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Unique reproductive biology of the broadcasting sea cucumber *Holothuria floridana*: facultative recruitment on adults inside nursery grounds

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ABSTRACT

Information on newly settled juveniles and recruitment processes in sea cucumbers is sparse for most commercial species, impeding protection of nursery habitats, which are key to management. Here, the ecology of early life stages of *Holothuria floridana* was investigated. In laboratory experiments, females released a small number of oocytes, which underwent abbreviated lecithotrophic development. During broadcast spawning, some of the negatively buoyant and sticky oocytes settled on the adults where they remained anchored up to the pentactula stage (encapsulated). Several wild adults collected in the Placencia Lagoon (Belize) each hosted ~2 propagules (eggs, blastulae, pentactulae, juveniles). The nursery habitat was characterized by high adult density, shallow seagrass beds close to mangroves, and mud dominated substrata. The present work illustrates a unique facultative offspring-adult association in a free-spawning lecithotrophic species of Holothuroidea, possibly representing a hybrid state between broadcasting and external brooding. This uncommon reproductive feature may ensure successful local recruitment in one of the most competitive habitats of the tropical Americas, where predation pressure is likely to be very high.

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Introduction

Reproductive and recruitment patterns are key ecological parameters for developing management strategies around commercially exploited marine species (Gillanders 2002; Fodrie and Levin 2008), including sea cucumbers (Purcell et al. 2012, 2013). It can help build an understanding of population dynamics and turnover rates, as well as identify areas that should be designated for marine protection (Caley et al. 1996; Slater et al. 2010a). Moreover, understanding juvenile ecology and habitat preferences may help scientists determine the ultimate carrying capacity of a given marine region and facilitate the eventual release of captive-reared juveniles to appropriate habitats, with an aim to increase their survival, as proposed for sea cucumbers by Bell and Nash (2004) and Purcell (2004).

The majority of echinoderms, including most sea cucumbers, are broadcast-spawners, with either feeding or non-feeding larvae developing in the plankton (Arnone et al. 2015). However, some species have adopted a brooding strategy to protect their offspring in various ways (Smiley et al. 1991), such as inside the coelom, in dorsal depressions, in folds of the sole, in chambers underneath dorsal plates, in interradial

pouches surrounding the tentacles, or under the sole (e.g. McEuen and Chia 1991; Giménez and Penchaszadeh 2010). Remarkably, a limited number of echinoderms have developed a mixed strategy to partition the risk between parental protection and dispersal, whereby embryos and larvae from the same clutch will either develop freely or be brooded, such as in the sea star *Henricia lisa* (Mercier and Hamel 2008). The reproductive strategy of a species has great incidence on its population dynamics.

Most of the existing knowledge on the spatial distribution of Holothuroidea relates to the adults, and is often gathered within broad assessments of the density, abundance, distribution or reproductive cycle of a species (e.g. Sloan and Von Bodungen 1980; Chao et al. 1994; Herrero-Perezrul et al. 1999; Mercier et al. 2000a; Shiell and Uthicke 2006). Adult stages of sea cucumbers are benthic; some species live on rocky or hard substrata while others live either on the surface or buried inside the sediment (Purcell et al. 2012). Adults of the focal species, *Holothuria floridana*, are commonly found inside coral reefs, seagrass beds and mangroves, generally on sand or coral rubble (Rogers 2013; Rogers

et al. 2018a, b). Comparatively less research has been undertaken to document and assess nursery grounds in a variety of marine species around the world (Roberts 2000; Miller and Christodoulou 2014; Olds et al. 2014). A nursery habitat is a subset of all habitats where juveniles occur (Beck et al. 2001); it can provide critical insight about the ecology of a species.

Information regarding juveniles of wild sea cucumbers is sparse for most species, including those of commercial interest. Shiell (2004) developed a list of 22 species of sea cucumbers in which juveniles between 2 and 8 mm long were documented. They were found in a variety of habitats, including seagrass beds and reef flats, and on diverse substrata such as coral rubbles, rocks, algae, sand and mud. The few studies dedicated to juveniles generally found them in localized areas and in low numbers (Shiell 2004) without strong support for the existence of a delimited nursery habitat. Bourjon and Desvignes (2018) found five juveniles of *H. difficilis* 20–50 mm long on a reef in Reunion Island (Indian Ocean) and Desbiens and Wolfe (2020) found six small juveniles (7–12 mm) of *Stichopus* sp. in coral rubble in Palau, western Micronesia. Setyastuti et al. (2018) documented a nursery of *H. leucospilota* in Indonesia with juveniles measuring 30–50 mm in length occurring at a density of 0.25 ind m^{-2} on sand, mixed coral rubble or shell fragments within seagrass beds (*Thalassia hemprichii* and *Enhalus acoroides*). For most species, sightings relate chiefly to intermediate-size juveniles, e.g. in the tropical sea cucumbers *H. atra* (10–40 mm), *Astichopus multifidus* (80–210 mm; although length of juveniles in this species is not clear), and *H. scabra* (10–40 mm, Mercier et al. 2000b) among others (see review by Shiell 2004). Similarly, in the temperate-polar species *Cucumaria frondosa*, juveniles less than 30 mm long have only rarely been reported in the wild in shallow water underneath rocks or pebbles (Hamel and Mercier 1996).

In one of the first exhaustive studies on juvenile sea cucumbers, Mercier et al. (2000b) described settled pentactulae and early juveniles (<9 mm) of *H. scabra* occurring in large numbers on the surface of seagrass leaves in Solomon Islands, close to the habitat where adults are also abundant on sandy-muddy substratum. The investigators noted that the size, location, and abundance of juveniles were correlated with habitat characteristics such as depth, granulometry and organic richness of the substratum, and with the nearby presence of seagrass beds. In another detailed study conducted in New Zealand, juveniles of *Australostichopus mollis* 30–60 mm long

were present (0.1–0.18 m^2) in areas with considerable adult densities (0.72–1.14 m^2). This habitat was characterized by elevated nitrogen content and phaeopigment:chlorophyll-*a* ratio, small grain size and the presence of large shell fragments (~10 cm), which may provide a unique settlement microhabitat for competent larvae (Slater and Jeffs 2010b). High densities of juveniles of *Apostichopus japonicus* (6.7 ind m^2) were also reported in the intertidal zone in Japan, in association with large amounts of the macroalgae *Sargassum* spp. and *Ulva* spp., moderate eutrophication and protection from environmental fluctuation (desiccation and wave energy) during low tide (Yamana et al. 2006). Factors such as water movement, food availability and seagrass density were observed to affect the distribution of juveniles of *H. tubulosa* (Bulteel et al. 1992). Juveniles were found in habitats devoid of adults in the species *Acaudina molpadioides* (at low tide on muddy flats), *Actinopyga echinates* (no specified habitat) and *Stichopus hermanni* (in algal beds) (James 2005). Young and Chia (1982) found that juveniles of the dendrochirotid *Psolus chitonoides* preferred to settle on or near the adults and suggested that gregarious settlement could play an important role in the recruitment process.

Based on field collections, the present study highlighted a direct and intimate association between the adults and the early life stages (embryos to juveniles) of the commercial sea cucumber *H. floridana* in coastal waters of Belize. In addition to outlining this unique behaviour, which is not among the reproductive features previously reported in the order Holothuriida, spawning and larval development were assessed in the laboratory, and the nursery habitat of *H. floridana* was surveyed and characterized.

Methods

Study areas and densities of adults

A large region of southern Belize (Central America) was explored for the presence of adults of *Holothuria floridana* (Figure 1), and subdivided in areas and habitats, including zones with and without runoff influence (various salinities). Survey data were used to identify and characterize areas that harboured populations of *H. floridana* and areas where *H. floridana* was not recorded (Figure 1 B). Densities of *H. floridana* were estimated per metre square based on visual surveys in February 2020 from in various sites covering a total area of ~3,000 m^2 and during other studies carried out in 2014, 2015 and 2016 throughout the Placencia Lagoon

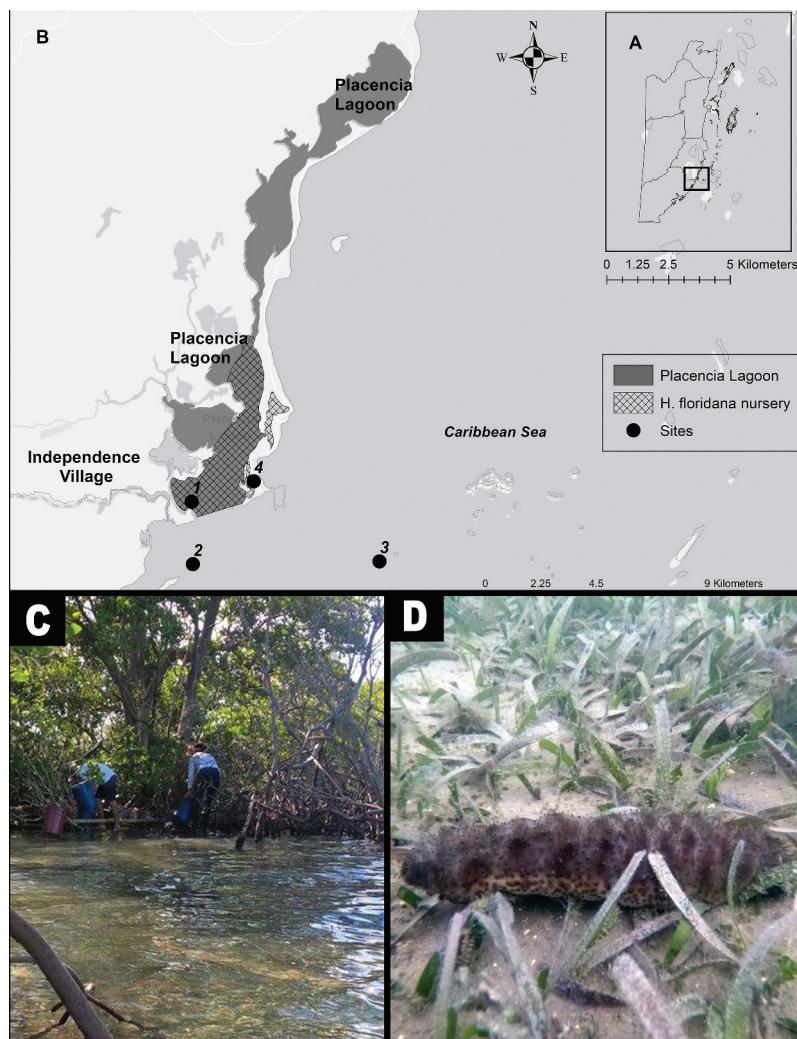


Figure 1. **A.** General view of Belize showing the study area (square). **B.** Investigated areas in southern Belize (close-up view): Site 1 (mouth of Placencia Lagoon); 2 (banks of Joe Caye); 3 (banks of Wagga Caye); 4 (channels within Placencia Lagoon); Placencia Lagoon (grey shaded area) and *H. floridana* nursery within the Placencia Lagoon (grid shaded area). **C.** Seagrass beds (*T. testudinum*) extending to mangroves in site 1. **D.** Adult of *H. floridana* resting in seagrass beds in site 1. *H. floridana* in D is about 21 cm long.

in ~15 different sites (Figure 1 B). Only sea cucumbers visible on the surface or partially buried in the sediment were counted (although there may have been others buried deeper in the substratum).

Collections

On 18 August 2016, 20 individuals of *H. floridana* were handpicked during free dives at the mouth of the Placencia Lagoon (Figure 1 B, Site 1) at a depth of 6 m. They were individually placed in plastic bags while underwater. The first 10 individuals were visually examined for the presence of large epibionts, which were removed and preserved individually in 70% ethanol. The other 10 individuals were carefully rinsed with seawater and everything detached from them was

preserved as above. The samples were then transferred to the University of Belize Environmental Research Institute laboratory for analysis and identification. Among them, embryos, larvae and juveniles that were suspected to be of *H. floridana* (preliminary examinations) were measured and photographed. The identification of the juveniles was later confirmed using ossicle morphology, as per Mcnab and Rogers (2017), while the embryos were identified based on their specific size and morphological characters, as per Rogers (2018) and the culture of embryos, larvae and juveniles performed in the laboratory (see method below). The oocytes and embryos of *H. floridana* (up to hatching at the late pentactula) are surrounded by a distinctive sticky outer layer (capsule) not found in any other sea cucumber from Central America. This unique character was used to confirm the identity of the early developmental stages

found associated with the wild collected adults. The corresponding age of all stages collected from the field was established based on cultures developed under laboratory conditions (see below for details).

On 2 February 2020, a total of 60 individuals of *H. floridana* were collected and preserved (see above). They were dissected and sexed using gonad smear to detect the presence of spermatozoa or oocytes. The ethanol in which they were preserved was sieved using 100 µm mesh and the material retained was examined for the presence of epibionts, especially small items (including sea cucumber propagules). Fifteen other individuals were collected from the banks of Joe Caye, among seagrass *Thalassia testudinum* and patch coral near the mouth of the Placencia Lagoon (Figure 1 B, Site 2) at a depth of 2.4 m; another 15 from the banks of Wagga Caye, also among seagrass *T. testudinum* and patch coral, further away from the Placencia Lagoon (Figure 1 B, Site 3) at depths of 2–6.1 m. Finally, 30 individuals were collected from a lagoon channel around mangrove patches inside the Placencia Lagoon close to Placencia Village (Figure 1 B, Site 4) at depths of 0.6–2.6 m. These collections of adult sea cucumbers were carried out with the marine research permit 066–19 from the Belize Fisheries Department. Adults collected in both 2016 and 2020 ranged from 11 to 35 cm in total length (mean 20.9 ± 5.4 cm) and ranged from 107.8 to 644.8 g in total weight (mean 296.1 ± 87.1 g).

Habitat characterization

During all sea cucumber collections, the depth was established and the habitat described, including substratum types (mud, sand, coral rubble) based on the description from Mumby and Harborne (1999), other organisms present (e.g. corals), and seagrass species. To understand the habitat where adults of *H. floridana* were found, general depths, salinities, temperatures, substrata and complementary species composition were also obtained from the Placencia Lagoon management plan (Wildtracks 2015), from the Placencia Lagoon report (Ariola 2003), from studies carried out in the lagoon by Short et al. (2006) and from this current study.

Spawning and fecundity

To better understand the ecology of embryos, larvae and juveniles of *H. floridana* found on the body wall of adults and to characterize the nature of the relationship between adults and offspring, laboratory experiments were developed. On the full moon of

April 2018, 60 adults of *H. floridana* were collected from the Placencia Lagoon and surrounding waters (Sites 1 and 2 described earlier) by snorkel between 1.5 and 2.4 m depths and transported to a nearby commercial shrimp hatchery in southern Belize. They were induced to spawn using temperature shock (26°C), based on Agudo (2006). Thereafter, all individuals were transferred to spawning tanks (2,500 L) at ambient temperature (34°C).

Males and females were kept in the same tank and, as females began to spawn, the broadcasting behaviour was assessed, e.g. slow or forceful release. Oocytes were visually examined as they were expelled in the water column. The following oocyte features were scored: buoyancy (whether they floated or sank); distribution (whether they remained separate or clustered together); stickiness (whether they adhered to each other or to any surface, including adult sea cucumbers). Some of the features observed in these cultures were used to identify the propagules found on wild-collected adults of *H. floridana*.

To calculate the realized fecundity (number of oocytes spawned), oocytes were collected from 20 broadcasting females in April, May and June and stored in 100 ml of filtered seawater. Three 4-ml aliquots ($n = 5$ replicates per female spawn) were sampled before counting oocytes under a stereomicroscope (described above). The total number of oocytes was then estimated based on the sample volume.

Development

Development was monitored from fertilization to the hatching of pentactula larvae and occurrence of early juvenile stages. These were used as reference to age the embryos and juveniles found in the wild. Oocytes from 20 females were pipetted immediately after their release and distributed in 20 separate 2000-ml beakers (~600 – 1000 oocytes per beaker). Artificial fertilization was achieved on three successive spawning trials, in April, May and June 2018 using a ratio of three spermatozoa per oocyte, as per Xiyin et al. (2004). After fertilization, excess spermatozoa were rinsed out of the culture vessels by filtration (100 µm mesh) and the water changed. Cultures were kept under natural photoperiod, with daylight provided through large windows and doors. The tanks were kept at 28°C; and 50% of the water was changed daily as per Agudo (2006) and Al Rashidi et al. (2012). The morphology of the developing embryos, larvae and juveniles was characterized, and photos taken under a stereomicroscope (Meiji EMZ-13TR) equipped with a camera (Canon EOS T6i 24MP DSLR).

Prevalence of adult-offspring associations in the Placencia Bay

The number of propagules (embryos, larvae and juveniles) in the study sites was estimated based on the density of adults in areas where *H. floridana* was observed (see above for details) and the surface area on which they were found, as well as the proportion of adults hosting propagules and the mean number of propagules on them. This value was estimated for Sites 1, 2 and 4 where the epibionts were found on the adults and extrapolated for the entire Placentia Lagoon surface offering environmental conditions susceptible to accommodate *H. floridana* (areas with salinities >21 psu and sand or mud and seagrass habitats).

Mucus collection

Wild collected individuals of *H. floridana* are covered with an abundance of mucus entangling several small organisms (plants and animals) as well as inorganic matter. Under laboratory conditions, the mucus of 16 individuals was removed, along with the organic and inorganic matter it contained, and sieved using 50 µm nylon screen to remove excess water before measuring its wet weight. Major organic and inorganic components were examined and characterized under the stereomicroscope described above.

Results and discussion

Despite the growing number of juveniles of various species of holothuroids being reported in the field, an intimate association between broadcast-spawning parents and their early propagules, as presented here, appears to be an unusual reproductive feature. However, this association is clearly facultative, as the offspring survived without the presence of adults, although the study highlighted a number of advantages and adaptations supporting the benefit of what might be tentatively described as an intermediate brooding strategy or intraspecific epibiosis, enhancing both the protection, feeding, growth and survival of epidermal recruits.

Association between adults and propagules

Adults of *Holothuria floridana* ($n = 80$) collected from the various sites measured between 11 and 35 cm in total length (20.9 ± 5.4 cm) and the 12 that hosted propagules measured 16–32 cm (21.3 ± 4.3 cm). There was no significant difference (Wilcoxon; $p < 0.05$) in the total lengths of adults that hosted

propagules and the lengths of those that did not. The minimum size of sea cucumbers that hosted propagules was 16 cm, slightly larger than the size at sexual maturity known for that species as per Ramos-Miranda et al. (2017), suggesting that they were primarily the more mature sea cucumbers, i.e. those involved in spawning.

The propagules found on sea cucumbers were at different stages of development, including fertilized oocytes, blastulae, pentactulae (early or late) and juveniles. They were either still on the body wall of males and females, or in the same collecting bag as the adult from which they detached during collection. Of a total of 20 adults collected in August 2016 (Site 1, Figure 1 B), five hosted propagules, which included four embryos, five larvae and three juveniles (Figure 2 A, B, C, D, and F). The first adult hosted one juvenile, measuring 9.1 mm mouth-anus estimated to be ~2 months (based on the laboratory cultures; Figure 2 E). This juvenile was found entangled in the mucus along the ventral surface of the body wall (Figure 2 F). One fertilized oocyte 265 µm in diameter, estimated to be ~2 h old (Figure 2 A) was also found associated with the same individual. A second individual harboured a juvenile (9.0 mm) and two fully formed pentactulae still inside their protective capsule (Figure 2 D) measuring 338 µm in length; both were ~74 h old. The third sea cucumber hosted another juvenile (9.1 mm estimated to be ~2 months old) but no embryos or larvae. A fourth adult hosted two embryos that were ~2 h old (fertilized oocyte, Figure 2 A) and ~49 h (blastula, Figure 2 B) and one larva ~66 h old (early pentactula; Figure 2 C), measuring 265, 311 and 325 µm in diameter, respectively. The fifth adult hosted one ~49 h old blastula measuring 259 µm and two early pentactulae ~66 h old measuring 315 µm and 317 µm. The data revealed that a single adult individual could host more than one stage of propagules, suggesting a prolonged relationship and recruitment over successive spawning bouts (since the entire development lasts 5–6 days in *H. floridana*). Due to the broadcast mode of spawning, it could not be determined whether the various propagules were the direct offspring of the host or of other nearby conspecifics.

From the 60 adults collected in 2020, a total of 11 juveniles ($9.0\text{--}9.5 \pm 0.2$ mm; ~2 months old) were found on seven adult males and females, suggesting that either sex can host propagules. More precisely, two adult males hosted two and three juveniles each. The two juveniles on the first male measured 9.0 and 9.4 mm and the three juveniles on the second male measured

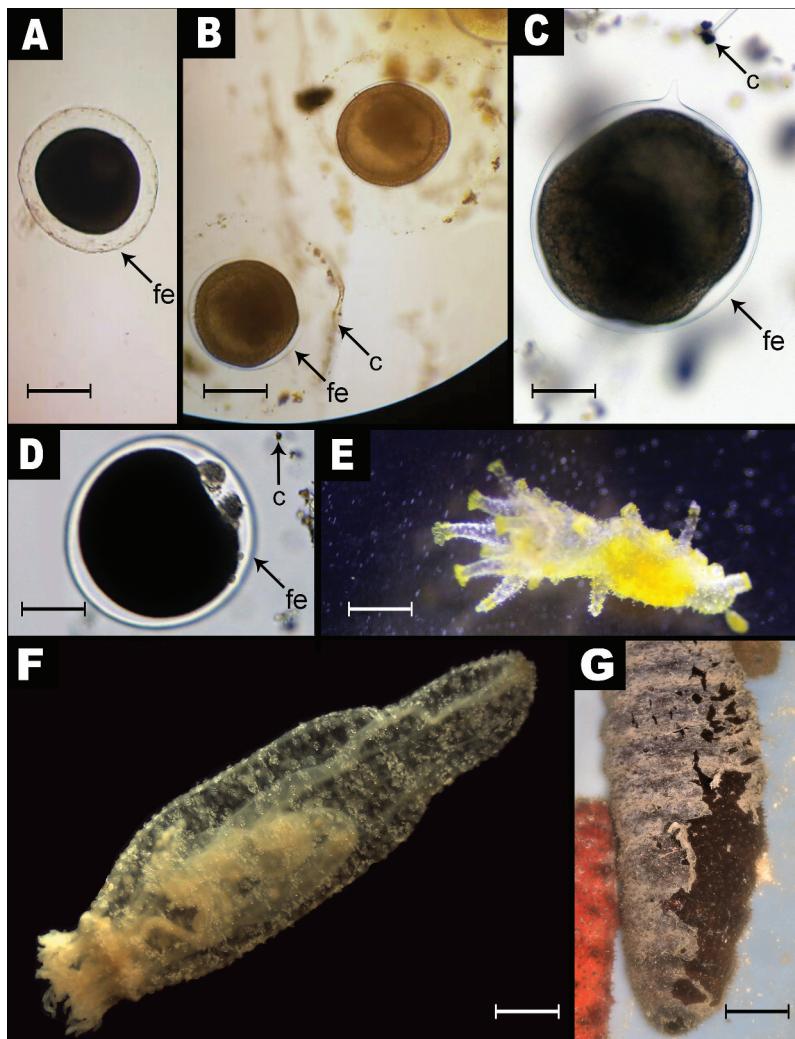


Figure 2. *Holothuria floridana*. **A)** ~2 h old fertilized oocyte showing fertilization envelope (fe); **B)** ~49 h old blastula showing fe and the sticky capsule (c); **C)** ~66 h old early pentactula showing fe and c; **D)** ~74 h old fully formed pentactulae showing fe and c; **E and F)** Juvenile with functional digestive system; still found on the body wall of adults; **G)** Mucus mixed with organic matter and epibionts falling off from the external body of an adult. The scale bar represents 132.5 µm in A; 103.6 µm in B; 52.5 µm in C; 67.6 µm in D; 2 cm in E; 1 mm in F; 3.3 cm.

9.1, 9.2 and 9.4 mm. Five adult females hosted 1–2 juveniles each. The first female hosted one juvenile measuring 9.3 mm; the second female hosted two juveniles measuring 9.2 and 9.3 mm; the third female hosted one juvenile measuring 9.2 mm; the fourth female hosted one juvenile measuring 9.5 mm and the fifth female hosted two juveniles measuring 9.4 and 9.5 mm.

Although the spawning posture is similar to that of other Holothuriida, the slow release of negatively buoyant oocytes in *H. floridana* differs from the forceful ejection seen in most other species, like *H. scabra* (Hamel et al. 2001). It more closely resembles spawning of *H. mexicana*, which has negatively buoyant oocytes (Emlet 2016). During spawning under laboratory conditions, females of *H. floridana* released oocytes at a slow pace, forming a stream of negatively buoyant gametes,

which rapidly sank to the bottom (reaching the substratum inside 10 s; Figure 3 A, B, C, D and E), as observed by Edwards a century ago (Edwards 1909). Negatively buoyant oocytes were described in a few other holothuroids, including *Cucumaria lubrica* and *C. pseudocurata* (McEuen 1988). In *H. floridana*, there was very little movement of the anterior part of the spawning individuals (Figure 3 A), allowing the propagules to remain in bundles or strings (Figure 3 D and E), and favouring sinking. This behaviour contrasts with that of several known broadcasting species of Holothuriida, Stichopodida and Dendrochirotida in which dispersal of gametes in the water column is favoured upon release by the movement of the whole anterior body or the tentacles (e.g. Costelloe 1985; McEuen 1988; Hamel and Mercier 1996; Mercier et al. 2007).

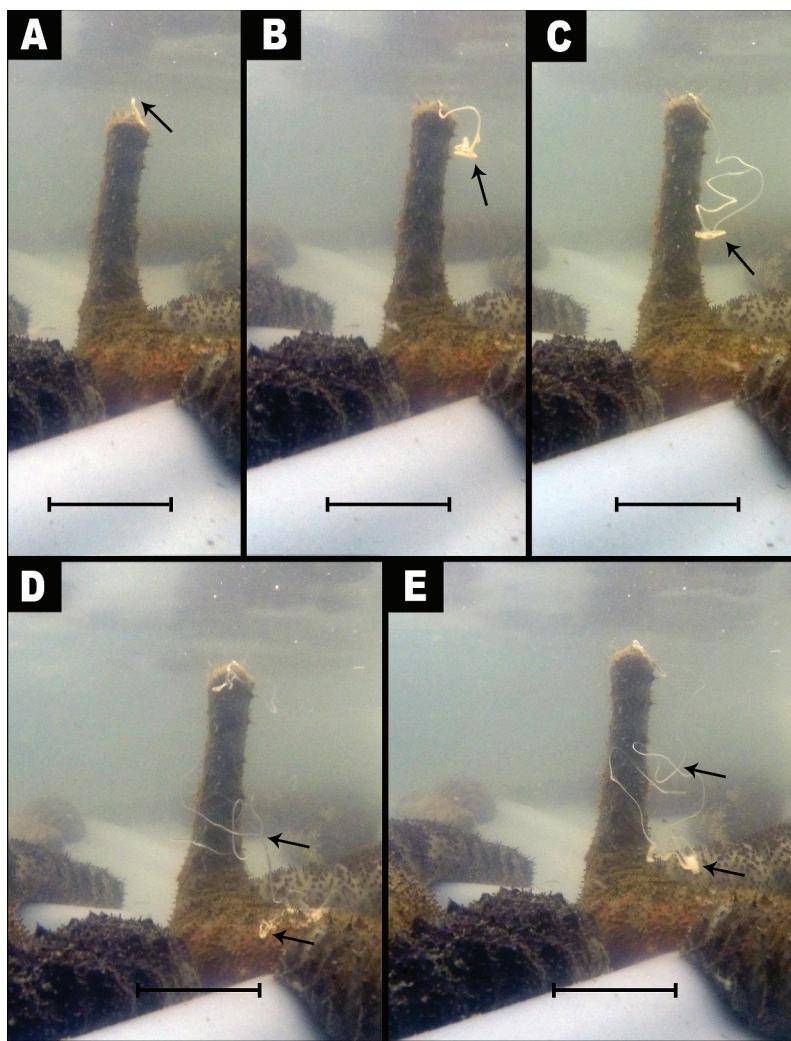


Figure 3. **A.** Female of *Holothuria floridana* broadcasting oocytes at the beginning of spawning (arrow); **B.** Negatively buoyant oocytes immediately start to sink; **C.** Negatively buoyant oocytes after 5 s; **D.** and **E.** Oocytes reached the dorsal body wall of the spawner within 10 s. The scale bar represents 6 cm .

As propagules of *H. floridana* contacted the substratum, three elements were determined to promote association with the adults: their sticky nature; their negative buoyancy; and the mucus covering the entire body wall surface of the adults (Figure 2). Fertilized oocytes had an average diameter of 265 µm and sank to the nearest available substratum, with none remaining in the plankton. Sticky oocytes have been described in other echinoderms like the sea star *Patiriella exigua* (Byrne and Anderson 1994), the brittle star *Amphiopodia occidentalis* (Emlet 2006) and the sea cucumber *C. pseudocurata* (McEuen 1988), but not in the sympatric *H. mexicana*, which has negatively buoyant oocytes of a similar size and has been suggested to hybridize with *H. floridana* by Engstrom (1980). The embryos and larvae of *H. floridana* continued to develop inside the fertilization envelope while surrounded by an outer capsule (Figure 2 B-D). Their stickiness made them adhere to each other and

to all surfaces, including the bottom of beakers or tanks, plastic pipettes, and body wall of sea cucumbers. The capsule remained present until hatching at the late pentactula stage, when the larvae developed feeding podia and the capacity to anchor to any substratum by themselves. A similar sticky outer capsule was described in other echinoderms (McEdward and Janies 1997), but not in holothuroids, although a 'layer of microvillae present outside the fertilization envelop' was observed in *H. mexicana* (Emlet 2016). In *H. floridana*, the sticky capsule could well be a special adaptation favouring the link between adults and propagules. The abundant mucus produced by the body wall (Figure 2 G) may also help capture the early propagules and retain them on the adults until the juveniles become autonomous (Figure 2 E, F).

Holothuria floridana undergoes abbreviated lecithotrophic development, without passing though the

auricularia or doliolaria larval stages observed in several other broadcasting species (e.g. Hu et al. 2013; Huang et al. 2018). The entire development lasted ~6 days (again similar to *H. mexicana*; Emlet 2016), from fertilization to the late pentactula (~340 µm long), which emerged from the protective capsule with the five primary tentacles fully developed, ready to begin life as a deposit feeder. In the sympatric *H. mexicana*, hatching was described to occur at the gastrula stage instead (Emlet 2016).

The mucus covering the body wall of adults represents about 5–10 g wet weight and holds large quantities of inorganic and organic matter, including phytoplankton (diatoms), tiny benthic organisms (bivalves, crabs, algae, gastropods), inorganic particles, and decaying matter. Both the mucus itself and the particles/organisms trapped in it could be a source of nutrients for the growing juveniles, in line with food sources described for other Holothuriida sea cucumbers (Massin 1982; Uthicke 1999; Roberts et al. 2000; Plotieau et al. 2013). It can be hypothesized that these juveniles may obtain refuge and quality food while retained on the adults. It is possible that juveniles found on the body wall of adults are protected from predation and/or suffocation (free demersal propagules at early developmental stages would likely be smothered by the abundant sediments in sea grass beds). While the juveniles of *H. floridana* survived without the presence of adults in laboratory conditions, they grew only to ~1.9 g in twelve months (i.e. 6.1 cm y^{-1}), which is slower than expected for a tropical sea cucumber in the order Holothuriida (e.g. Battaglene et al. 1999b; Hamel et al. 2003). By comparison, *H. scabra* grew at a rate of ~0.39 g day $^{-1}$ (Battaglene et al. 1999a) and *H. atra* at 0.12–1.43 g day $^{-1}$ (Seeruttun et al. 2008) under hatchery conditions. Here, based on the known spawning time for this region (July; Edwards 1909), the presence of juveniles up to 9 mm long on individuals of *H. floridana* in August suggests that growth on adults is faster than in independent cultures. Overall, findings suggest that this association could be beneficial but facultative.

The realized fecundity measured here for *H. floridana* was 821.35 ± 136.7 oocytes; another study estimated 12,000–150,000 oocytes (Luis Felaco, personal communication, 15 December 2020). These values are lower than in most other species from the same family, which may spawn hundreds of thousands to millions of oocytes during one event (Conand 1993; Mercier and Hamel 2009). This difference suggests that *H. floridana* relies on an intermediate strategy, mixing low fecundity and abbreviated nonpelagic lecithotrophic development, possibly leading to local recruitment. Values of fecundity described in holothuroids exhibiting pelagic

lecithotrophic development can be similar, higher, or lower. For example, fecundity in *H. floridana* is lower than in the tropical species *Pseudocolochirus violaceus*, which was determined to produce between 9×10^4 to 5×10^5 oocytes (Kumara et al. 2013), but higher than in the deep-sea species *Ypsilothuria talismani* that produces a maximum of 50 oocytes per individual (Tyler and Gage 1983). Overall, it is similar to the fecundity of *C. frondosa* with 8,000–60,000 oocytes per adult female (Gianasi et al. 2020). Most species of Holothuriida are planktotrophic and display longer development times expected to favour dispersal over larger scales (Conand 1993; Toral-Granda and Martínez 2007; Morgan 2009; Uthicke et al. 2009).

It remains difficult to determine whether the reproductive biology of *H. floridana* exemplifies a shift towards a dual strategy involving broadcasting and external brooding. Externally brooding species retain developing embryos either in dorsal depressions, as in *Lissothuria nutriens* (Wooton 1949), in brood chambers beneath the dorsal calcareous plates, as in *Psolus ephippifer* (Thomson 1878), in large interradial pouches surrounding the tentacular crown, as in *P. koehleri* (Vaney 1925) and *P. charcoti* (Ekman 1925), on the ventral sole, as in *P. antarcticus* (Ludwig 1897), or in pockets of the ventral sole, as in *Psolidium incubans* (Ekman 1925). An intermediate state towards a dual reproductive strategy has been documented in the deep-sea asteroid *Henricia lisa* which broods most propagules, while a few escape but still develop into juveniles (Mercier and Hamel 2008). Inversely here, a minority of oocytes were found trapped in the mucus covering the adult body wall in a facultative adult/offspring association, whereas the majority presumably developed on nearby substrata, including sea grass leaves, coral fragments, rocks, or mud, which are abundant in this habitat.

McEuen (1988) indicated that the female of *C. pseudocurata* released demersal oocytes that were moved by the podia towards the ventral surface where they were brooded. Similarly, females of *C. lubrica* were seen to capture oocytes and brood them among podia underneath the ventral surface (Engstrom 1982). The temperate sea cucumber *Rhabdomolgus ruber* was suggested to exhibit a form of brood protection provided via the secretion of a protective egg capsule, but data are limited (Eckelbarger and Riser 2013). In *H. floridana*, the oocytes seem to haphazardly adhere on the body wall surface with no involvement of the podia or other active participation of the adult, which can be either male or female. Also unusual in *H. floridana* is that hatching from this capsule occurs at the late larval stage, probably helping propagules remain firmly attached to the adult during the most vulnerable stages, from the

first few cleavages up to the early pentactula. Such assumptions are consistent with the preliminary observations made by Edwards (1888, 1909). This strategy is possibly a transition between pelagic and brooded development as the oocytes and developing propagules present some characters normally associated with brooding (McEdward and Janies 1997) but the species lacks any organized brooding mechanisms such as the involvement of the podia or the presence of a brooding site/chamber. Moreover, Sewell (1994) and Chia (1974) mentioned that characteristics associated with brooding species are lecithotrophy, a small size and hermaphroditic tendencies, among which only one character (lecithotrophy) applies to *H. floridana*, which is otherwise a large sea cucumber (maximum of 47 cm long) with separate sexes.

Characterization of the nursery habitat

In general, the Placencia Lagoon is shallow (to a maximum of 5.8 m depth), influenced by tides, and freshwater runoff in some locations (Ellison and Farnsworth 1993); it extends between mainland Belize and the Placencia Peninsula (Figure 1 B). The lagoon is 3.4 km at its widest, 20 km long, and covers a surface area of about 30 km² (Ariola 2003). The salinity fluctuations in the Placencia Lagoon are controlled by semi-diurnal tides, which have an amplitude of ~30 cm (Rützler and MacIntyre 1982). Annual rainfall varies from 2032 to 3048 mm and there are three major freshwater runoff areas, including the Santa Maria Creek, August Creek and Big Creek (Ariola 2003). During various collection efforts previously carried out in the Placencia Lagoon (Rogers et al. 2018a, b), individuals of *H. floridana* were found only from the mid-section outwards, towards the estuary, where the lagoon meets the sea, in areas where salinities were always >21 psu (Figure 1 B, shaded area).

Propagules on the adults of *H. floridana* were found in Sites 1, 2 and 4, all of which held habitats where the temperature was 30–31°C, salinity was 32–33 psu (with minimum value of 28 psu) and dissolved oxygen levels were 7.2–7.4 mg l⁻¹ (with a minimum recorded value of 6 mg l⁻¹). Moreover, salinity ranges in areas of the Placencia Lagoon where adults of *H. floridana* were found (Figure 1 B) ranged from 20 to 33 psu over the annual cycle. Both adults and juveniles were found to tolerate salinity ranges of ≥21 – 35 psu under aquaculture conditions (Rogers et al. 2019), similar to *H. scabra* (Mercier et al. 1999), defining areas of the Placencia Lagoon with potentially suitable environmental conditions.

The sediment in Sites 1, 2, and 4 where the adult-offspring association was detected (Figure 1 B, C and D) consisted of fine mud, silt and sand distributed patchily, or of seagrass meadows (*T. testudinum*, *Halophila bainii*, *Halodule beaudettei* and *Syringodium filiforme*; Figure 1 B, C and D) extending between the prop roots of red mangrove (*Rhizophora mangle*) on the peninsula and mainland sides. Mercier et al. (1999) also found several individuals of the holothuroid *H. scabra* <10 mm long on leaves of seagrass (*E. acoroides* and *T. hemprichii*) in the Solomon Islands, suggesting that they can offer both settlement substrata and food supply, and constitute nursery areas. Juveniles of *H. mexicana* (<50 mm) in Puerto Rico were found on mangrove roots either among algae or between oysters, in clumps of *Halimeda* sp., and on the seagrass *T. testudinum* (Cutress 1996). Juveniles of other species, including *Astichopus multifidus* and *Actinopyga agassizii* in Cuba, were found in seagrass meadows; those of *A. mauritiana* were found on reef flats and those of *A. echinutes* were found in coral rubble in New Caledonia (Shiell 2004). Like the latter tropical sea cucumber species, *H. floridana* share similar habitats for their early juveniles, suggesting the importance of protecting such habitats, especially to ensure successful recruitment.

Estimate of recruitment on adults in the Placencia Lagoon

Areas of the Placencia Lagoon with a salinity >21 psu and substrata that can provide habitat to *H. floridana* (i.e. seagrass, sand and mud) were estimated to spread over 14.1×10^6 m² (Figure 1 B) representing about half of the lagoon or a total surface area of 30 km² (Ariola 2003). Based on the density of adults found in those favourable areas of the lagoon (between 1 and 10 ind m⁻²; mean of 6 ind m⁻²), the abundance of *H. floridana* and the proportion of adults that hosted offspring (~15%), it can be deduced that offspring recruitment on adults in the habitable Placencia Lagoon could be between 4.2 and 42.4×10^6 (mean of 25.4×10^6). When combined to unknown settlement on all other available substrata, these data suggest that Placencia Lagoon is an important source of recruitment for *H. floridana*.

Knowing and understanding the behaviour, habitat and ecology of *H. floridana* is important in highly touristic areas like the Placencia Lagoon. Critical threats identified include dredging, mangrove clearance, water pollution, destructive boating habits and unsustainable fishing practices (Wildtracks 2015). All these activities can be detrimental to the habitats and the

sea cucumbers (adults and juveniles) in this proposed nursery area. A three-fold management goal for the Placencia Lagoon was proposed, including protecting mangrove forests and fringing ecosystems, seasonally and permanently inundated wetlands, endangered species and nursery habitats for marine life (biological); creating buffer zones for flood and wind control and to ease effects of climate change (safety); increasing tourism revenues through promotion of the Placencia Lagoon as an environmentally conscious community and eco-tourism destination (economic) and maintaining traditional rights-based low-impact fishing (traditional use) (Wildtracks 2015). The designation of the Placencia Lagoon as a Wildlife Sanctuary (Wildtracks 2015), for instance, may be one way to bolster protection. Considering the fishing activities around *H. floridana* in Belize and other countries of Central America, it is not impossible that the population decrease (Rogers et al. 2018b), especially in the shallowest depth of their distribution, as shown in *H. floridana* in Mexico by Hernández-Flores et al. (2015), could become a limiting factor for successful recruitment of that species.

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