Observations of reproduction in captivity by the Dougherty Plain Cave Crayfish, *Cambarus cryptodytes*, (Decapoda: Astacoidea: Cambaridae)

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Biologists have long been interested in organisms that live permanently below ground for a host of reasons, which include studies of extremophiles, evolutionary process, optic system and pigment degeneration, simplified food webs, and trophic dynamics. Subterranean organisms can be broken into simple non-taxonomic assemblages including stygobionts (obligate groundwater inhabitants) and troglobionts (obligate terrestrial cave inhabitants). One of the exciting aspects to this field of study includes the fact that many species living in caves could be described as "transitional" in that the morphology of a given species does not yet (and may never) represent the full suite of characters typically associated with permanent life below ground (e.g., loss of the visual system, loss of pigment, resistance to starvation, etc.) in a condition known as "troglomorphy." The study of stygobiotic organisms has advanced such that live specimens are more frequently being maintained in the laboratory for biological study and experimentation. Included in this group are stygobiotic crayfishes of eastern North America. While live specimens have been maintained in the laboratory for a number of reasons (e.g., waiting for males to change to Form 1 condition (reproductive readiness) for species identification) we are unaware of any previously documented captive breeding of a stygobiotic and troglomorphic crayfish species in the lab; however, there is a report of a stygobiotic and non-troglomorphic crayfish having been bred in captive conditions, *Procambarus milleri* (Radice and Loftus 1995). Here, we report on

successful reproduction in laboratory conditions of a stygobiotic and troglomorphic crayfish endemic to the Floridan Aquifer of Georgia and Florida.

The Dougherty Plain Cave Crayfish (also known as the Apalachicola Cave Crayfish), *Cambarus cryptodytes* Hobbs, 1941, inhabits portions of the Floridan Aquifer below Georgia and Florida. The species was described by Hobbs (1941) from a well in Jackson County, Florida, and is a troglomorphic species with reduced pigmentation, degenerate eyes, and elongate antennae and legs (**Figure 1**). The Dougherty Plain Cave Crayfish is often syntopic with the Georgia Blind Salamander, *Eurycea wallacei* (Carr, 1939), and can serve as a predator of the salamander (Sutton and Relyea 1971; Means 1992; Fenolio et al. 2013). Little is known of the reproductive biology or ecology of the Dougherty Plain Cave Crayfish; however, one exception was a paper by Opsahl and Chanton (2006) wherein they used stable isotope analysis to suggest that in remote corners of the Floridan Aquifer, with little surface input, chemosynthesis might fuel the base of the food web and ultimately provide energy (via food items) to *C. cryptodytes* and *Eurycea wallacei*. Reproductively ready males (Form I males) have been collected in September and October (Hobbs 1981). To our knowledge, no ovigerous females have been collected and there have been no successful attempts at captive reproduction. Our goal was to establish a laboratory facility where we could acclimate a small number of wild-collected adult Dougherty Plain Cave Crayfish and attempt reproduction in captivity.

Figure 1. The Dougherty Plain Cave Crayfish (*Cambarus cryptodytes*) inhabits the Floridan Aquifer below Florida and Georgia, USA. Photo by Danté B. Fenolio.

Previous success with the captive reproduction of groundwater-inhabiting crayfish

Radice and Loftus (1995) reported on the captive reproduction of the Miami Cave Crayfish, *Procambarus milleri* Hobbs, 1971 (**Figure 2**). This species still retains pigment, has functional eyes, and is more robust than most troglomorphic crayfish (Caine 1978). Reproductive output for the Miami Cave Crayfish is reported as "several dozen to over 100" for a given female's reproductive output (Radice and Loftus 1995). While this reproductive output is more common in surface crayfish species, it is higher than that reported for other stygobiotic crayfishes in the wild (45 eggs on average in *Orconectes inermis inermis*; Jegla 1969; Hobbs and Barr 1972).

Figure 2. The Miami Cave Crayfish (*Procambarus milleri*) is found in groundwater below the city of Miami, Florida. The species is the only stygobiotic crayfish known to breed in captivity. Note the presence of eyes and pigment, which render this species, in general, non-troglomorphic. Photo by Danté B. Fenolio.

Laboratory housing for cave crayfish

We assembled 24 aquaria (37.85 L) on two enamel coated stainless steel racks (**Figure 3**). The floor of each aquarium was drilled with a diamond drill bit, producing a 2.54-cm diameter hole, which was fit with a bulkhead fitting. A standpipe made from 2.54-cm diameter PVC pipe was placed into the bulkhead (inside each aquarium), rising to within 7.62 cm of the top of the aquarium. In this fashion, the standpipe is used as an overflow drain and maintains the water level below the top rim of each aquarium. The

aquaria were tiered with four per shelf. Each tier spills into the aquarium below until the bottom shelf drains into the central filter sump. A 265-L plastic filter sump was accommodated between the racks. Two stackable plastic crates were placed into the filter sump. These crates each hold limestone rocks from the locality at which the cave crayfish were collected. The rock serves as (1) a filter substrate for nitrifying bacteria, (2) a buffer to produce the appropriate pH and (3) a source for trace elements. An Iwaki® brand PM21 pump was used to pump water from the filter sump back to the top shelves of aquaria on each rack (**Figure 4**). Ball valves were added inline so that the return flow into each aquarium could be individually adjusted. With the system full of water and the water level within 15 cm of the top of the filter sump, the total volume of the system is approximately 960 L. We used a TECO® brand TC20 inline water chiller, maintaining the water at a constant 20°C (68°F)**.**

Figure 3. This is the laboratory housing for the set of Dougherty Plain Cave Crayfish that bred in captivity (without water in the system here). Note the three shelves, each with 4 37.85L aquaria. The top two shelves spill into the tanks below them through standpipes and bulkhead fittings. The bottom shelf drains through PVC pipe to the central filtration unit (blue tub). The white unit is the TECO® brand TC20 inline water chiller, maintaining the system at a constant 20°C. Photo by Danté D. Fenolio.

Establishing a breeding colony

Twelve Dougherty Plain Cave Crayfish (4 males, 8 females) were collected by cave divers in February 2012 (**Figures 5 & 6**) from four localities (two from each) in Jackson Co., Florida (Hole in the Wall, Jackson Blue, Black Hole, and Maunz Spring) and four more individuals from one locality in Dougherty Co., Georgia (Radium Spring). Numbers

were limited by the permits and out of concern regarding the collection of a species of potential conservation concern. Sizes of crayfish ranged 3–6 cm total length (tip of anterior point on carapace to end of telson). Crayfish were initially acclimated one individual per aquarium for several days. Each aquarium contained 10 PVC pipe segments (2.54 cm diameter and 8 cm in length) as cover. No other objects were included in aquaria. Each individual was sexed and labeled. A variety of foods were offered to crayfish including: San Francisco Bay Brand® frozen bloodworms ("red mosquito larvae") and glassworms ("white mosquito larvae"), and Bio-Pure® brand frozen *Daphnia* and frozen brine shrimp. New Life Spectrum® brand "small fish formula" sinking pellet food was also offered. All food items were accepted by the crayfish which were offered food every three days. Subsequently, live "black worms" (*Tubifex tubifex*) were purchased from a commercial dealer and were accepted as a food item. We provided the various food items in an attempt to diversify the diet and provided these different items in equal proportions.

Figure 4. The bottom corner of the sump filter where the Iwaki® brand pump draws water from the filter and pumps it back to the top shelves of aquariums. A second bulkhead accommodates a quick drainage system for water changes. The bulkhead can be opened and the filter water drains into a storm drain below. For ease of replacement, note that there are union valves between the pump and the filter box. If the pump burns out, the unit can be removed via the union and replaced quickly and easily. Photo by Danté D. Fenolio.

Figure 5. Cave divers were used to secure breeding stock of the Dougherty Plain Cave Crayfish and Georgia Blind Salamander (*Eurycea wallacei*). Photo by Danté B. Fenolio.

Figure 6. Cave divers explored flooded cave waterways and hand collected crayfish and salamanders for studies involving genetics as well as disease surveys. Live animals were also collected to establish captive colonies of both species. Photo by Benjamin Martinez.

Results of reproductive pairings

We attempted to pair males and females based on size, not locality; similarly sized crayfish were paired together to avoid potential aggression and cannibalism. Two of four males were form I, two were form II. Four pairs were introduced to one another on 2 May 2012, three months after collection. By 8 May 2012, three of four females had cut their corresponding male in half and were found feeding on them. In two of the three cases, the male had been slightly larger than the female but the outcome was the same. The one remaining pair appeared compatible (male was form I) and remained in close proximity to one another until 4 July 2012 when this final male was also found cut in half, with the female feeding on him. No copulation was observed and the pair was together for 65 days. This female had a total length (tip of rostrum to the tip of the telson) of 4 cm, while the male measured 4.3 cm.

Female in berry

On 15 July 2012, the female that had been paired with a male for 65 days was observed to have deposited seven (2 mm diameter) orange/yellow eggs, which were attached to her swimmerets beneath her abdomen (**Figure 7**). The female continued to feed while brooding her eggs. The eggs did not appear to change in size or color until the week of August 20–26. At this time, structures could be seen in the eggs resembling legs and an abdomen. On 28 August 2012 (44 days after the eggs had been deposited), all seven of the eggs hatched (**Figure 8A,B**). The large yolks from the eggs were clearly visible through the carapaces of the 3 mm long hatchling crayfish (**Figure 8B**). Over the next 12 days, hatchlings would occasionally drop from the mother's abdomen, crawl freely around the aquarium, and then climb back onto her body beneath her abdomen. On some occasions, hatchlings were seen crawling on top of the female's abdomen. How the juveniles that released from their mother found her again is not known. The female crawled around the aquarium with slow movements and when she was still, juvenile would climb back onto her body. At 12 days post-hatching, the last of the hatchlings released from her body and no longer crawled back and onto the female. The hatchlings fed on the same foods that had been offered to the adults. A timeline of captive reproduction of Dougherty Plain Cave Crayfish is provided in **Table 1**.

Improvement of the standpipes

The standpipes in the aquaria had been outfitted with PVC strainers but the diameter of the holes in the strainer proved too large (**Figure 9, left**). The hatchlings all managed to fit through the strainer and into the filter box (where they were sometimes observed). A solution to the problem was found by placing nylon mesh over the opening of the standpipes (**Figure 9, right**) such that the diameter of the mesh was too small to allow the escape of hatchling crayfish.

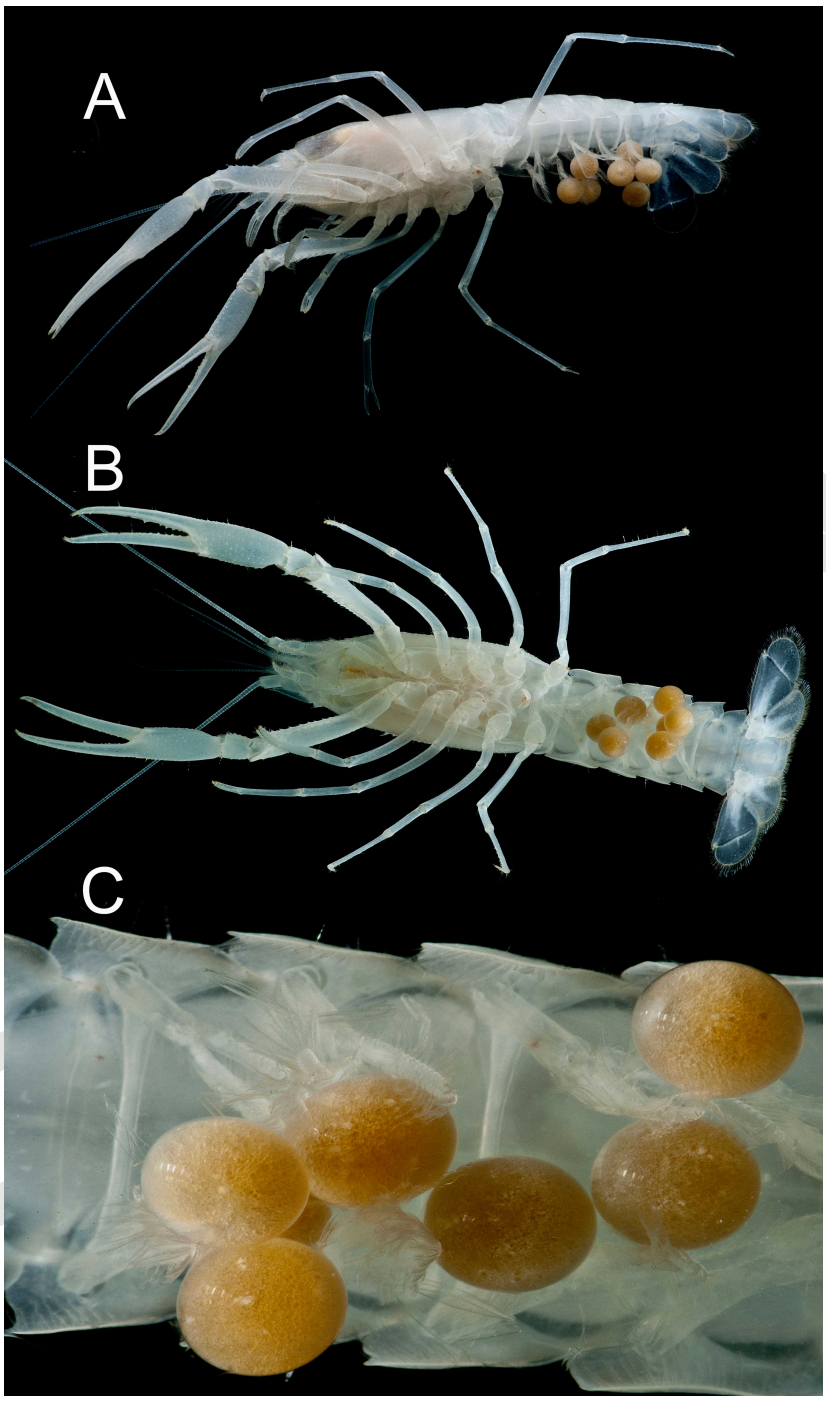

Figure 7. Lateral (A) and ventral (B) views of the female Dougherty Plain Cave Crayfish, *Cambarus cryptodytes*, in berry with seven eggs; (C) Close-up of the seven eggs. Photos by Danté B. Fenolio.

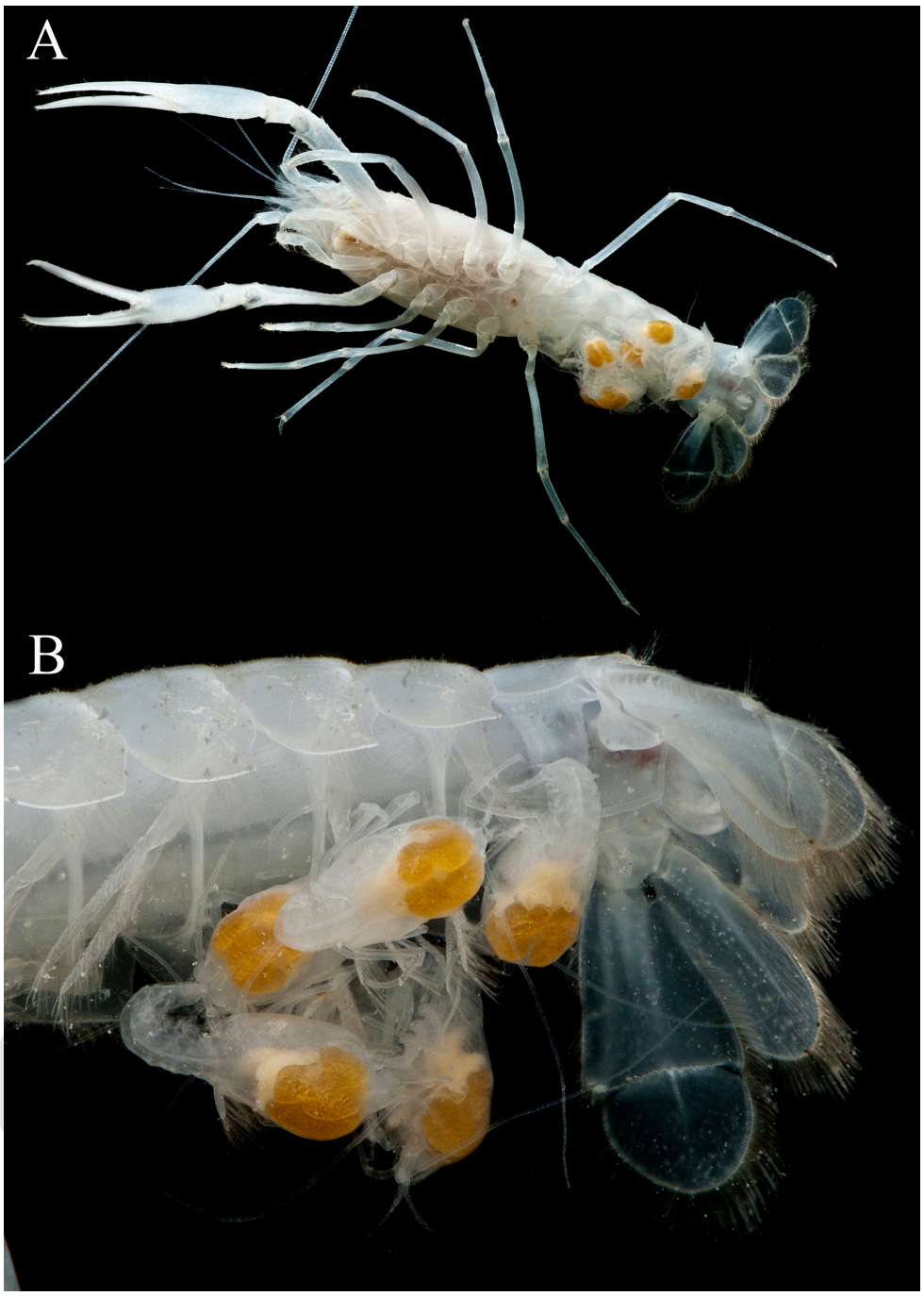

Figure 8. (A) Female Dougherty Plain Cave Crayfish with seven hatchlings. (B) Close-up of the hatchlings attached to the underside of the female's tail. Photo by Danté B. Fenolio.

Table 1. Notes on the captive breeding of the Dougherty Plain Cave Crayfish, *Cambarus cryptodytes*.

Date	No. eggs/juveniles below abdomen	Notes
2 May 2012	—	Initial 4 pairings.
8 May 2012	—	Only 1 pair remains (all other males eaten)
4 July 2012	—	Male in last pair eaten
15 July 2012	7	Female lays eggs and attaches them to underside of her abdomen.
27 August 2012	7	Eggs hatch, 44 days development @ 20°C.
28 August 2012	7	The bright orange/yellow color of the yolk is clearly visible beneath the carapace of the hatchling.
29 August 2012	6	One hatchling drops off of abdomen and is crawling around aquarium.
30 August 2012	6	
31 August 2012	6	
1 September 2012	2	One free swimming hatchling is clearly eating blood worm.
2 September 2012	0	
3 September 2012	0	
4 September 2012	5 @ 9:30 am	5 hatchlings back beneath female's abdomen!
4 September 2012	5 @ 4:45 pm	
5 September 2012	3 @ 10:00 am	Two hatchlings dropped off again.
5 September 2012	7 @ 4:30 pm	All hatchlings back beneath abdomen!
6 September 2012	1 @ 10:00 am	All but one hatchling have dropped off again.
6 September 2012	5 @ 5:00 pm	More changes in the number of hatchlings beneath abdomen.
7 September 2012	3 @ 10:00 am	Hatchlings crawling all over female's body: 1 on carapace, 1 below the carapace.
7 September 2012	2 @ 5:00 pm	
8 September 2012	0	
9 September 2012	0	
10 September 2012	0	No hatchlings beneath abdomen for 3 days.

Figure 9. The openings in the strainer on the PVC standpipe to the left proved too large allowing hatchling crayfish to escape into the filter box. The nylon mesh covering for the standpipe to the right is the method now employed to prevent the escape of small crayfish from aquaria. Photo by Danté B. Fenolio.

Conclusions

Our observations represent the first report of an ovigerous female and evidence for a successful reproduction in captivity of the Dougherty Plain Cave Crayfish. Although we cannot be absolutely certain that the male-female pair copulated, as we did not directly witness the event, the male was in Form I condition and lived in close association with the female in the same piece of PVC pipe for 65 days. This is 10.5 times longer than the other three attempted pairings. However, we cannot rule out that the female may have stored sperm from a previous copulation before she was captured and brought into captivity. Regardless, our study demonstrates that females can be held in captivity, deposit eggs, and those eggs can be successfully reared to hatching. One hypothesis for the observed behavior is that predation by females of males may be related to living in a generally energy-limited environment. Dr. Tom Poulson (pers. comm) hypothesized that this might be an example of sexual cannibalism, similar to that observed in other terrestrial invertebrate species, such as praying mantis, where the female consumes the male after copulation. The energy provided by ingesting the male can then be invested to the developing eggs. However, we did not observe evidence of developing ova in the

three females that ingested their potential mates within the first six days of pairing. Consequently, another hypothesis is that cannibalism of males is an artifact of living in captivity. More laboratory investigations are needed to determine if the behavior is actually sexual cannibalism, as verification from observations of wild crayfishes by cave divers is unlikely.

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