest-guarding hellbender, including time of the strike on each videotape, whether the strike was successful, and prey type.

| Video | Time of strike h:min:sec | Successful (Yes or No) | Prey type |
|--------------|-------------------------------------|-----------------------------------|------------------|
| 1 | 0:42:35 | No | Crayfish |
| 1 | 2:31:59 | Yes | Cyprinid Fish |
| 1 | 4:27:06 | No | Cyprinid Fish |
| 1 | 4:27:15 | Yes | Cyprinid Fish |
| 3 | 1:27:17 | Yes | Cyprinid Fish |
| 3 | 6:32:17 | No | Crayfish |
| 10 | 0:22:44 | No | Crayfish |
| 10 | 1:47:53 | No | Crayfish |
| 2, 3, 4 | variable | Yes | Eggs (7) |
| 2, 10 | variable | No | Unidentified (2) |

Table 5. Number of fish from four families observed at the nest over the course of the study, and their respective orientations with respect to the nest.

| Family | Orientation | | | Total |
|---------------|--------------------|----------------|-------------|--------------|
| | Toward | Lateral | Away | |
| Centrarchidae | 31 | 3 | 4 | 38 |
| Cyprinidae | 6 | 3 | 4 | 13 |
| Percidae | 0 | 2 | 1 | 3 |
| Ictaluridae | 0 | 2 | 0 | 2 |
| Total | 37 | 10 | 9 | 56 |

Table 6. Number of Centrarchid (Centrarchidae) and Non-Centrarchid (Cyprinidae, Percidae, Ictaluridae) fish that were oriented Toward, Lateral to, or Away from the nest.

| Group | Toward | Lateral | Away | Total |
|------------------|---------------|----------------|-------------|--------------|
| Centrarchid | 31 | 3 | 4 | 38 |
| Non- Centrarchid | 6 | 7 | 5 | 18 |
| Total | 37 | 10 | 9 | 56 |

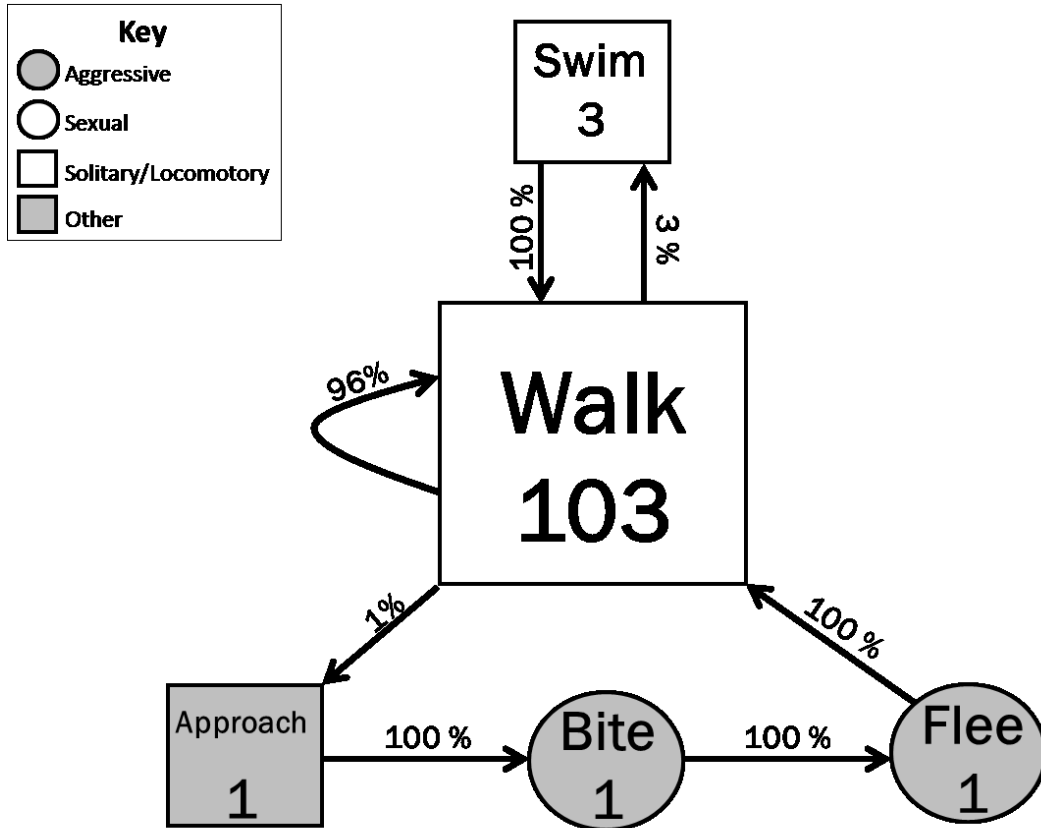


Figure 4. Behavioral transitions during the Pre-oviposition period (21 September 2012). Frequency of behavioral actions (inside boxes) and % time initiated action was followed (arrow) by another action. Shape sizes are indicators the frequency in which the initiating behavior occurred (e.g., larger boxes indicate the behavior occurred more often than smaller boxes).

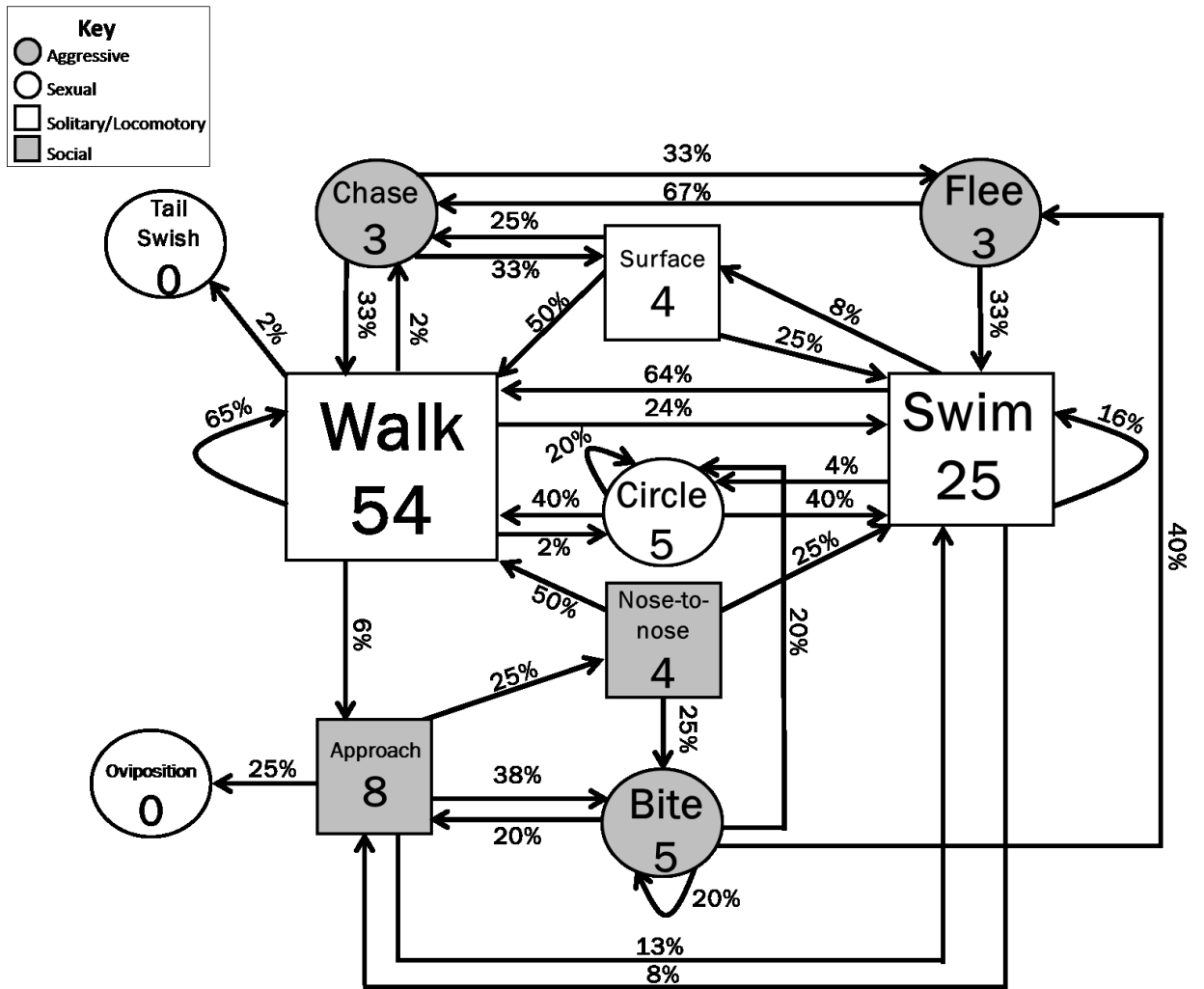


Figure 5. Behavioral transitions during Oviposition Event A (22 September 2012), which included two instances of oviposition by different females in the same nest box. Frequency of behavioral actions (inside boxes) and % time initiated action was followed (arrow) by another action. Shape sizes are indicators the frequency in which the initiating behavior occurred (e.g., larger boxes indicate the behavior occurred more often than smaller boxes). Zeros indicate that the behavior did not initiate a sequence within the 5-min designated time frame.

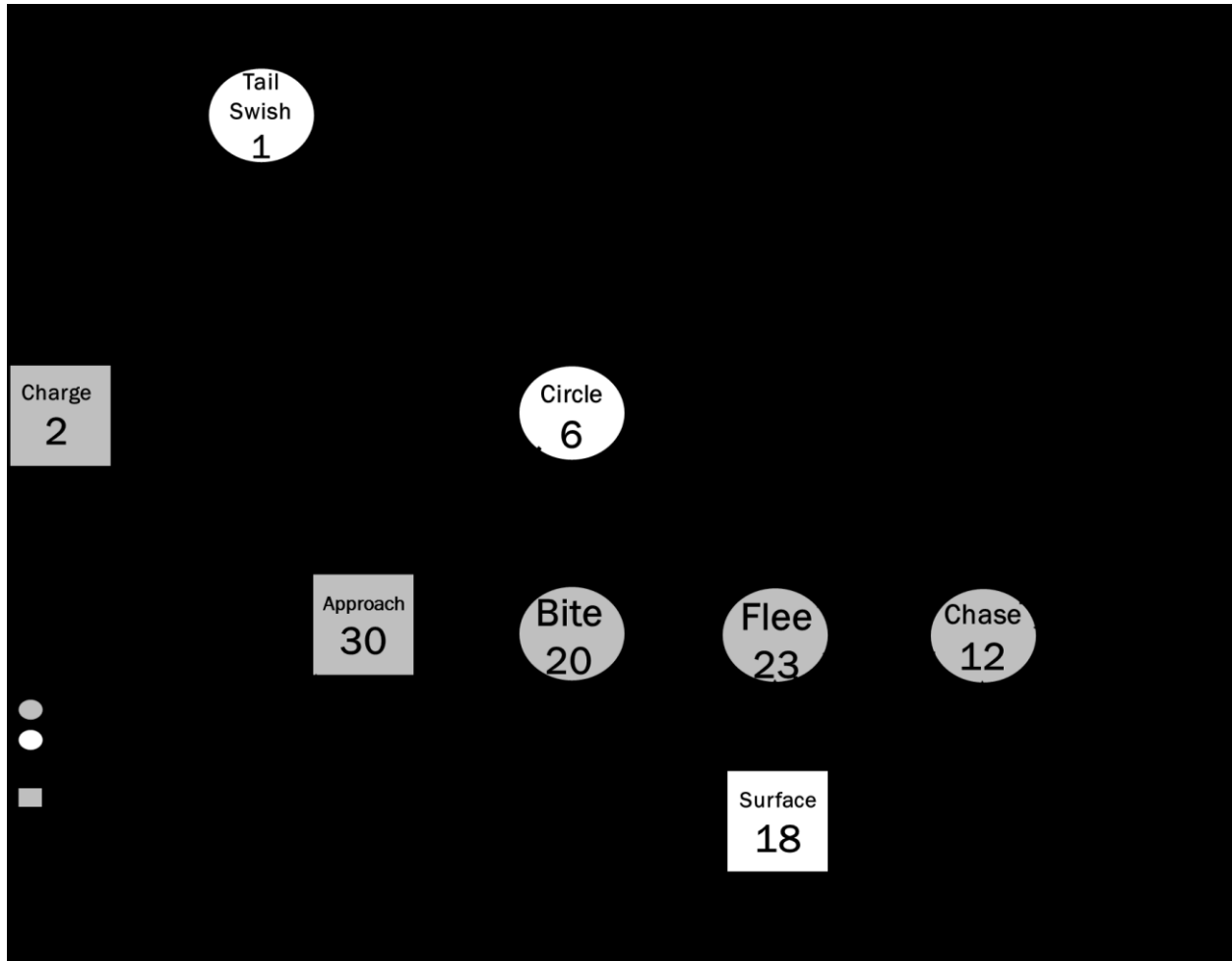


Figure 6. Behavioral transitions during the Inter-oviposition period (23 September 2012). Frequency of behavioral actions (inside boxes) and % time initiated action was followed (arrow) by another action. Shape sizes are indicators the frequency in which the initiating behavior occurred (e.g., larger boxes indicate the behavior occurred more often than smaller boxes).

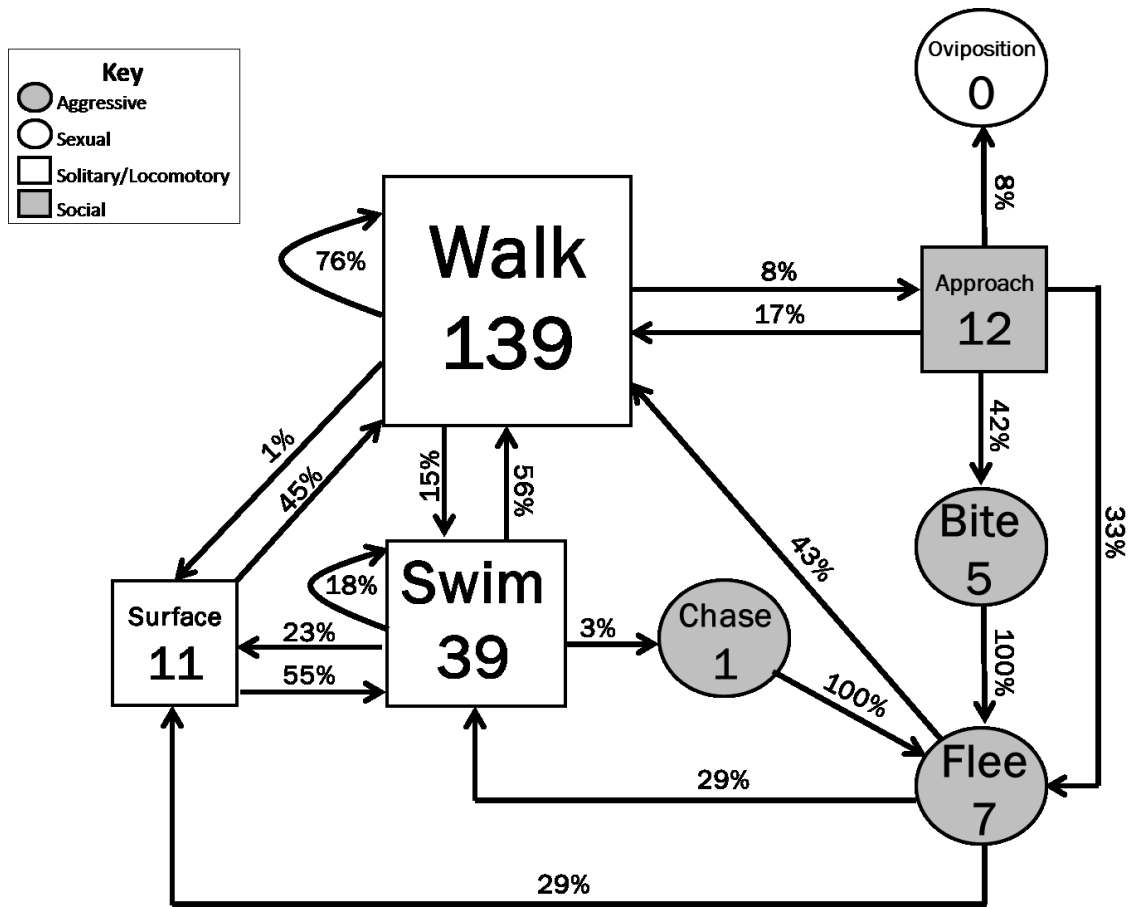


Figure 7. Behavioral transitions during Oviposition Event B (24 September 2012). A third female deposited eggs that were fertilized by the same male that fertilized both clutches in Oviposition Event A. Frequency of behavioral actions (inside boxes) and % time initiated action was followed (arrow) by another action. Shape sizes are indicators the frequency in which the initiating behavior occurred (e.g., larger boxes indicate the behavior occurred more often than smaller boxes). Zeros indicate that the behavior did not initiate a sequence within the 5-min designated time frame.

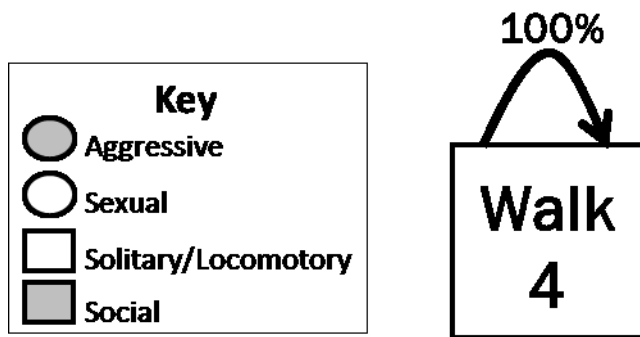


Figure 8. Behavioral transitions during Post-oviposition. Walking was the only behavior performed on 25 September 2012. Frequency of behavioral actions (inside box) and % time initiated action was followed (arrow) by another action.

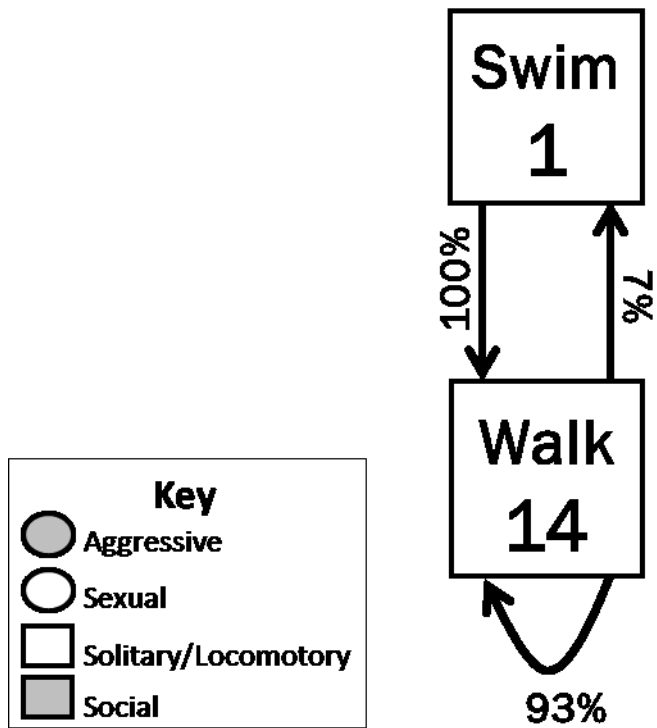


Figure 9. Behavioral transitions during Post-oviposition. Walking and Swimming occurred on 26 September 2012. Frequency of behavioral actions (inside box) and % time initiated action was followed (arrow) by another action.

Rocking and Tail Fanning Behavior

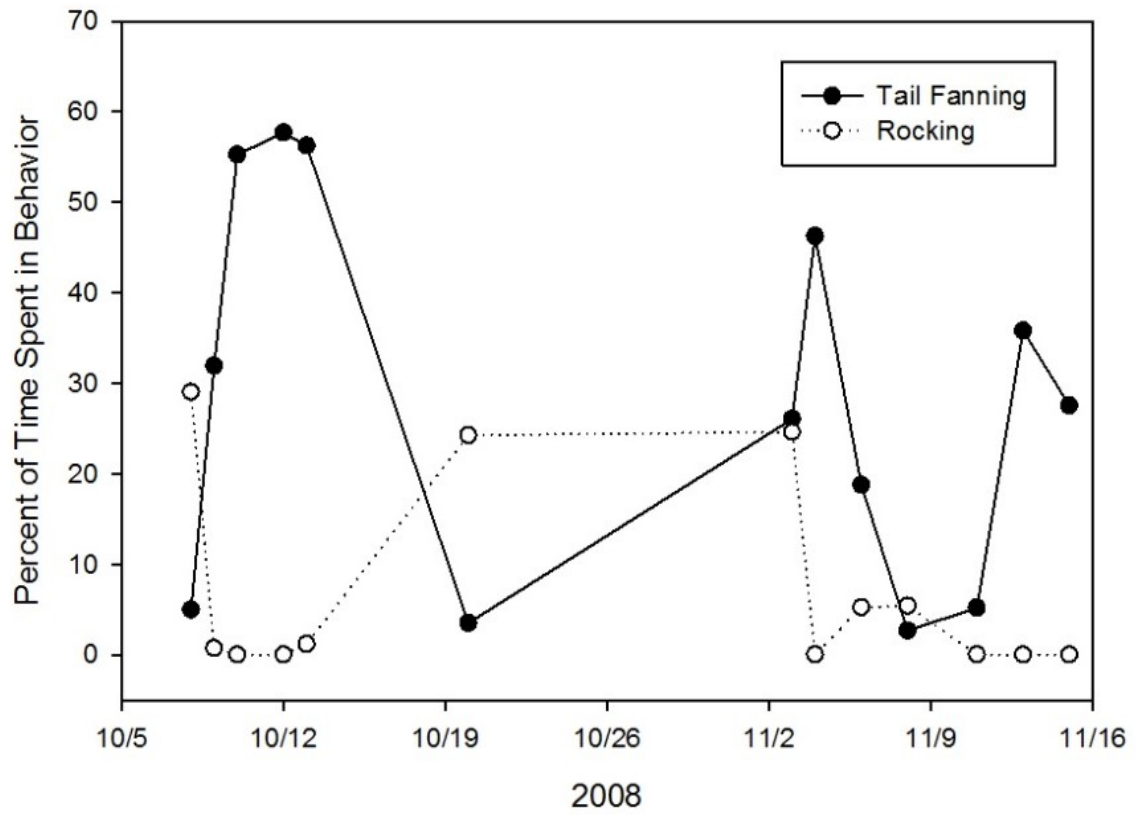


Figure 10. The proportion of observable time that a nest-guarding hellbender spent rocking and tail-fanning between 5 October and 16 November of 2008.

Videos with 1.5 Hours or More Viewable Footage

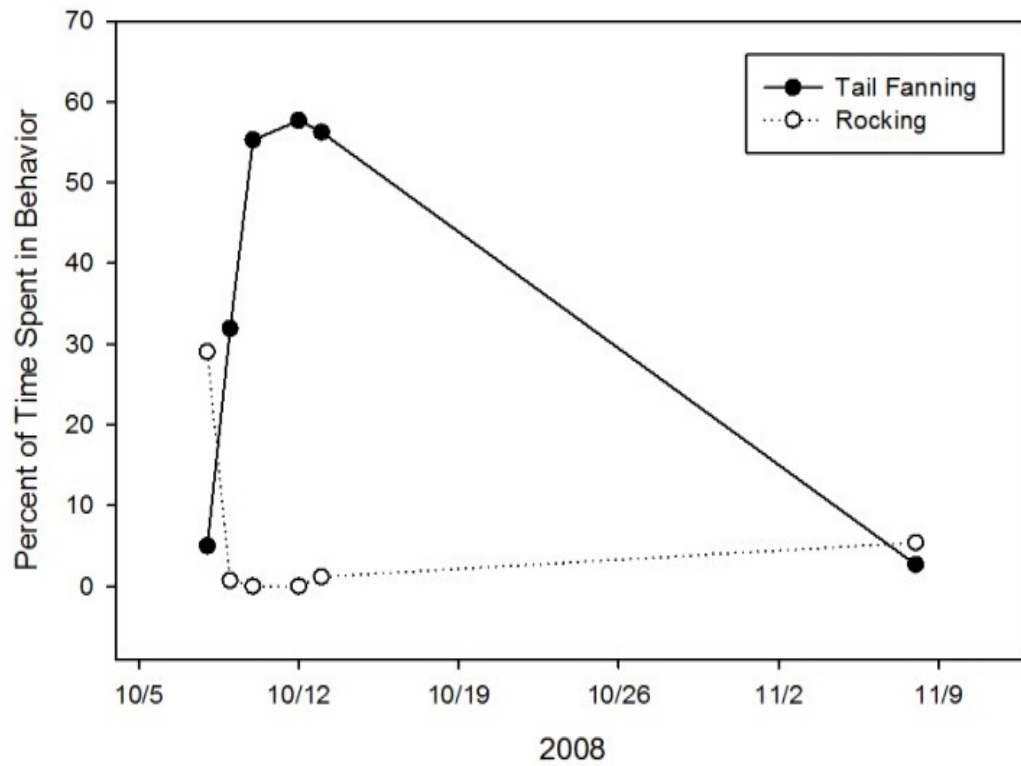


Figure 11. Proportion of observable time that the nest-guarding male spent in rocking (body moving from side to side) and tail-fanning (rhythmic, lateral movement of tail over eggs) between 5 October and 16 November of 2008 in videos with 1.5 hours or more of viewable footage. Videos represented are 1, 2, 3, 4, 5, and 10.

Nest Entrance Behavior

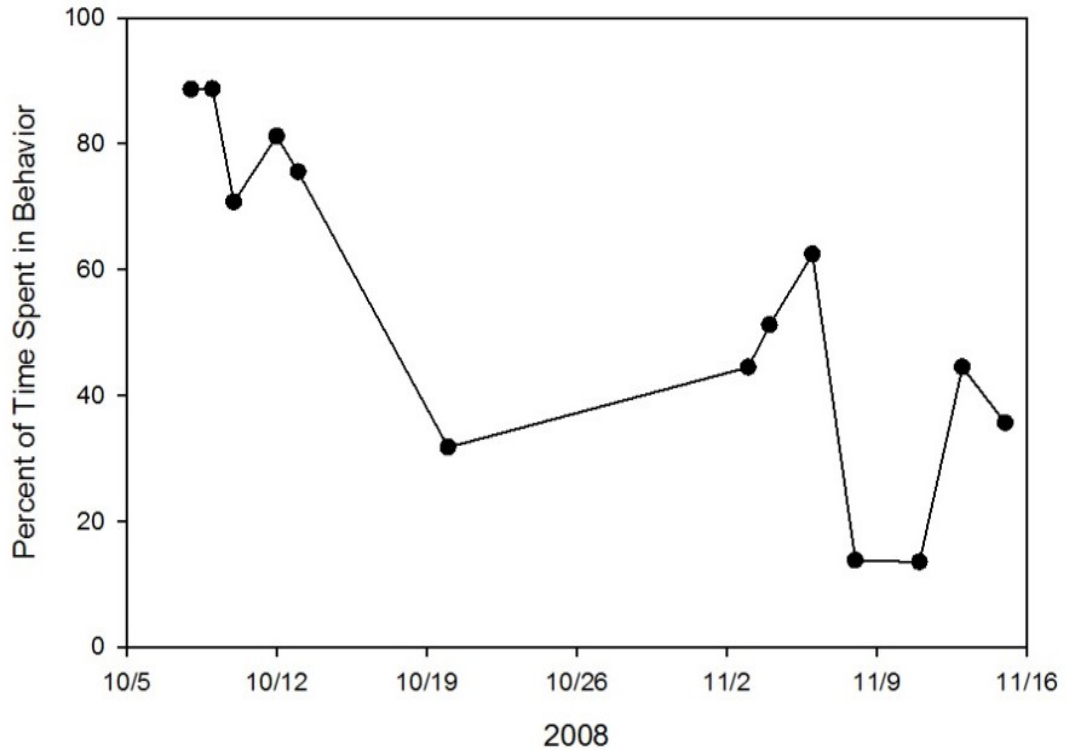


Figure 12. Percent of observed nest-occupancy time that the male hellbender was visible at the nest entrance.

Correlation Analysis of Nest Entrance Behavior and Fish Observations

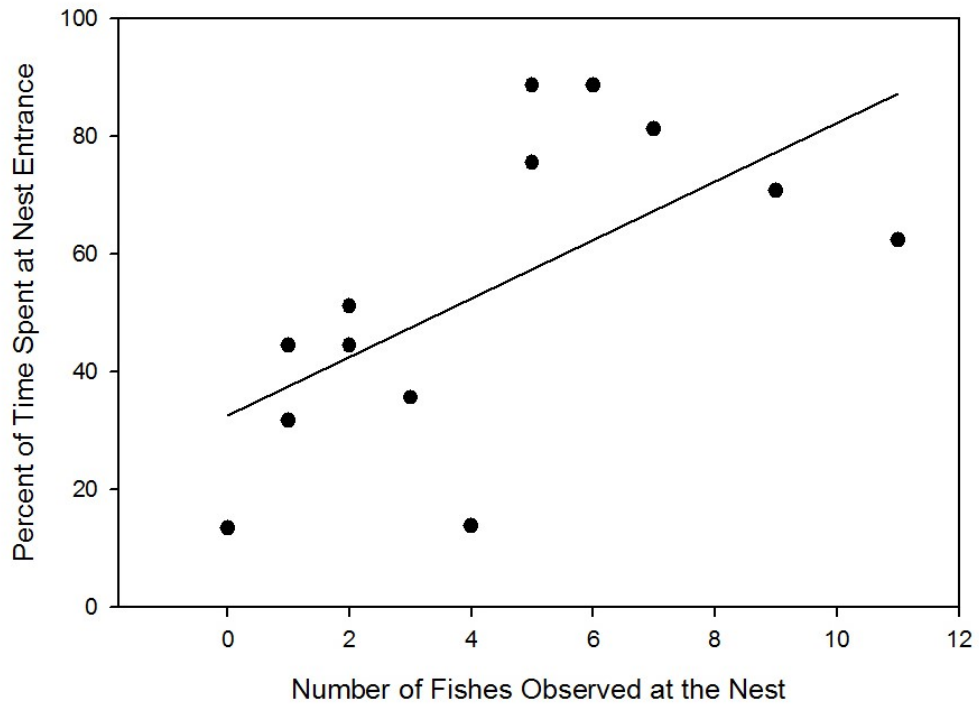


Figure 13. Percent of time spent at the nest entrance as a function of the number of fishes observed at the nest.

DISCUSSION

Courtship and Oviposition in Captivity at the Saint Louis Zoo

Descriptions of the reproductive behavior of hellbenders is limited both in natural habitats due to their secretive nature and in captivity, where the first successful reproductive event occurred relatively recently (Ettling et al. 2013). This study provides the first quantitative ethological analysis of the behavior of hellbenders immediately prior to and during an oviposition event. These data collected from video recordings of the captive reproductive events reported in Ettling et al.'s (2013) study, help to fill in the details of sequences of behavior previously reported in anecdotal observations, with both similarities and differences.

As reported in numerous previous studies (Smith 1907, Peterson 1988, Bishop 1941), males began defending nesting sites prior to the oviposition period. In nature, males typically defend a “den” site consisting of a depression located under a flat cover rock, or within crevices or holes in the bedrock (Bishop 1941, Pflingsten and Downs 1989). Although flat rocks were available, the hellbenders in our study defended only the nest boxes.

Hellbenders were generally most active during 1:00—7:00 hr, and oviposition occurred 2:00—7:00 hr. My findings are consistent with other studies that suggest aggression increases during the breeding season (Smith 1907; Peterson 1988; Foster et al. 2009). Although my data span a limited period, the increase in aggression was abrupt, with increases in both number and type of overt acts. The day before oviposition included only three agonistic acts (1 each of approach, bite and flee), whereas the day of

oviposition included 38 acts of six overt behaviors (chase, flee, nose-to-nose, approach, circle, bite). Locomotory activity also became more intense, with swimming (as opposed to walking) comprising only 3% of Pre-oviposition locomotory movements, increasing to 28% on the day of oviposition. It is not known whether the observed increase in aggression and movement intensity is as abrupt in the field or whether the very low level of aggression and movement intensity on the day before oviposition is typical of a more extended Pre-oviposition period. In any case, I recommend that managers of captive breeding facilities carefully monitor hellbenders for increased aggression and swimming activity as a possible indicator of imminent oviposition. Both locomotory activity and agonistic behavior remained high through the second oviposition event, and were particularly pronounced during the day between the oviposition events.

Most anecdotal field observations of aggression and the apparent territorial spacing of males in the field suggest that aggression has three primary functions: male-to-male competition for breeding sites (Alexander 1927, Hillis and Bellis 1971, Nickerson and Mays 1973), (2) male attempts to coerce females to enter or leave their nest sites or (3) male attempts to protect their eggs from oophagy (Smith 1907). However, the aggressive acts that I observed in the artificial streams were mostly female-female, with females apparently competing to occupy the oviposition sites. Female-female aggression associated with reproduction may be more common than previously thought; few overt aggressive acts have been observed in the field, and the contestants are rarely definitively identified with respect to sex (e.g., Nickerson and Mays 1973). Alternatively, female-female aggression could be a result of the specific conditions/densities within the artificial stream.

It is possible that the dramatic reduction of population sizes of Ozark hellbenders in recent decades (e.g., Wheeler et al. 2003) has also resulted in alterations in the frequency or intensity of aggressive behavior in natural habitats. For example, limitation of available receptive females or fertile males (see Unger and Mathis 2013) may have resulted in more intense competition. Alternatively, lower population densities may have led to an overall reduction in aggressive encounters. The latter seems unlikely since fresh wounds, in at least some cases resembling conspecific bite marks, have been reported in post-decline (~ early 1980's: Wheeler et al. 2003) populations (Pfungsten 1990; Wheeler et al. 2003; Miller and Miller 2005; Williams and Groves 2014).

In addition to documentation of the amount and type of aggressive behavior that occurred during each period, the kinematic analysis also allows for inferences about whether there are consistent transitions from one behavior to the next. Although variability of transitions was high, some general patterns were apparent from the data. Not surprisingly, Flight (rapid swimming away from another hellbender) was typically the result of being bitten or chased. Both Swimming and Chasing appear to be energetically costly because they were frequently followed by surfacing behavior. Circling behavior, which occurred 11 times, has been reported during courtship in a taxonomically wide range of salamanders (e.g., Plethodontidae: Cupp 1971; Salamandridae: Bruni and Romano 2010), frequently leading to oviposition. However, in my observations, circling consistently led only to locomotory behavior or more circling. Other physical interactions (bite, nose-to-nose, and oviposition) were immediately preceded simply by Approach, where one hellbender swam toward the other without changing speed (as opposed to the relatively rare Charge, which was characterized by

increased speed). Tail Swishing by the male was observed by the male on only two occasions and so may not play as strong a role as the tail undulations that are a part of courtship of some other salamander taxa (Houck and Arnold 2003). Females have been reported to lay eggs in the same nest in the wild (Nickerson and Mays 1973), and this study confirms that this behavior can also happen under captive-rearing conditions.

Natural Nest Guarding in the North Fork of the White River

Although nest guarding by male hellbenders has been documented in numerous reports (e.g., Smith 1907, Bishop 1941, Nickerson and Mays 1973, Larson et al. 2013, Unger and Mathis 2013), very few details are available, and no long-term systematic analyses have been reported. Nest occupancy by the male during the 38 day observation period was remarkably high. The hellbender rarely left the nest unguarded, with the average time spent away from the nest being less than 5 min (maximum of 18.5 min). The proportion of the time at the nest that the male spent with his head at the front of the nest varied throughout the season, with the highest percentage (about 90%) at the beginning of the season, declining to about 20–40% over the next 5–6 wks. The threat of egg predation by other hellbenders appears to be particularly high during and shortly after the oviposition period when eggs trailing from the females' cloacae or floating in the water may draw attention to the nests (e.g., Smith 1907, Peterson 1988). Although I did not observe the male interact with other hellbenders, aggressive defense of eggs by guarding males against other hellbenders has been described (Smith 1907, Bishop 1941), and eggs have been found in the stomachs of both males and females during this period (Smith 1907, Peterson 1985). Therefore, I infer that one function of the male's position

at the nest opening is vigilance against potential egg predators. The hellbenders presence at the nest opening was positively correlated with the presence of fishes, which could be either due to vigilance against nest predators or attraction to potential prey (see below).

The video data confirm that male hellbenders are opportunistic foragers while at the nest entrance, with eight apparent foraging attempts observed. However, only about a third of attacks were successful. All of the strikes were directed toward either crayfish or cyprinid fishes, which is in agreement with the results of numerous studies of hellbender diets (Smith 1907; Netting 1929; Nickerson and Mays 1973; Nickerson et al. 1983; Peterson 1985; Peterson et al. 1989). Although, crayfish (*Orconectus* sp.) are generally cited as the primary prey type (Smith 1907; Alexander 1927; Netting 1929; Swanson 1948; Nickerson and Mays 1973; Wiggs 1976), no strikes at crayfish were successful. Our limited data suggest that the prevalence of crayfish in hellbender diets may be due to their abundance in the habitat rather than their ease of capture. It should be noted, however, that crayfish are predators of salamander eggs (e.g., Gamradt and Kats 1996), and so strikes at crayfish may be a result of nest defense rather than foraging per se.

I observed seven instances of egg predation by the guarding male. Smith (1907) suggested that male hellbenders consume their own eggs because of their value as food. However, in some other species of salamanders, egg consumption by guarding individuals has been observe to be removal of fungal-infected (nonviable) eggs from the nest (e.g., Tilley 1972; Okada et al. 2015), a behavior that Okada et al. (2015) termed hygienic filial cannibalism. This behavior may contribute to hatching success by decreasing the likelihood of fungal infections spreading to healthy, non-contaminated eggs. I could not determine whether the eggs that were consumed were healthy.

Tail-fanning and rocking behaviors were performed in relatively high frequencies, with tail-fanning being performed up to 60% of the time that the male was at the nest entrance. These behaviors were also reported for guarding males of the Japanese Giant Salamander, *Andrias japonicus* (Okada et al. 2015, Takahashi et al. 2016). Tail-fanning by brooding *A. japonicus* (Okada et al. 2015) and rocking by nonbrooding hellbenders (Harlan and Wilkinson 1981) was higher when oxygen concentrations were low, suggesting that these behaviors function to increase dissolved oxygen (DO) levels to both the hellbender and the eggs. Low DO levels have been linked to poor survival of fish embryos (Hale et al. 2003). Egg agitation could provide more DO to the developing embryos and enhance the hatching success. When egg agitation was initiated in rearing developing eggs at the RGCHC in the absence of a guarding male, hatching success increased substantially (Chawna Schuette, personal communication).

Centrarchids comprised over half of the total observations of fishes near the nest during the recording period. Based on their frequent orientation toward the nest, centrarchids appeared to have a higher level of interest in the nest than the other fish species. Some centrarchids are known to engage in nest-raiding behavior (Pflieger 1997) so their primary role may be as potential egg predators rather than as potential prey. Reviews of diet studies generally do not list centrarchids as prey (e.g., Nickerson and Mays 1973, Petranka 1998).

For the remaining fish taxa (Cyprinidae, Ictaluridae, Percidae) there was no consistent orientation patterns with respect to the nest, with individuals being oriented toward, lateral, and away from the nest in similar frequencies. In at least some members of these families, fishes are known to raid the nest of other fish and consume eggs (Page

1983; Walsh and Burr 1985; Fletcher 1993; Pflieger 1997), and at least some species can consume salamander eggs despite their large membranes (Drake et al. 2014). However, their vulnerability to predation from the defending male may have deterred them from showing strong interest in the nest.

Further Implications

Paternal care of eggs in salamanders is relatively rare, occurring in only the Cryptobranchidae, Hynobiidae, and Sirenidae (Nussbaum 2003; Reinhard et al. 2013). Based on both the energetic costs and risks of injury in securing a territory (e.g., Hopkins and DuRant 2011, Miller and Miller 2005) and the low rate of foraging success during the egg guarding period (this study), I infer that parental care is costly for male hellbenders. Males and females both clearly benefit from increased survival of eggs via decreased predation from other hellbenders (e.g., Smith 1907) and predatory fishes (this study). I hypothesize that male-male aggression associated with the onset of the breeding season may also play a role in mate selection by females. Success during male-male contests prior to courtship could be an indicator of an individual's ability to protect developing eggs from both intraspecific and interspecific egg predation. Choosey females would benefit by selecting males that are likely to be better at protecting her eggs. My observations of female-female aggression for priority access to a guarding male support the hypothesis that females are competitive, at least in this captive situation. Females also may prefer to lay their eggs in nests of males that have already proved successful, as reported by Bishop (1941) and as seen in our observations at SLZ, which may increase the benefits for successful territorial males. The dramatic population

declines observed for Ozark hellbenders (e.g. Wheeler et al. 2003) could substantially impact the costs and benefits associated with territoriality and egg guarding in hellbenders, but the type of effects that may result are not clear. Smith (1907) noted congregations of up to twelve individuals during the breeding season, with substantial male-male aggression associated with securing of territories by males. Such aggregations are likely to be much smaller currently. Potentially a proportionally larger number of high quality nesting sites than were available in the past might lead to less male-male aggression for spawning sites and more direct competition for females. With this constraint, should female hellbenders be less likely to discriminate against poorer quality males (e.g. a male in any condition would be better than not choosing a male at all)? Will females be selected to be more competitive for the few high quality males? Although I observed female-female aggression, female aggression is not documented in the literature; however, a possible explanation may be because of the difficulty of determining sex of specific individuals in the field when reproductive activity is high. It is likely that decreasing population sizes will affect the evolution of courtship and paternal care in hellbenders, but specific predictions require additional data on shifting costs and benefits for both males and females.

REFERENCES

- Adams, M.J., D.A. Miller, E. Muths, P.S. Corn, E.H.C. Grant, L.L. Bailey, G.M. Fellers, R.N. Fisher, W.J. Sadinski, H. Waddle, and S.C. Walls. 2013. Trends in amphibian occupancy in the United States. *PLoS One* 8:e64347. doi: 10.1371/journal.pone.0064347.
- Alavi, S.M.H., and J. Cosson. 2006. Sperm motility in fishes: (II) Effects of ions and osmolality. *Cell Biology International* 30:1–14.
- Alexander, W.P. 1927. The Allegheny hellbender and its habitat. *Buffalo Society of Natural Science* 7:13–18.
- Bernstein, H. 1953. Structural modifications of the amphibian skin. *Proceedings of the Pennsylvania Academy of Science* 27:204–211.
- Bishop, S.C. 1941. The salamanders of New York. *New York State Museum Bulletin* 243:1–365.
- Bodinof, C.M., J.T. Briggler, R.E. Junge, J. Beringer, M.D. Wanner, C.D. Schuette, J. Ettlign, R.A. Gitzen, and J.J. Millspaugh. 2012. Postrelease movements of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). *Herpetologica* 68:160–173.
- Bonislawska, M., J. Szulc, and K. Formicki. The effect of water salinity on the motility of spermatozoa of the brook trout, *Salvelinus fontinalis* (Actinopterygii: Salmoniformes: Salmonidae). *Acta Ichthyologica et Piscatoria* 45:143.
- Briggler, J.T. 2016a. Hellbender propagation efforts. Missouri Department of Conservation, Jefferson City, Missouri. *Science Notes* 11:3.
- Briggler, J.T. 2016b. Hellbender recovery actions. Missouri Department of Conservation, Jefferson City, Missouri. *Science Notes* 11:3.
- Briggler, J.T., and Ackerson, J.R. 2012. Construction and use of artificial shelters to supplement habitat for hellbenders (*Cryptobranchus alleganiensis*). *Herpetological Review* 43:412.
- Browne, R.K., H. Li, Z. Wang, S. Okada, P. Hime, A. McMillan, M. Wu, R. Diaz, D. McGinnity, and J.T. Briggler. 2013. The giant salamanders (Cryptobranchidae): Part B. Biogeography, ecology and reproduction. *Amphibian and Reptile Conservation* 5:30–50.
- Bruner, H.L. 1914. Jacobson's organ and the respiratory mechanism of amphibians. *Morphologisches Jahrbuch* 48:63–82.

- Bruni, G., and A. Romano. 2011. Courtship behaviour, mating season and male sexual interference in *Salamandrina perspicillata* (Savi, 1821). *Amphibia-Reptilia* 32:63–76.
- Burgmeier, N.G., S.D. Unger, T.M. Sutton, and R.N. Williams. 2011. Population status of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. *Journal of Herpetology* 45:195–201.
- Cogger, H. 1999. *Reptiles and Amphibians*. Time Life Books, London, UK.
- Cooke, A.S. 1981. Tadpoles as indicators of harmful levels of pollution in the field. *Environmental Pollution Series A, Ecological and Biological* 25:123–133.
- Crowhurst, R.S., K.M. Faries, J. Collantes, J.T. Briggler, J.B. Koppelman, and L.S. Eggert. 2011. Genetic relationships of hellbenders in the Ozark highlands of Missouri and conservation implications for the Ozark subspecies (*Cryptobranchus alleganiensis bishopi*). *Conservation Genetics* 12 637–646.
- Cupp, P.V., Jr. Fall courtship of the green salamander, *Aneides aeneus*. *Herpetologica* 1971:308–310.
- Drake, D.L., T.L. Anderson, L.M. Smith, K.M. Lohraff, and R.D. Semlitsch. 2014. Predation of eggs and recently hatched larvae of endemic Ringed Salamanders (*Ambystoma annulatum*) by native and introduced aquatic predators. *Herpetologica* 70:378–387.
- Ettling, J. A., M.D. Wanner, C.D. Schuette, S.L. Armstrong, A.S. Pedigo, and J.T. Briggler. 2013. Captive reproduction and husbandry of adult Ozark hellbenders *Cryptobranchus alleganiensis bishopi*. *Herpetological Review* 44:605–610.
- Feder, M. E., and W.W. Burggren. 1985. Cutaneous gas exchange in vertebrates: design, patterns, control and implications. *Biological Reviews* 60:1–45.
- Fletcher, D.E. 1993. Nest association of dusky shiners (*Notropis cummingsae*) and redbreast sunfish (*Lepomis auritus*), a potentially parasitic relationship. *Copeia* 1993:159–167.
- Floyd, T.M., and S. Unger. 2016. *Cryptobranchus alleganiensis* (Eastern Hellbender) reproductive behavior and habitat. *Natural History Note. Herpetological Review* 47:273–274.
- Foster, R.L., A.M. McMillan, and K.J. Roblee. 2009. Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State. *Journal of Herpetology* 43:579–588.

- Gamradt, S.C., and L.B. Kats. 1996. Effect of introduced crayfish and mosquitofish on California Newts. *Conservation Biology* 10:1155–1162.
- Guimond, R.W. 1970. Aerial and aquatic respiration in four species of paedomorphic salamanders: *Amphiuma means means*, *Cryptobranchus alleganiensis alleganiensis*, *Necturus maculosus maculosus*, and *Siren lacertian*. PhD Dissertation, University of Rhode Island, Kingston, Rhode Island, USA.
- Guimond, R.W., and V.H. Hutchison. 1973. Aquatic respiration: An unusual strategy in the hellbender *Cryptobranchus alleganiensis alleganiensis* (Daudin). *Science* 182:1263–1265.
- Hale, R.E., C.M. St. Mary, and K. Lindström. 2003. Parental responses to changes in costs and benefits along an environmental gradient. *Environmental Biology of Fishes* 67:107–116.
- Harlan, R.A., and R.F. Wilkinson. 1981. The effects of progressive hypoxia and rocking activity on blood oxygen tension for hellbenders, *Cryptobranchus alleganiensis*. *Journal of Herpetology* 15:383–388.
- Hillis, R.E., and E.D. Bellis. 1971. Some aspects of the ecology of the hellbender, *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. *Journal of Herpetology* 5:121–126.
- Hocking, D.J., and K.J. Babbitt. 2014. Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology* 9:1–17.
- Hopkins, W.A. and S.E. DuRant. 2011. Innate immunity and stress physiology of eastern hellbenders from two stream reaches with differing habitat quality. *General and Comparative Endocrinology* 174:2107–2115.
- Houck, L.D., and S.J. Arnold. 2003. Courtship and mating behavior. Pp. 383–424 *In* *Reproductive Biology and Phylogeny of Urodela*. Sever, D.M. (Ed.) Science Publishers, Inc., Enfield, New Hampshire, USA.
- Huheey, J.E. and A. Stupka. 1967. *Amphibians and reptiles of Great Smoky Mountains National Park*. University of Tennessee Press, Knoxville, Tennessee, USA.
- Humphries, W.J. 2007. Diurnal seasonal activity of *Cryptobranchus alleganiensis* (Hellbender) in North Carolina. *Southeastern Naturalist* 6:135–140.
- Larson, K.A., B.G. Gall, and J.T. Briggler. 2013. The use of gastric transmitters to locate nests and study movement patterns of breeding male Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) *Herpetological Review* 44:434–439.

- Lehner, P.N. 1998. Handbook of ethological methods. Cambridge University Press, Cambridge, UK.
- Martin, P., and P.P.G. Bateson. 2007. Measuring behaviour: an introductory guide. Cambridge University Press, Cambridge, UK.
- Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern hellbender status assessment report. Natural Resources Research Institute, Technical Report 9:1-41.
- Miller, B.T., and J.L. Miller. 2005. Prevalence of physical abnormalities in eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) populations of middle Tennessee. Southeastern Naturalist 4:513–520.
- Missouri Department of Conservation (MDC). 2016. Missouri species and communities of conservation concern checklist. Missouri Natural Heritage Program, Missouri Department of Conservation. Jefferson City, Missouri, USA.
- Netting, M.G. 1929. The food of the hellbender *Cryptobranchus alleganiensis* (Daudin). Copeia 1929:23–24.
- Nickerson, M.A., and C.E. Mays. 1973. The hellbenders: North American "giant salamanders". Milwaukee Public Museum Special Publications in Biology and Geology 1:1–106.
- Nickerson, M.A., R.E. Ashton Jr., and A.L. Braswell. 1983. Lampreys in the diet of hellbender *Cryptobranchus alleganiensis* (Daudin), and the Neuse River waterdog *Necturus lewisi* (Brimley). Herpetological Review 14:10.
- Noble, G.K. 1925. The integumentary, pulmonary, and cardiac modifications correlated with increased cutaneous respiration in the amphibia: A solution of the 'hairy frog' problem. Journal of Morphology 40:341–416.
- Nussbaum, R.A. 1985. The evolution of parental care in salamanders. Miscellaneous Publications of the Museum of Zoology 169:1–50.
- Nussbaum, R.A. 2003. Parental care. Pp. 527–612 *In* Reproductive Biology and Phylogeny of Urodela. Sever, D.M. (Ed.) Science Publishers, Inc., Enfield, New Hampshire, USA.
- Okada, S., Y. Fukuda, and M.K. Takahashi 2015. Paternal care behaviors of Japanese giant salamander *Andrias japonicus* in natural populations. Journal of Ethology 33: 1–7.
- Page, L.M. 1983. Handbook of darters. T. F. H. Publications, Inc., Neptune City, New Jersey, USA.

- Peterson, C.L. 1985. Comparative demography of four populations of the hellbender, *Cryptobranchus alleganiensis*, in the Ozarks. Ph.D Dissertation, University of Missouri, Columbia, Missouri, USA. 158 pp.
- Peterson, C.L. 1988. Breeding activities of the hellbender in Missouri. *Herpetological Review* 19:28–29.
- Peterson, C.L., J.W. Reed, and R.F. Wilkinson. 1989. Seasonal food habits of *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae). *The Southwestern Naturalist* 34:438–441.
- Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington D.C., USA.
- Pfingsten, R. A. 1990. The status and distribution of the hellbender, *Cryptobranchus alleganiensis*, in Ohio. *Herpetological Review* 21:48–51.
- Pfingsten, R.A., and F.L. Downs. 1989. Salamanders of Ohio. Ohio State University College of Biological Sciences, Columbus, Ohio, USA.
- Pflieger, W.L. 1997. The fishes of Missouri, revised edition. Missouri Department of Conservation. Jefferson City, Missouri, USA. 372 pp.
- Pollet, I., and L.I. Bendell-Young. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. *Environmental Toxicology and Chemistry* 19:2589–2597.
- Reinhard, S., S. Sebastian, and A. Kupfer. 2013. External fertilization and paternal care in the paedomorphic salamander *Siren intermedia* Barnes, 1826 (Urodela: Sirenidae). *Zoologischer Anzeiger* 253:1–5.
- Smith, B.G. 1907. The life history and habits of *Cryptobranchus allegheniensis*. *The Biological Bulletin* 13:5–39.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
- Swanson, P.L. 1948. Note on the amphibians of Vernango County, Pennsylvania. *American Midland Naturalist* 40:362–371.
- Tago, K. 1927. Notes on the habits and life history of *Megalobatrachus japonicus*. *Congrès International de Zoologie, tenu à Budapest 1927*:828–838.

- Takahashi, M.K., S. Okada, and Y. Fukuda. 2016. From embryos to larvae: seven month-long paternal care by male Japanese giant salamander. *Journal of Zoology* 2016. doi:10.1111/jzo.12433.
- Tilley, S.G. 1972. Aspects of parental care and embryonic development in *Desmognathus ochrophaeus*. *Copeia* 1972:532–540 .
- Tonione, M., J.R. Johnson, and E.J. Routman. 2011. Microsatellite analysis supports mitochondrial phylogeography of the hellbender (*Cryptobranchus alleganiensis*). *Genetica* 139:209–219.
- U.S. Fish and Wildlife Service (USFWS). 2011. Endangered and threatened wildlife and plants; endangered status for the Ozark hellbender salamander. *Federal Register* 76:50. CFR Part 17.
- Unger, S.D. and A. Mathis. Larval growth and the potential for head-starting of Eastern and Ozark hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C. a. bishopi*). *Herpetological Review* 44:547–550.
- Walsh, S.J., and B.M. Burr. 1985. Biology of stonecat, *Noturus flavus* (Siluriformes: Ictaluridae), in central Illinois and Missouri streams, and comparisons with Great Lakes populations and congeners. *The Ohio Journal of Science* 85:85–96.
- Wells, K.D. 2007. Complex life cycles and the ecology of amphibian metamorphosis. Pp. 599–644 *In* The ecology and behavior of amphibians. University of Chicago Press, Chicago, Illinois, USA.
- Welsh, H.H., and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California’s redwoods. *Ecological Applications* 8:1118–1132.
- Wheeler, B. A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: a 20+-year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation* 109:151–156.
- Whitford, W.G., and V.H. Hutchison. 1965. Gas exchange in salamanders. *Physiological Zoology* 38:228–242.
- Wiggs, J.N. 1976. Food habits, starvation, and growth in the hellbender (*Cryptobranchus alleganiensis*). M.A. Thesis, Southwest Missouri State University. Springfield, Missouri, USA. 39 p.
- Williams, L. A., and J.D. Groves. 2014. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in eastern hellbenders (*Cryptobranchus a. alleganiensis*) in western North Carolina, USA. *Herpetological Conservation and Biology* 9:454–467.

APPENDICES

Appendix A. Data Matrices.

Matrix 1. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during the Pre-oviposition period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 21 September 2012 Period: Pre-oviposition

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| BT | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| WK | 0 | 99 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 103 |
| SW | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| AP | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| FL | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| CS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SF | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| BT | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| WK | 0.00 | 0.96 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| SW | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| AP | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| FL | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SF | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Matrix 2. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during the Oviposition Event A period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 22 September 2012 Period: Oviposition Event A

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------------|
| BT | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 5 |
| WK | 0 | 35 | 13 | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 54 |
| SW | 0 | 16 | 4 | 2 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 25 |
| AP | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 8 |
| FL | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| CS | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SF | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| CL | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 5 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NN | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------------|
| BT | 0.20 | 0.00 | 0.00 | 0.20 | 0.40 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 1.00 |
| WK | 0.00 | 0.65 | 0.24 | 0.06 | 0.00 | 0.02 | 0.00 | 0.00 | 0.02 | 0.00 | 0.02 | 0.00 | 1.00 |
| SW | 0.00 | 0.64 | 0.16 | 0.08 | 0.00 | 0.00 | 0.00 | 0.08 | 0.04 | 0.00 | 0.00 | 0.00 | 1.00 |
| AP | 0.38 | 0.00 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.25 | 1.00 |
| FL | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CS | 0.00 | 0.33 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SF | 0.00 | 0.50 | 0.25 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CL | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 1.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NN | 0.25 | 0.50 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

Matrix 3. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during the Inter-oviposition period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 23 September 2012 Period: Inter-oviposition

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------------|
| BT | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| WK | 0 | 218 | 82 | 22 | 0 | 3 | 1 | 1 | 3 | 0 | 0 | 0 | 330 |
| SW | 0 | 76 | 20 | 2 | 0 | 0 | 1 | 9 | 2 | 0 | 0 | 0 | 110 |
| AP | 20 | 3 | 0 | 2 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| FL | 0 | 6 | 6 | 0 | 0 | 6 | 0 | 5 | 0 | 0 | 0 | 0 | 23 |
| CS | 0 | 7 | 1 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| CR | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| SF | 0 | 3 | 11 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 18 |
| CL | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--------------|
| BT | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| WK | 0.00 | 0.66 | 0.25 | 0.07 | 0.00 | 0.01 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 1.00 |
| SW | 0.00 | 0.69 | 0.18 | 0.02 | 0.00 | 0.00 | 0.01 | 0.08 | 0.02 | 0.00 | 0.00 | 0.00 | 1.00 |
| AP | 0.67 | 0.10 | 0.00 | 0.07 | 0.13 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| FL | 0.00 | 0.26 | 0.26 | 0.00 | 0.00 | 0.26 | 0.00 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CS | 0.00 | 0.58 | 0.08 | 0.00 | 0.17 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CR | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| SF | 0.00 | 0.17 | 0.61 | 0.06 | 0.00 | 0.06 | 0.00 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CL | 0.00 | 0.50 | 0.33 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TS | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| NN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Matrix 4. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during the Oviposition Event B period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 24 September 2012 Period: Oviposition Event B

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|----|-----|----|----|----|----|----|----|----|----|----|----|-------|
| BT | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| WK | 0 | 106 | 21 | 11 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 139 |
| SW | 0 | 22 | 7 | 0 | 0 | 1 | 0 | 9 | 0 | 0 | 0 | 0 | 39 |
| AP | 5 | 2 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 12 |
| FL | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 7 |
| CS | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SF | 0 | 5 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| CL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| BT | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| WK | 0.00 | 0.76 | 0.15 | 0.08 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| SW | 0.00 | 0.56 | 0.18 | 0.00 | 0.00 | 0.03 | 0.00 | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| AP | 0.42 | 0.17 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 1.00 |
| FL | 0.00 | 0.43 | 0.29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.29 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CS | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SF | 0.00 | 0.45 | 0.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| CL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Matrix 5. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during 25 September 2012 of the Post-oviposition period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 25 September 2012 Period: Post-oviposition

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| BT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WK | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| SW | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SF | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| BT | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| WK | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| SW | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| AP | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SF | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Matrix 6. Matrices used to count behavioral transitions (top) and their respective frequencies (bottom) during 26 September 2012 of the Post-oviposition period. The first column contains the initiating behavior and the first row identifies the behavior that followed the initiating behavior. Identified behaviors were Bite (BT), Walk (WK), Swim (SW), Approach (AP), Flee (FL), Chase (CS), Charge (CR), Surface (SF), Circle (CL), Oviposition (OV), Tail swish (TS), and Nose-to-nose (NN).

Date: 26 September 2012 Period: Post-oviposition

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| BT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| WK | 0 | 13 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| SW | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| AP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SF | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TS | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | BT | WK | SW | AP | FL | CS | CR | SF | CL | OV | TS | NN | Total |
|----|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| BT | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| WK | 0.00 | 0.93 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| SW | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| AP | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| FL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CR | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SF | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CL | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| OV | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Appendix B. Electrofishing Sampling Data. Data were collected by MDC Fisheries Biologists near the natural nest site in the North Fork of the White River. Samples were approximately 3 hours long and data are expressed as Catch per Unit Effort (fish per hour).

| Species | Catch per Unit Effort (Fish/Hour) | |
|---------------------|-----------------------------------|------|
| | 2008 | 2009 |
| Black Crappie | 0.4 | 0 |
| Black Redhorse | 28.5 | 48.7 |
| Chain Pickerel | 0 | 0.7 |
| Golden Redhorse | 5.9 | 8.0 |
| Largemouth Bass | 5.9 | 6.0 |
| Northern Hog Sucker | 18.1 | 26.7 |
| Ozark Bass | 86.7 | 50.0 |
| Rainbow Trout | 0.7 | 1.7 |
| Shorthead Redhorse | 4.1 | 1.7 |
| Smallmouth Bass | 20.0 | 21.3 |
| Spotted Bass | 0 | 2.7 |
| Yellow Bullhead | 6.3 | 0.7 |