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NATURAL HISTORY CHARACTERISTICS OF *SYNAPTULA HYDRIFORMIS,* AN APODID SEA CUCUMBER FROM OYSTER POND, SAN SALVADOR ISLAND, THE BAHAMAS

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ABSTRACT

Synaptula hydriformis (Echinodermata, Holothuroidea, Apodida, Synaptidae: Lesueur, 1824) is a small, hermaphroditic sea cucumber commonly found creeping over the benthic pearl oyster communities in Oyster Pond, San Salvador Island, The Bahamas. Unusual features include possession of an ovo-testis; internal self-fertilization; embryonic brooding accompanied by matrophagy (juveniles feed on their mother's coleomic cells and tissues); and viviparous, live-birth of juveniles with adult characteristics. Through live-culture and paraffin histology, life history stages were characterized. The ovo-testis was found to contain both sperm and eggs supporting its characterization as a simultaneous hermaphrodite. Three unique color morphs were described: solid brown, brown striped, and translucent. In living transparent specimens, we are able to observe juveniles ambulating and feeding within the mother's body cavity. Autogamy (self-fertilization) and vivipary (live-birth) mark the synaptid family as unusual among sea cucumbers. Other families of sea cucumber tend to release gametes into the water column where they undergo external fertilization and develop into free-swimming planktonic larvae. We developed a method for inducing both synchronous spawning and birthing. Subsequently, a successful technique for culturing juvenile sea cucumbers was established. Developmental stages were characterized for the larvae. We found evidence for direct development, bypassing both auricularia and doliolarian stages and skipping the metamorphosis typical of other holothuroideans. We discuss *Synaptula* in the context of other hermaphroditic organisms that seem well-adapted for colonizing the island's inland, anchialine pond communities.

INTRODUCTION

While investigating invertebrate communities in the anchialine ponds of San Salvador Island in The Bahamas, we frequently encountered a small, "naked sea cucumber" identified as *Synaptula hydriformis* living in Oyster Pond. These soft-bodied echinoderms bear little resemblance to other, hard-bodied members of the phylum, (sea stars, brittle stars, and urchins) and have adapted a life style that seems to have converged with that of small benthic worms. At first viewed as a nuisance (they clung to wetsuits and booties with minute, hook-like ossicles embedded in their gelatinous skins), we soon became fascinated by their natural history and set out to investigate (and manipulate) their reproductive life cycle.

Synaptula hydriformis was first described by the accomplished French naturalist Charles-Alexandre Lesueur in 1824 during his association with William Maclure and Robert Owen, the remarkable founders of an intellectual commune established in New Harmony, Indiana. Lesueur first encountered *Synaptula hydriformis* on an expedition he made with William Maclure in the winter of 1815-1816 to the West Indies, in a rich mangrove community on the north side of the island of Guadeloupe. The species has been referred to by many different names, including *Holuthuria hydriformis, Holuthuria viridis, Synaptula vivipara, Synapta viridis, Synapta pourtalesii, Leptosynapta hydriformis, Leptosynapta pourtalesii, Heterosynapta viridis, Synapta vivipara, Synapta picta,* and *Chondrocloea vivipara* (Heding, 1928).

The second significant mention of *Synaptula hydriformis* involves a specimen collected by the U.S. Fish Commission on an expedition to The Bahamas in 1886 from the very first ship ever designed specifically for marine research, the *USS Albatross*. While exploring San Salvador Island (formerly Watling's Island), the *Albatross* obtained a second specimen of this sea cucumber. This was stored in the Smithsonian Museum, and later identified as *Synaptula hydriformis* (Lesueur 1824) when Hubert Clark uncovered it in the 1900s. Clark performed extensive (beautifully illustrated) research on this self-fertilizing, internally brooding species and wrote prolifically of its physiology, taxonomy, and embryology (Clark, 1897, 1907). The Smithsonian obtained a more recent sample from The Bahamas in 1996, collected by Charlene D. Long (American Natural History Museum). The most-studied specimens were collected from Bermuda, Panama, Jamaica, and the Florida Keys. Since Clark's impressive monograph, there are only modest references to the species until Jennifer Frick (Smithsonian Marine Station, Fort Piece, FL) took up the study of their ovo-testis and matrophagy (or matrotrophy: feeding on, or deriving nourishment from ones' mother) in the late 1990s for her Ph.D. Frick collected specimens from Indian River Lagoon and Lake Surprise on Key Largo (Frick et al., 1996; Frick, 1998).

Synaptids are interesting from a variety of perspectives. Unlike most other sea cucumbers, the apodid species lack tube feet for motility. They adhere to the substrate through surprisingly clingy dermal hooks called ossicles and ambulate using their feeding tentacles to pull themselves along, while feeding on the substrate. As self-fertilizing hermaphrodites, they are well-adapted for colonizing novel habitats. A single specimen could theoretically establish an entire population without a mating partner. This has implications for genetic diversity. It is possible that the entire population within our field site is comprised of only a few genetic "clones". The life-style of embryonic brooding and matrophagy is also interesting. If laboratory cultures of the transparent adults can be established, and experimental triggers for fertilization and parturition developed, this organism offers promise as a novel model organism for the study of invertebrate (echinoderm) embryology. Their ability to undergo live-birth also provides an intriguing novelty rarely seen outside of the chordate phylum: a mechanism by which juveniles living within the mesoderm-lined coelomic space of their mothers are shunted to the animal's posterior, expressed into the outside world within a mesoderm/ectoderm "birthing sac", and released through a developmentally programmed (reversible?) rupture of that sac during birth. It seems likely that juveniles from multiple, asynchronous reproductive cohorts are delivered (exhibiting "superfetation") as evidenced by varying sizes and states of development of the progeny expressed at the time of birth from a single individual. It would be interesting to learn whether or not larval forms can be sorted and retained when older juveniles are birthed, or if they too are discharged without regard to their developmental maturity.

METHODS

Collection and laboratory culture of *Synaptula hydriformis*

Specimens were collected by hand while snorkeling over beds of scaly pearl oysters in Oyster Pond, San Salvador Island, The Bahamas. It is perhaps worth noting, that synaptids insinuate themselves deeply into the internal spaces of calcareous-algal aggregates that form biotic outcrops which also house a variety of bivalves and living sponge. It is often, unfortunately necessary to open such outcrops in order to extract living specimens. We would endorse an attitude of respectful prudence when it is necessary or desirable to collect living specimens. Individual specimens were isolated and maintained in finger bowls with seawater, and fed small droppers-full of sedimentary floc collected from Oyster Pond (a loose bottom sediment rich in microbial material that the *Synaptula* appear to feed on). Floc was maintained under full-spectrum lights to promote algal growth.

Induction of birthing and spawning

Adult *Synaptula* were placed in 90 mm petri dishes half-filled with seawater and floated on an ice-water bath for ten minutes. These gave birth within 6-18 hours (exhibiting a lot of individual variation). Juveniles of numerous developmental stages from a single adult were observed, confirming "superfetation" (multiple broods maintained within the same adult). We also used this cold-shock treatment to induce ovulation and internal spawning, which we confirmed by subsequently decapitating and expressing the coelomic contents onto a microscope slide. By doing this at various times after the cold shock, successive developmental of stages were recovered. It is not clear that this was a highly efficient method for obtaining larval stages, and we recently learned that Frick employed a light/dark transition to induce spawning. She held them in continuous illumination and then exposed them to darkness for approximately one hour. She then returned them to light, and egg release began within 5 minutes (Fricke et al., 1996).

Photography

Live images were taken with hand-held iPhones, focused through the microscope ocular lens, or deployed as makeshift macro-lens cameras. In one case the live image was taken with an Olympus BH40 microscope using bright field optics and a DP-71 digital camera. Fixed histological specimens were examined using the Olympus BH40 microscope.

Paraffin Histology

Sea cucumbers were collected in January 2017. Specimens were fixed in Bouin's fixative, dehydrated, and embedded in paraffin as described by Humason (1979). Eight to nine micron-thick sections were made and slices were stained in Gomori's trichrome stain (Fisher Scientific Inc.). Slides were examined using an Olympus BH40 microscope using brightfield optics. Images were taken with a DP-71 digital camera.

RESULTS

Synaptula hydriformis color morphs

Three distinct color morphs were identified from Oyster Pond on San Salvador Island in The Bahamas: a solid brown, a "tiger-striped" form, and a pale white or translucent (colorless) variety (Figure 1). The relative abundances of the various color morphs from a sample of 329 specimens were 108 brown, 118 striped, and 103 translucent.

Figure 1: Three color morphs of Synpatula hydriformis: brown (left), striped (center), and translucent (right). Note, individuals are typically ~5 cm long.

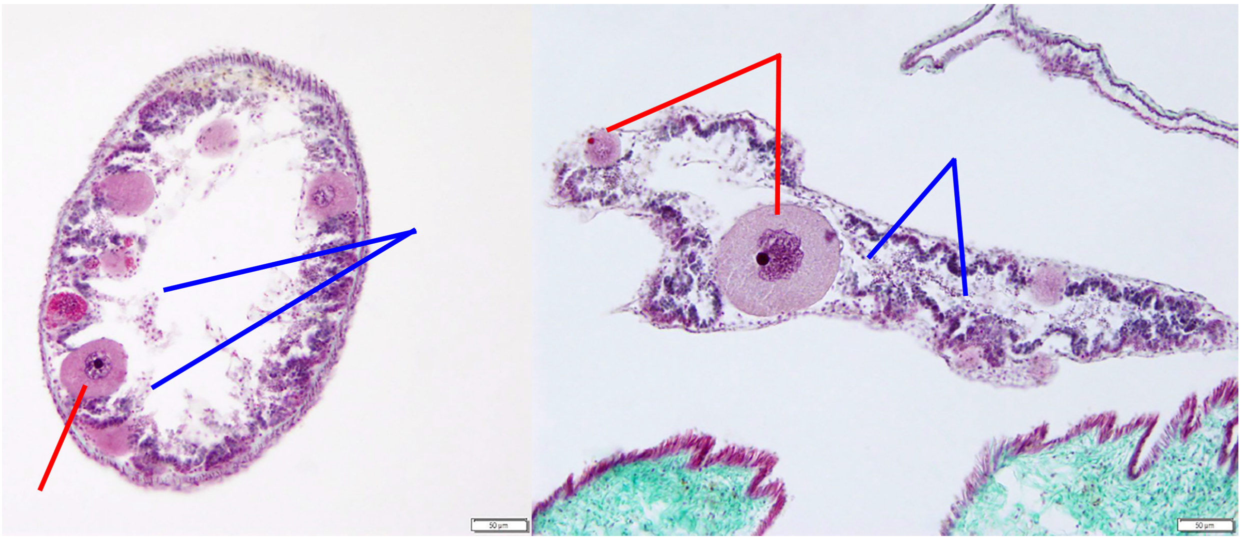

Figure 2: Paraffin histology, and Gomori's triple-stain sections of Synaptula ovo-testis. Red lines point to developing oocytes. Blue lines point to bundles of developing sperm cells. Scale bars = 50 um.

Histological observations of the gonad

Synaptula specimens were fixed, embedded in wax, sectioned, and stained using Gomori's tri-chrome. Examples appear in Figure 2. We could readily discern both developing sperm bundles and maturing oocytes within the same gonad, confirming this organ as an "ovo-testis".

Live observations of vivipary and matrotrophy

Translucent specimens allowed us to observe live juveniles ambulating and feeding within the parent's body cavity (Figure 3). Juveniles were fully formed and exhibited the classic holothurian "feed-walking" behavior in which they use their sticky, para-oral tentacles to both pull themselves along the substrate (in this case, their parent's body cavity) and feed. After drawing themselves forward, each tentacle is inserted into the oral cavity, and adherent food/substrate is "wiped off" by action of an oral sphincter as the tentacle is retrieved for its next step. Given the variety of sizes of juveniles, from 200 microns up to nearly 1 cm, it was clear that the juveniles were indeed growing within the parent's body cavity, deriving nourishment from their parental host (coelomocytes and possibly undeveloped eggs, Frick, 1998).

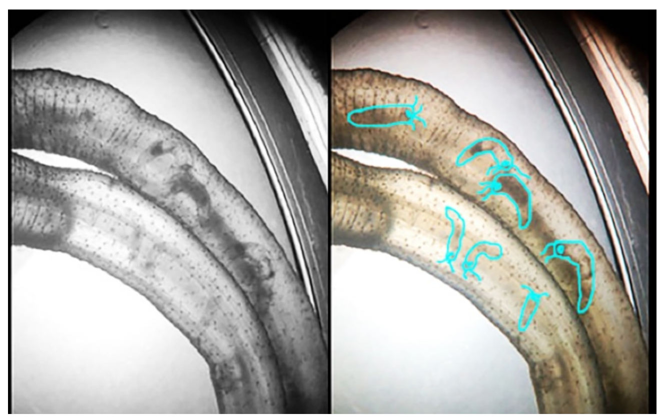

Figure 3: Translucent bodies of Synaptula hydriformis under low magnification microscopy. The left image is un-retouched. The right image highlights the outlines of juveniles in blue.

Parturition

Adult specimens were isolated into 90 mm petri dishes half-full of seawater. Individual dishes were floated on an ice-slurry for ten minutes and restored to room temperature (76° F). Within 6-18 hours, multiple juveniles were expressed from the posterior cloaca of the adults. The process of birthing (at least by this artificial induction) appears to involve eversion of a membrane sack from the cloaca (Figure 4) that gradually fills with juveniles.

Figure 4: Five juveniles have been expressed into the birthing sac. Arrow indicates cloaca.

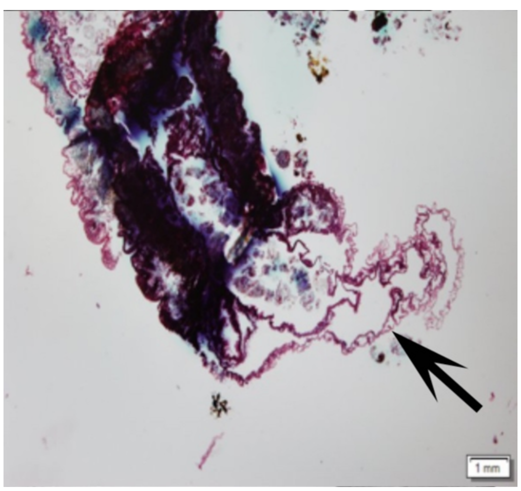

Figure 5: Complex double-membrane of birthing sac emerging from adult's cloaca (paraffin histology).

The membrane sack appears to be comprised of two membranes (Figure 5). We suspect that these twin membranes represent mesoderm and endoderm, respectively, as juveniles that are living inside a mesodermally-lined coleomic space are separated from the outside world by both the coelomic lining and either ectoderm or endoderm near the cloacal opening. Eversion of this dual-membrane "birthing sac" is an extraordinary event. At some point, an oval aperture appeared in the sac, through which juveniles were expressed into the seawater medium. Juveniles expressed in this manner have been kept alive on Oyster Pond floc culture for up to 52 days (Figure 6). It should be noted that cold-shock induced birthing (especially appearance of the "birthing sac") may differ from birth that occurs in nature. A "natural birth" video by Alvaro Migotto (2017) from the University of Sao Paulo shows juveniles (and embryos) being expelled from the cloaca directly.

Figure 6: Two juveniles freshly born by cold-shock induced parturition. Image shows posterior of intact adult and two minute, live juveniles approximately 1-2 mm in length (arrows).

Superfetation

Cross-section histology revealed embryos and juveniles of conspicuously different reproductive cohorts within a common coelom. This suggests that multiple rounds of ovulation and self-fertilization can occur on a rolling schedule without intervening births—a process known as superfetation (Figures 7 and 8).

Figure 7: An embryo (upper right) and a much larger juvenile (middle) within a single coelomic space.

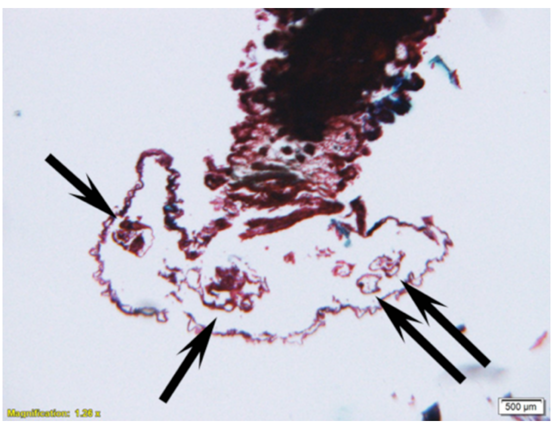

Figure 8. Four (or more) embryos and juveniles of different sizes (ages?) being expressed during parturition (arrows).

Induction of ovulation, self-fertilization, and embryogenesis

To avoid multiple environmental shocks accompanying collection and transport before lab work, and the consequent confusion of timing, we treated isolated adult *Synaptula* to an ice-shock in the field at the time of capture. Back in the lab, at various times after this initial shock, *Synaptula* adults were decapitated and coelomic fluids expressed by squeezing. In this way, juveniles (variable ages) and embryos from a variety of stages more tightly correlated with time of induction were obtained and photographed, resulting in a near complete gallery of developmental stages (Figure 9). In one specimen, 75 juveniles were found inside the adult body cavity. Although this technique of inducing gamete release and fertilization appeared to work, the frequency of early (presumably "induced") embryos produced seemed low. Since then, we have learned that Fricke deployed a light/dark entrainment to induce ovulation, triggered by a "lights-on" signal (Fricke et al., 1996).

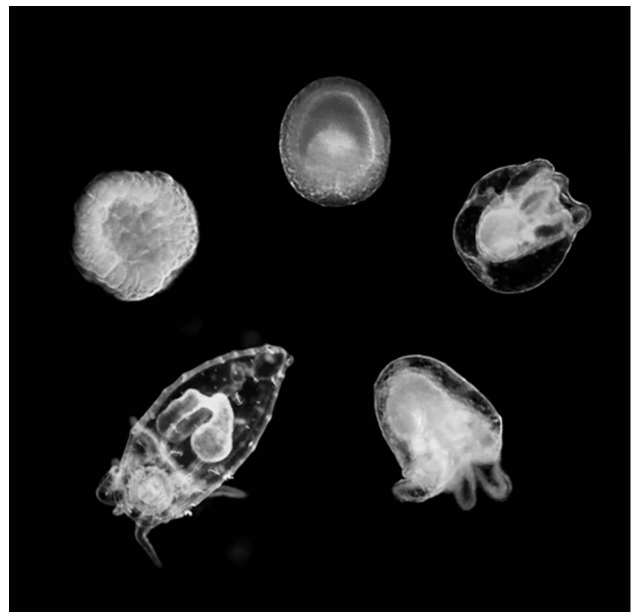

Figure 9: Embryos at various stages of development surgically expressed from shocked adults. Stages represent (beginning at center-left and traveling clockwise): (1) blastula, (2) gastrula, (3) complete juvenile with 5 tentacle buds, (4) tentacle elongation, and (5) appearance of ossicles (tiny endoskeletal hooks in the outer skin). Timing for stages: blastula: 3 hrs; gastrula: 6.5 hrs; tentacle buds: 12 hrs; ossicles: 24 hrs, post-fertilization.

DISCUSSION AND CONCLUSIONS

Synaptula hydriformis exhibit a broad repertoire of fascinating reproductive habits including simultaneous hermaphroditism, internal self-fertilization (they exhibit almost a clonal form of propagation), direct development from gastrula to the adult body form without the intervening metamorphosis seen in most echinoderm larvae, matrotrophy (feeding on maternal cells or products), superfetation (brooding multiple cohorts of young from independent spawning events), and live birth. If nothing else, this study promises to expand our vocabularies. Our findings largely confirm earlier discoveries from the elegant works of both Clark (1897, 1907, 1933), Frick (1998) and Frick et al. (1992, 1996). Regarding matrophagy (or matrotrophy), a recent review by Ostrovsky et al. (2016) suggests that matrophagy has actually arisen five times independently within the holothuroidea, including the clade that encompasses both *Synaptula* and *Chiridota* (a second, less common apodid cucumber also found in Oyster Pond).

Since little other work exists on the species, it is nice to refresh these observations for a new generation of naturalists. The things that we may have contributed uniquely to this body of work on *Synaptula hydriformis* include the development of an ice-shock technique for inducing parturition, a photo-microscopic gallery of embryonic stages, and images of the remarkable "birthing sac" observed in our laboratory-induced cultures.

The discovery of a translucent morph of *Synaptulid* sea cucumbers, along with their direct pattern of development, raises the possibility of recruiting this species as a new model organism for the study of invertebrate embryology. Its pattern of self-fertilization also raises interesting questions about the genetic diversity of our population and highlights its ability to colonize. This is interesting in that several organisms from the inland ponds appear to exhibit hermaphroditic (or even parthenogenetic) life history patterns pre-adapting them for colonization. These include the scaly pearl oyster (*Pinctada longisquamosa*: a sequential hermaphrodite), most of the gastropods, and the mangrove killifish, *Rivulus marmoratus* (a self-fertilizing hermaphrodite we found in Mermaid Pond and another of the interior ponds). It would be interesting to explore whether these isolated anchialine pond environments are unusually rich in self-fertilizing species, reflecting their potentially rare episodes of colonization.

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