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ORIGINAL PAPER

Spawning and larval development of the mesophotic octocoral *Swiftia exserta* in aquaria

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Abstract

The 2010 *Deepwater Horizon* oil spill injured mesophotic and deep-sea environments over a vast area. In order to restore mesophotic and deep-sea coral species impacted by the spill, information on fundamental ecosystem processes such as reproduction is needed. During expeditions in 2021 and 2022, fragments of the mesophotic octocoral *Swiftia exserta* were collected from the northern Gulf of Mexico and transported to aquaria at federal facilities in South Carolina, Florida, and Texas. In fall of 2021 and 2022, several of these fragments spawned in captivity, providing an opportunity to learn about their reproduction and inform future restoration activities. Broadcast spawning occurred on 19 and 20 October, 2021, and on 20 days from 29 September to 7 November, 2022. These spawning events permitted detailed observations of spawning behavior and timing, and yielded over 2,400 oocytes. Individual spawns were preceded by a distinctive "spawning posture" in the polyps, lasting between five minutes and two hours, and may have been cued by light. *Swiftia exserta* larvae settled and developed at comparable rates to other broadcast spawning octocorals, becoming swimming planulae by three days post spawn (dps) and starting to settle by 14 dps. These observations represent the first such records for *S. exserta* and, more broadly, for any mesophotic coral in the Gulf of Mexico, providing important insights for the restoration of these species. This investigation lays the foundation for future work to explore the influences of seasonal environmental variables, such as light and temperature, on spawning and reproductive seasonality in this species.

Keywords Octocoral reproduction · Mesophotic corals · Spawning · Larval development · Coral husbandry · Restoration · Benthic communities

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Introduction

In 2010, the *Deepwater Horizon* (*DWH*) disaster released approximately 3.2 million barrels of oil into the deep Gulf of Mexico (GOM; McNutt et al. 2012). This resulted in injury to over 770 square miles of mesophotic and deep benthic habitats extending up the continental slope from the wellhead to the continental shelf and into coastal habitats across the northern GOM (Montagna et al. 2013; Fisher et al. 2014). A Natural Resource Damage Assessment documented and quantified injuries to mesophotic and deep benthic communities (Fisher et al. 2014; Montagna et al. 2013; DWH NRDA Trustees 2016), and further investigations reported impacts to mesophotic octocorals, such as *Swiftia exserta*, in particular (Etnoyer et al. 2016; Silva et al. 2016; Frometa et al. 2017). Efforts to restore the corals injured by the *DWH* oil spill are underway (Open Ocean Trustee Implementation Group 2019), and an understanding of the ecology of these species is needed to implement targeted direct restoration approaches.

Swiftia exserta (Ellis & Solander, 1786) is a branching plexaurid octocoral found throughout the GOM at depths of 10 to 200 m (Goldberg 2001) that features prominently on mesophotic rocky reefs (Etnoyer and Cairns 2017). As in other regions, mesophotic and deep gorgonian assemblages in the GOM are key structural components of benthic communities, providing important ecosystem services and supporting associated species (Weaver et al. 2001; Krieger and Wing 2002; Buhl-Mortensen and Mortensen 2005; Stone et al. 2014). In addition to capturing suspended particulate material while feeding, the branching morphologies of the colonies create physical relief, modifying water flows, affecting sedimentation, and generating microhabitats for demersal fishes and epibenthic invertebrate species (Krieger and Wing 2002; Buhl-Mortensen and Mortensen 2005; Peccini and MacDonald 2008; Kahng et al. 2011). Despite its prevalence in the northern GOM and status as a primary target of direct restoration following the *DWH* oil spill (Frometa and Etnoyer 2022), little is known about *S. exserta* reproduction. Information on the reproductive ecology of *S. exserta* can help inform conservation and restoration activities.

Among octocorals, gonochorism is the dominant reproductive strategy, with species employing one of three modes: broadcast spawning, internal, or external brooding (reviewed in Kahng et al. 2011). While octocorals are capable of asexual, vegetative propagation, all species examined to date also reproduce sexually (reviewed in Kahng et al. 2011). Populations of octocoral species do not always exhibit an equal sex ratio (1:1 females: males), as sex ratios are often female-biased, including several reports of extremely rare or absent males (Brazeau and Lasker 1989; Coffroth and Lasker 1998; Kahng et al. 2011). Some octocoral species reproduce periodically or seasonally, with discrete, often annual spawning periods (Ribes et al. 2007 and references therein; Orejas et al. 2007), while others reproduce aperiodically or quasi-continuously, producing multiple cohorts of gametes throughout the year (Dahan and Benayahu 1997; Eckelbarger et al. 1998; Kahng et al. 2008). Octocoral reproduction has been linked to environmental factors such as water temperature (Santangelo et al. 2003; Ribes et al. 2007; Rossi and Gili 2009; Kahng et al. 2011; Gomez et al. 2018) and lunar rhythms (Kahng et al. 2011; Coelho and Lasker 2016a). However, for many octocoral species, the relationship between environmental cues and spawning remains unknown. Moreover, most knowledge about the reproductive biology of octocorals comes from shallow-water species, with very little being known for mesophotic or deep-water species.

In addition to fundamental aspects such as reproductive mode and seasonality, studying the dynamics of larval development can provide valuable insights for restoration, although these remain unknown for most mesophotic and deep-water coral species (Waller et al. 2023). From shallow species and the few examples of mesophotic and deep-water octocorals with available data, larval development seems to vary by species, reproductive mode, and locality (Kahng et al. 2011; Waller et al. 2023). For internally brooding species, embryogenesis occurs inside the polyps of the parent colony, and the resulting planulae are released to crawl or swim to their recruitment location (Kahng et al. 2011). In broadcast spawning and externally brooding species, embryogenesis takes place either in the water column (broadcast spawning species) or on the surface of the parent colony (externally brooding species; Kahng et al. 2011).

In octocoral species studied so far, the development from a fertilized oocyte to a planula larva can take place in a matter of days, leading to observations of metamorphosis and recruitment within one week of spawning (as in *Plexaura kuna*, Lasker and Kim 1996 and *Dendronephthya hemprichi*, Dahan and Benayahu 1998). However, larval development of other species can take longer, with planulae formation within 48–72 h and settlement within 3–5 weeks of spawning (*Rhytisma fulvum*, Benayahu and Loya 1983; *Paramuricea clavata,* Linares et al. 2008; *Alcyonium acaule*, Teixido et al. 2016). Octocoral species may produce swimming (pelagic) or crawling (demersal) larvae, and these mobility characteristics, in combination with reproductive mode, contribute to variation in larval dispersal. Local larval retention has been linked with brooding species that produce demersal larvae, while broadcast spawning species that produce pelagic larvae have been associated with the capacity for broader larval dispersal (Harrison and Wallace 1990; Stimson 1978; Gutiérrez-Rodríguez and Lasker 2004;

Figueiredo et al. 2014; Coelho and Lasker 2016b). These variations in larval dispersal capacity have implications for population connectivity and, therefore, an understanding of larval dispersal potential and dynamics is a key component of a successful long-term restoration strategy (Linares et al. 2008; Coelho and Lasker 2016b; Banaszak et al. 2023).

Spawning and larval development in corals often takes place over a brief timeframe that, if not witnessed directly, is easily overlooked and difficult to predict. The bias of the current body of work toward shallow species can be partially attributed to their accessibility to divers for repeated observations. Although corals in the deep sea and mesophotic zones are less accessible due to their deeper habitats, they are no less important to understand, particularly as they are threatened by anthropogenic impacts. The collection and maintenance of coral colonies or fragments from these deeper environments in aquaria can offer an opportunity for close observation and experimentation that would not otherwise be feasible.

In this study, we report observations of spawning in captivity by *S. exserta* fragments collected from mesophotic depths in the northern GOM. To our knowledge, this study is the first to document spawning or describe any aspect of reproduction in this species. We also report on other features of reproduction, including sex ratio, larval development, and settlement. This work aims to address key knowledge gaps with relevance to the restoration of this and other mesophotic octocoral species in the GOM and provide a foundation for future investigations of specific larval characteristics, as well as comparisons to other members of mesophotic and deep benthic communities.

Materials & methods

Collection of *Swiftia exserta*

During October of 2021 and June, July, and September of 2022, expeditions in the northern GOM used remotely operated vehicles (ROVs) to collect fragments of *Swiftia exserta* colonies from mesophotic (60–100 m) depths (Table 1; Fig. 1). These expeditions targeted the GOM regions of the Pinnacles Trend and De Soto Canyon Rim (PT&DSR), and the Flower Garden Banks National Marine Sanctuary (FGBNMS) expansion areas (Expansion of Flower Garden Banks National Marine Sanctuary 2021). Despite revisions to the genus *Swiftia* (McFadden et al. 2022), the identity of *S. exserta* from these sampling sites in the northern GOM has been confirmed using multiple methods including molecular phylogenetic analysis (Frometa et al. 2021) and was recently affirmed (McFadden et al. 2022). There were missions to PT&DSR in October 2021, June 2022, and October 2022, and missions to FGBNMS in July and September 2022. The cruises to PT&DSR, PS-22-08, and PS-22-22, nicknamed the "Submerged Acquisition of Living Tissue" (SALT) cruises, took place on the R/V *Point Sur* and utilized the ROV *Mohawk* (University of North Carolina, Wilmington; UNCW). The expeditions in FGBNMS, MT-22-MERCI1, and MT22-MERCI2, nicknamed the "Mesophotic Expedition for Restoration of Corals and other Invertebrates" (MERCI) cruises, took place on the R/V *Manta*, with collections by the ROVs *Beagle* (Marine Applied Research & Exploration; MARE) and *Mohawk* (UNCW). In total, samples from 44 live *S. exserta* colonies were collected to populate aquaria in three U.S. government laboratories (Table 1 and Online Resource 1). These samples will hereafter be referred to as "specimens" to indicate

Table 1 Summary of *S. exserta* collections for husbandry on expeditions to the northern Gulf of Mexico in 2021 and 2022

Region	Locality	Dates	Vessel	ROV	# S. exserta specimens collected for husbandry	Facility	Fragments subsampled between labs?
DSR	Pensacola Edge 01	10/4/21–10/8/21	RV *Point Sur*	*Mohawk*	8	HML (5) WARC (8)	Yes
PT&DSR	Pensacola Edge 01 Salt Ridge 3 Mountaintop Reef Boulder Field 4 Boulder Field 3	5/31/22–6/11/22	RV *Point Sur*	*Mohawk*	18	HML (6) WARC (12)	No
FGBNMS	East Flower Garden Bank Bright Bank Bright Bank Pinnacles	7/24/22–7/29/22	RV *Manta*	*Beagle*	6	SEFSC (6)	No
FGBNMS	Elvers Bank	9/11/22–9/16/22	RV *Manta*	*Mohawk*	12	SEFSC (6) WARC (12)	Yes

Regions include Pinnacles Trend (PT) and De Soto Rim (DSR) and the Flower Garden National Marine Sanctuary (FGBNMS). The labs where specimens were kept were the Hollings Marine Laboratory (HML), the Southeast Fisheries Science Center (SEFSC), and the Wetland and Aquatics Research Center (WARC). Collected specimens were sometimes further subdivided to provide husbandry fragments to multiple labs. The number of specimens held at each lab is indicated in the parentheses following the lab name

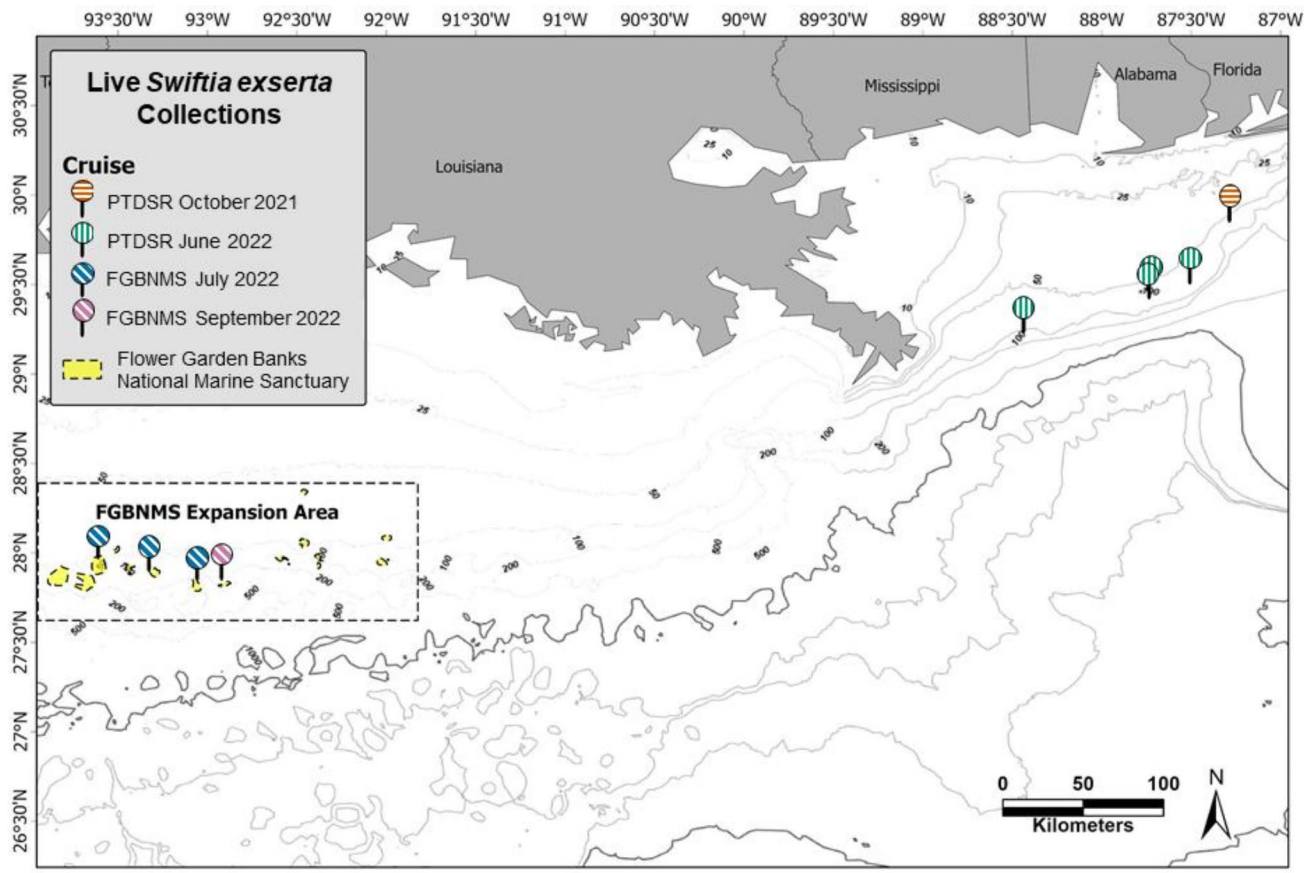

Fig. 1 Live *Swiftia exserta* collection locations in 2021 and 2022. Markers indicate the locations where samples were collected in PT&DSR (Pinnacles Trend and De Soto Canyon Rim) in October 2021 (green vertical pattern) and June 2022 (orange horizontal pattern), and in FGBNMS (Flower Garden Banks National Marine Sanctuary) in July 2022 (blue diagonal pattern) and September 2022 (pink diagonal pattern)

their individual identities (i.e. samples collected from different source colonies). Some of the live *S. exserta* specimens were further divided into multiple fragments to be distributed to multiple aquaria (Online Resource 2). Therefore, the term "fragment" does not carry a connotation of unique source colony identity, as multiple fragments may be collected from a single source colony or generated from a single specimen through subdivision.All *S. exserta* fragments were maintained in 3.8 L canisters on board for the remainder of the expeditions, with twice-daily water changes using bottom water collected by CTD casts.

Colony size measurement

Sizes of *S. exserta* colonies that were sampled for husbandry were estimated in situ using digital stills from ROV video recorded during sampling on all cruises. Using 10-cm lasers for calibration, the linear extension at the tallest point of the colony was measured using ImageJ (Schneider et al. 2012). Images with calibration lasers in place were not available for all samples, so the colony sizes reported in Online Resource 3 correspond to 35 of the 44 colonies sampled for husbandry on PS-22-08, PS-22-22, MT-22-MERCI1, and MT-22-MERCI2.

Histological sampling

Subsamples of *S. exserta* fragments held at WARC that were suspected to be involved in the spawning event in 2021 ($n=6$ individuals) were preserved in 10% buffered formalin after active spawning had concluded, then transferred to 70% ethanol after 24 h and shipped to the University of Maine Darling Marine Center in Walpole, ME for histological analysis. In 2022, subsamples were taken from most fragments collected for husbandry during the 2022 field season, preserved in 10% buffered formalin for 24 h, then transferred to 70% ethanol. Additional *S. exserta* samples were collected at sea for sex ratio analysis (total histology samples including samples of husbandry specimens: $n=60$), preserved as described in formalin and transferred to ethanol for shipping and storage. After preservation, the 2022 samples were shipped to the National Oceanographic

and Atmospheric Administration (NOAA) Hollings Marine Laboratory (HML) for histological analysis. Samples were handled in much the same way at both the Darling Marine Center and HML: they were decalcified in 1.5% ethylenediaminetetraacetic acid or RDO Rapid Bone Decalcifier® (Globe Scientific) and dehydrated in a graded ethanol series to 70% ethanol. At the Darling Marine Center, further dehydration to absolute ethanol, clearing in toluene, and paraffin infiltration occurred by hand, while at HML, samples were dehydrated to absolute ethanol, cleared with xylene, and infiltrated with paraffin using a HistoCore Pearl® automatic tissue processor (Leica Biosystems). Following wax infiltration, samples were embedded in paraffin blocks and sectioned using a rotary microtome. The 6-µm-thick sections were then mounted on slides and stained with hematoxylin and eosin.

Maintenance in aquaria

Upon returning to port, coral fragments were immediately transported either by car or shipping overnight to federal facilities, where they were gradually acclimated to aquaria for long-term residence. Coral fragments were transported and maintained live in aquaria at three U.S. government labs: the NOAA HML in Charleston, SC, the NOAA Southeast Fisheries Science Center (SEFSC) in Galveston, TX, and the U.S. Geological Survey Wetland and Aquatic Research Center (WARC) in Gainesville, FL. At each facility, coral fragments were maintained in closed aquarium systems consisting of two 325.5 L insulated black ABS plastic tanks with a 132.5 L insulated sump (783.6 L total per system). At SEFSC, some corals were also held in a 208.2 L glass aquarium with a 75.7 L glass sump. Fragments were divided between tanks according to collection region, such that corals in a single tank were all from the same region. These systems were maintained at 21–23 °C, to match the temperature of the collection location. These temperatures were consistent throughout the year, with no attempt to recreate seasonal temperature fluctuation. In 2021, aquarium facilities were lit with ambient light as needed for work, but in 2022, following observations of spawning in 2021, light exposure emerged as a focal area for investigators and adjustments were made at some facilities to enable better lighting control. At HML, the clear acrylic lids permitted ambient overhead lighting to reach the corals during working hours, generally 08:30 to 17:30, in both 2021 and 2022. At SEFSC, the tanks were exposed to ambient light, but in 2022 they were shielded with black plastic sheeting. As a result, although the room was lit from 07:30 to 16:00, six days of the week in both years, in 2022 the PAR in the tanks was 0, except when uncovered for maintenance, observation, and feeding, when it measured between 2 and 4. At WARC, the tanks experienced ambient lights in 2021 and 2022, which came on automatically from 08:00 to 18:00, with a PAR of 5–10 in the tanks measured in 2022.

At all facilities, coral fragments were fed a mixture of frozen rotifers, copepods (*Cyclopoida* sp.), live *Artemia* sp. nauplii, Polyp Lab® Reef Roids®, and powdered spirulina. This mixture was suspended in seawater and target fed to each fragment by pipette either twice (HML and SEFSC) or five times daily (WARC), six days a week. At HML, the spawning tank contained eight females, one known male, and one specimen of unknown sex. At SEFSC, one spawning tank contained four females and two males, and the other contained five females and one male. At WARC, one spawning tank contained seven females and five males, and the other contained four females, three males, two non-reproductive individuals, and eight specimens of unknown sex. Additional details of coral collection and distribution among lab facilities can be found in Online Resources 1 and 2.

Spawning activity in federal labs

Observations of spawning in aquaria from *S. exserta* samples collected in 2021 were unexpected, so few observations were recorded. In 2022, however, *S. exserta* fragments were monitored daily for evidence of spawning (gametes in the water column) or indications that spawning might soon take place (spawning posture assumed by polyps). If spawning was observed, the aquarium pumps were turned off, the released oocytes were collected and, if fertilized, the resulting larvae were carefully monitored. A "spawning event" was defined as the period of observed gamete release. If polyps had assumed a "spawning posture," with thickened, shortened tentacles (Fig. 2b, c), the aquarium pumps were turned off and that colony was monitored closely until it resumed a normal posture or released gametes. As a result of reliance on observation to identify spawning in aquaria, the frequency of spawning events and number of oocytes collected may have been influenced by observer effort and availability. If spawning went unnoticed, the circulation of the system would quickly flush any gametes out of the aquarium, removing not only the products of the spawn but also all evidence that a spawning event had occurred. Thus, the frequency of spawning and total number of oocytes collected may be an underrepresentation of total spawning by *S. exserta*.

There were differences among the labs in the ways that individual spawns were managed, oocytes and larvae were treated, and observations were recorded (summarized in Table 2). In all cases, spawned oocytes were collected by pipette and deposited in secondary containers to watch for signs of fertilization. Oocytes that had not divided after

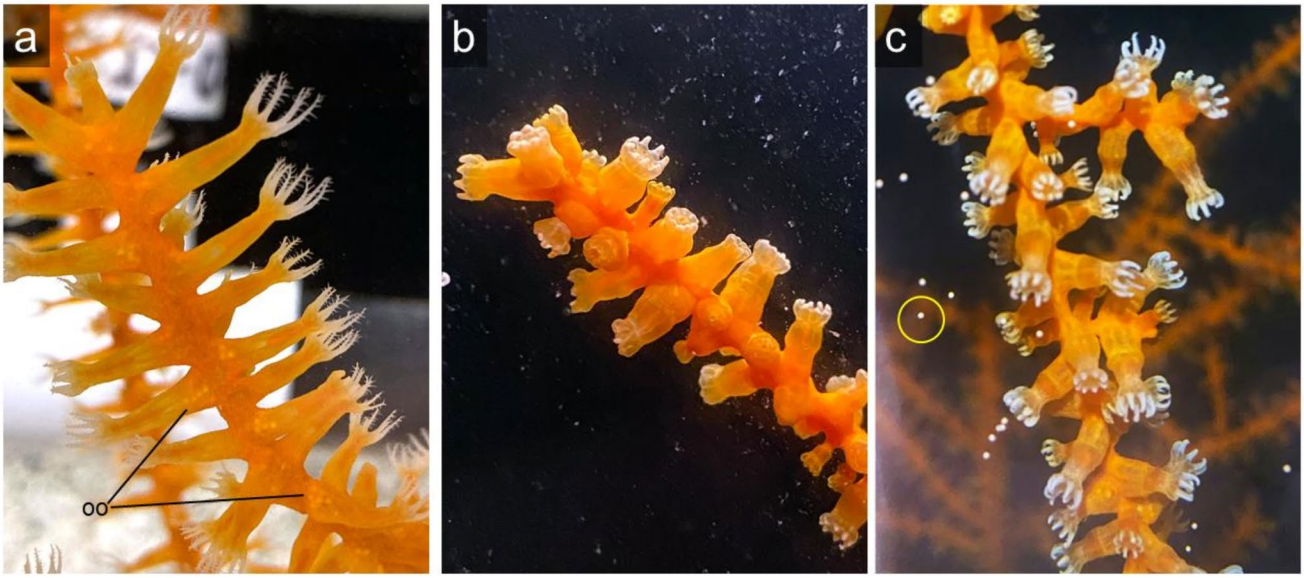

Fig. 2 Spawning activity in *Swiftia exserta*. (**a**) Polyps of a female colony of *S. exserta* with unreleased oocytes visible inside the polyps ("oo" indicates oocytes). (**b**) *Swiftia exserta* polyps in spawning posture. Tentacles are retracted/clubbed, and polyp trunk is retracted and thickened. (**c**) *Swiftia exserta* polyps in spawning posture releasing oocytes (one oocyte circled in yellow)

Table 2 Spawning in federal labs by *Swiftia exserta* in 2021 and 2022

Year	Facility	Early larvae		Water changes		Observations	Settlement offered		Post-settlement	Feeding
		Container	Environmental control	Amount	Frequency		Type/container	timing		
2021	HML	50-mL conical tube	Floated in adult aquarium	30–40%	Daily	Daily	Rubble from adult system, 50-ml conical tube	7 dps	N/A	N/A
	WARC	50-mL conical tube	Cooler in aquarium room maintained at 20–22 °C	50%	1–2 days	4–6 h until fertilization confirmed, then daily, every 1–3 days after settlement	Rubble from adult system. Settlement bin in 75.7 L "nursery" aquarium	9 dps	Settlement tiles moved out of bin onto racks in nursery aquarium	Frozen rotifers, copepods (Cyclopoida sp.), live *Artemia* sp. nauplii, Polyp Lab® Reef Roids®
2022	SEFSC	250-mL cups	Floated in adult aquarium	30–40%	daily	4–6 h until fertilization confirmed, then daily	Aragonite tiles. Settlement bin suspended in main aquarium	4 dps	Settlement tiles moved to main aquarium	Frozen rotifers, copepods (Cyclopoida sp.), live *Artemia* sp. nauplii, Polyp Lab® Reef Roids®
	WARC	200-mL cups, divided into groups of 20 ooctes/cup	Cooler in aquarium room maintained at 20–22 °C	50%	1–2 days	4–6 h until fertilization confirmed, then daily, every 1–3 days after settlement	Rubble from adult system and CCA-conditioned tiles. Settlement bin suspended in main aquarium	12 dps	Settlement tiles moved to racks in main aquarium	Frozen rotifers, copepods (Cyclopoida sp.), live *Artemia* sp. nauplii, Polyp Lab® Reef Roids®

Methods of managing spawning by *S. exserta* and the resulting larvae varied across federal facility and spawning year. Spawning occurred at the Hollings Marine Laboratory (HML), the Southeast Fisheries Science Center (SEFSC), and the Wetland and Aquatic Research Center (WARC). Settlement containers consisted of ~1-L plastic or acrylic containers with 105- to 200-µm mesh windows. Dps = days post spawn

12 h were discarded. When the larvae became elongated and less active, they were moved to customized settlement bins installed in the husbandry aquaria with adult fragments. Substrates for settlement varied across lab facilities and were provided at different times (Table 2). At HML, rubble from the main system was provided at seven days post spawn (dps). At SEFSC, larvae were presented with aragonite tiles to settle on when they were moved to the settlement bins. At WARC, rubble from the main system and tiles conditioned with crustose coralline algae (CCA) were provided at nine dps. In 2022, a separate substrate choice experiment was initiated at WARC with results to be reported in a separate, forthcoming publication. Following settlement and metamorphosis into primary polyps, the larvae were offered food in the form of frozen rotifers, copepods (*Cyclopoida* sp.), live *Artemia* sp. nauplii, and Polyp Lab® Reef Roids®.

Results

Sizes of husbandry source colonies in situ

Of the 44 *Swiftia exserta* colonies sampled for husbandry, 35 were measured from images captured in situ prior to sampling. These colonies ranged from 17.30 cm to 153.30 cm of linear extension at their tallest point, reported as "height" (Online Resource 3). The average colony height was 71.28 cm, with a standard deviation of 33.63 cm. Of the colonies measured, 10 were directly observed to spawn, eight females and two males. These colonies ranged in height from 38.29 cm to 153.3 cm, with an average height of 103.27 cm and a standard deviation of 37.75 cm. Thus, the smallest colony whose fragments were directly observed to spawn in aquaria was 38.29 cm in height. The smallest colony characterized as "reproductive" by the presence of gametes was 29.49 cm. All colony heights are reported in Online Resource 3. This evidence suggests that female *S. exserta* colonies of at least 29.49 cm in height are sexually mature.

Histological result & sex ratio

Six adult coral fragments collected from Pensacola Edge in PT&DSR in October 2021 and held at WARC were subsampled following spawning events on 19 and 20 October of that year and examined using histology (Fig. 3). All but one of the subsamples had evident gametes, indicating that four were female, one was male, and one was non-reproductive. Of the 84 *S. exserta* fragments collected in the 2022 field season for husbandry or reproductive analysis, 75 were sexed either using histology or the observed release of gametes. The remaining nine were not subsampled for histology or observed to release gametes, and their sexes remain unknown. In total, 49 females, 20 males, and six apparently non-reproductive fragments were identified by histological examination or observed gamete release. Thus, the observed sex ratio for these *S. exserta* individuals stands at 8.2: 3.3: 1 female: male: non-reproductive fragments. All of the apparently non-reproductive fragments were collected in June, five months before spawning, and the spermatocysts contained in fragments identified as male indicated spermatogenesis had only recently begun. This may indicate that spermatogenesis takes place over a relatively short timeframe (several months), and may not have begun at the time of collection in June. If all non-reproductive fragments are assumed to be males collected before the advent of spermatogenesis, the sex ratio becomes 1.9:1, female: male fragments. Of the 16 fragments that were observed to spawn in captivity, 14 were female and two were male, based on histological investigation and observed release of gametes.

Spawning events

Spawning was observed in a subset of *S. exserta* fragments in both 2021 and 2022. All spawning events occurred in the fall (September and October). In 2021, spawning behavior in tanks containing *S. exserta* fragments collected from Pensacola Edge in the PT&DSR region (Fig. 1) was observed on the morning of October 19th at HML and WARC, and again on the morning of October 20th at WARC only. Spawning was evidenced by observations of corals assuming spawning posture (Fig. 2b, c) and/or oocytes in the surrounding water (Fig. 2c). Spawned oocytes were approximately 350–400 μm in diameter. In females with visible oocytes (Fig. 2a), spawning posture occurred prior to spawning and was maintained several hours beyond the period when oocytes were actively being released. Fragments involved in the spawning event had returned to normal polyp behavior within 24 h. As fragments were held together in large tanks, rather than in isolation, and due to monitoring limitations, it was not possible to determine with confidence how many fragments contributed to each spawning event beyond those directly observed releasing oocytes. At HML, four oocytes were collected, with two preserved immediately in 10% buffered formalin and two retained for further observation. From the two days of spawning at WARC, approximately 150 oocytes were collected, with the majority transferred to 50-mL centrifuge tubes. Of the 150 oocytes collected, 32 developed into larvae and were transferred to individual 10-mL centrifuge tubes for further observation.

In the fall of 2022, *S. exserta* spawning was observed at SEFSC and WARC, but not at HML, on 20 days, with the first spawn on 29 September and the last spawn on 7 November (Fig. 4). Again, spawning was noted after the

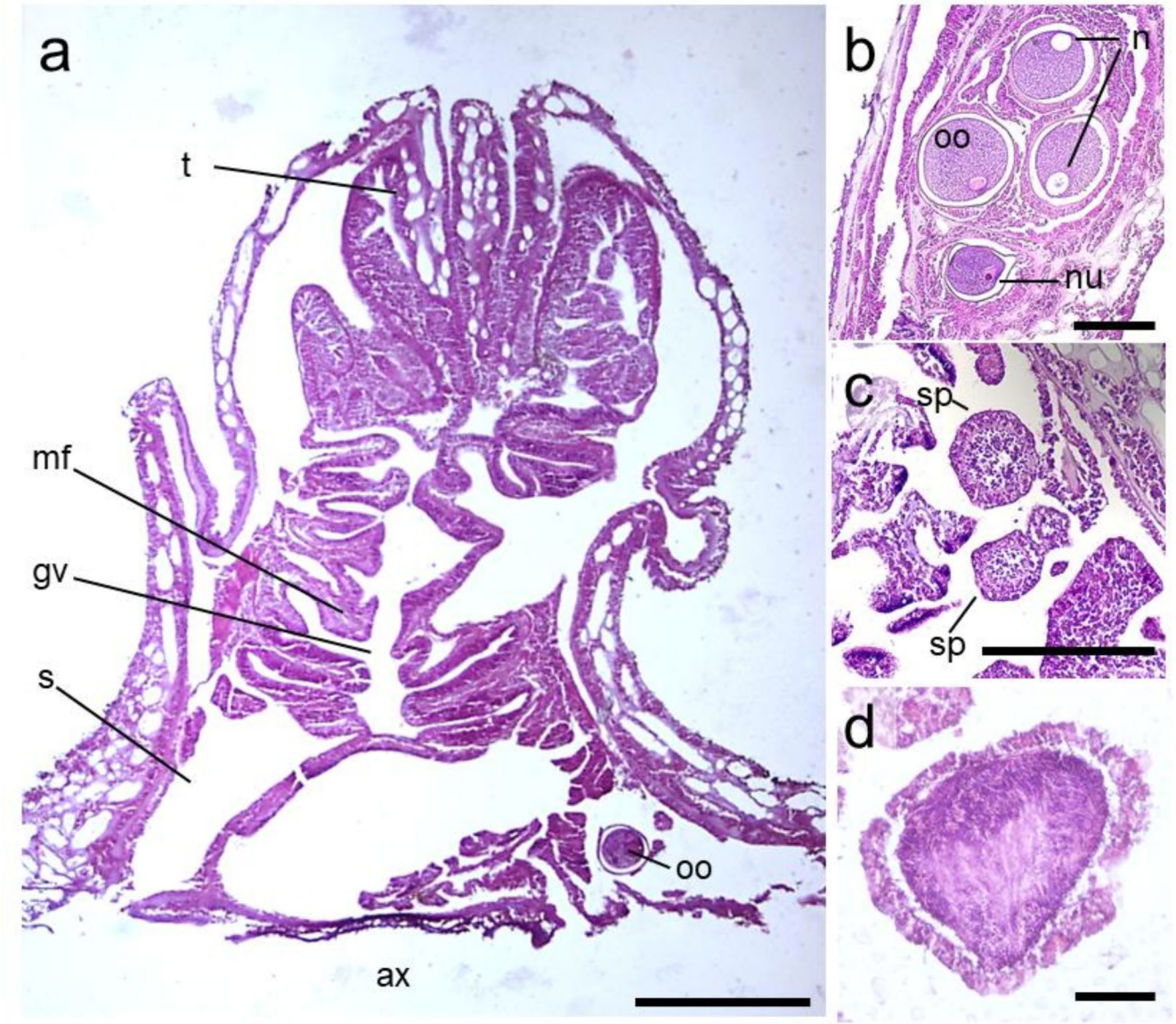

Fig. 3 Polyps and gametes of *S. exserta* as examined using histology and light microscopy. (**a**) Longitudinal section showing full polyp of *S. exserta*. t: tentacles, mf: mesenterial filaments, gv: gastrovascular cavity, s: septa, oo: oocyte, ax: space left after axis removed. Scale bar = 100 µm. (**b**) Cluster of previtellogenic oocytes. oo: oocytes, n: nucleus, nu: nucleolus. Scale bar = 250 µm. (**c**) Early-stage spermatocysts (sp). Scale bar = 250 µm. (**d**) Single late-stage spermatocyst. Scale bar = 50 µm

observation of spawning posture and the appearance of oocytes in the water. Spawning in 2022 was observed by fragments collected up to five months prior, with no repeat spawning or spawning behavior observed in any of the fragments collected in 2021. Over the course of the 2022 spawning period, 2,267 oocytes were collected.

Fragments of *S. exserta* collected from Boulder Field 4 in the PT&DSR region in June 2022 spawned at WARC on 17 days, with participation from fragments of at least three colonies, one of which was observed to spawn on 15 occasions (see Online Resource 2 for details). Over the course of these spawns, spawning posture was observed in advance of nine spawning events (Online Resource 2). Fragments of *S. exserta* collected from Elvers Bank in the FGBNMS in September 2022 spawned at WARC on 10 days, with participation by fragments of at least six colonies, including fragments of two colonies that were observed to spawn five times. Over the course of the 2022 period, 1,394 oocytes were collected from WARC, with an approximately 90% fertilization rate.

Spawning by fragments of two colonies of *S. exserta* collected in July 2022 from Bright Bank and Bright Bank Pinnacles in the FGBNMS was observed in aquaria at SEFSC on six days. From each of these spawns, up to 42 oocytes

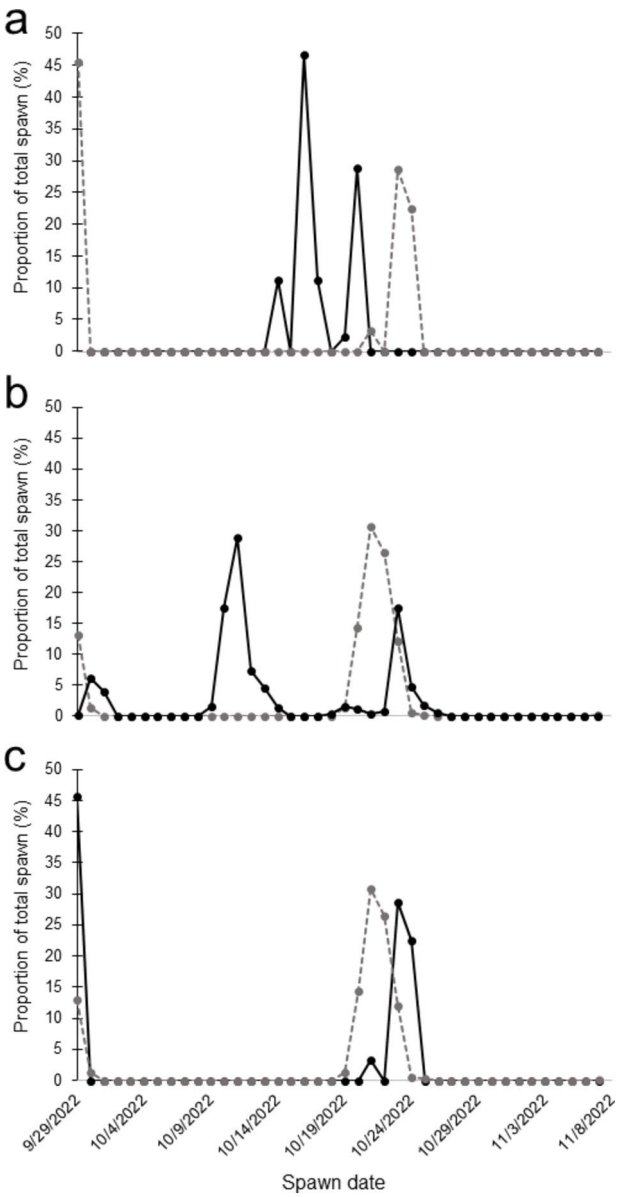

Fig. 4 Spawning effort in *Swiftia exserta* fragments collected in 2022 and held at federal facilities, displayed as the percentage of total oocytes collected from spawning fragments. (**a**) Spawning by fragments housed at the NOAA Southeast Fisheries Science Center collected from the FGBNMS (Flower Garden Banks National Marine Sanctuary) region. Data from fragments collected in June 2022 is shown by the solid line and data from fragments collected in September 2022 is shown by the dashed grey line. (**b**) Spawning by fragments held at the USGS Wetland and Aquatic Research Center collected from the PT&DSR region in June 2022, shown by the solid line, and the FGBNMS region in September 2022 shown by the dashed grey line. (**c**) Spawning by specimens collected from the FGBNMS in September 2022, subdivided at sea into two fragments and distributed to the NOAA Southeast Fisheries Science Center (solid line) and USGS Wetland and Aquatic Research Center (dashed grey line). No spawning was observed at the NOAA Hollings Marine Laboratory in 2022

were collected, resulting a total of 105 collected oocytes (see Online Resource 2 for full details). A single colony of *S. exserta* collected in September 2022 from Elvers Bank in the FGBNMS was also observed to spawn at SEFSC, and it spawned at least four times between 29 September 2022 and 24 October 2022. Over the course of these spawns, 768 oocytes were collected with 25–350 oocytes collected from each individual spawn. Although sperm release was not always observed, two *S. exserta* fragments were seen releasing sperm during spawning events. Across *S. exserta* spawning events at SEFSC in 2022, a total of 873 oocytes were collected, with near complete fertilization success.

Spawning start time, the time that gamete release was first observed, was recorded at WARC for *S. exserta* fragments collected in the PT&DSR region for 14 of 17 spawning events and 9 out of 10 spawning events for *S. exserta* fragments collected from FGBNMS (Johnstone et al. 2023; Online Resource 2). Across both WARC and SEFSC, spawning duration ranged from one hour and 40 min to three hours and 10 min after lights were turned on in the aquarium facility (Online Resource 2). The duration of spawning (first and last oocyte release) ranged from 5 to 32 min at WARC, and 30 min to 100 min at SEFSC. For fragments collected from PT&DSR, oocytes from 12 spawning events were confirmed to be fertilized and proceeded through cell division, while six spawning events did not yield fertilized oocytes (of the 488 total oocytes collected, 25 were unfertilized). In contrast, spawned oocytes from all spawning fragments collected from FGBNMS were confirmed to be fertilized despite only two events with corresponding observations of sperm release at WARC. In one instance, oocytes released by a fragment collected from Boulder Field 4 in PT&DSR were exposed to water from a tank holding spawning fragments collected from Elvers Bank in the FGBNMS. These oocytes were ultimately fertilized, and the resulting larvae were clearly labeled and raised in the main aquarium system following settlement as described for other larvae in 2022.

Polyps of the fragments collected from Elvers Bank in the FGBNMS in September 2022 were noted to have assumed a spawning posture ahead of six spawns at WARC, usually between one and two hours ahead of spawning, but as late as 13 min before spawning on one occasion. Polyp posture appeared to differ among male and female fragments and often was adopted by only a portion of the total number of polyps present on a fragment. The timing of spawning posture was not recorded at SEFSC.

Larval production & developmental timeline

In 2021, larval development was closely observed (Figs. 5 and 6). Assuming fertilization during the spawning events, developing embryos reached the 16-cell stage within four

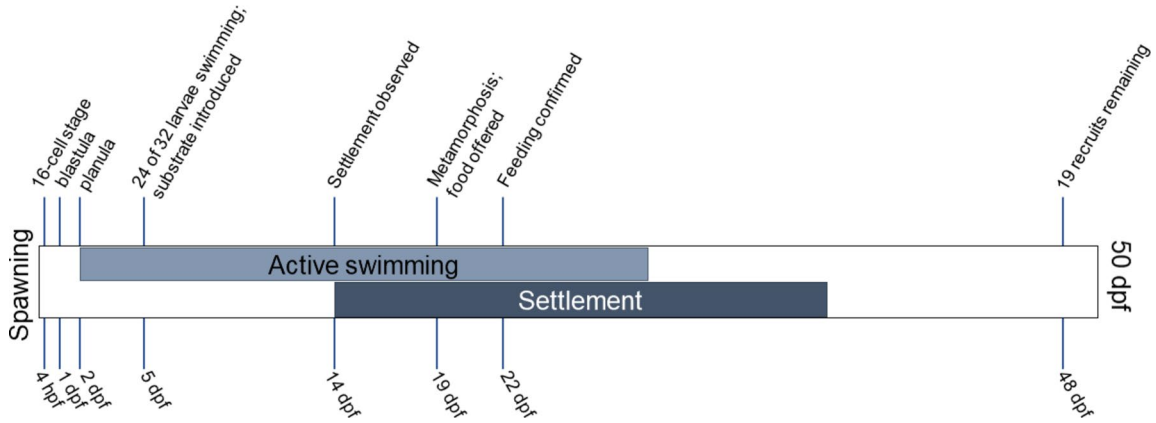

Fig. 5 Developmental timeline of *Swiftia exserta* larvae spawned in federal labs in 2021. *Swiftia exserta* embryos reached the 16-cell stage within four hours post fertilization (hpf) and became blastulae by one day post fertilization (dpf), becoming planulae by two dpf. By five dpf, at least 24 of the original 32 embryos had elongated into peanut-shaped planula larvae. Substrate was introduced at this point and the first settlement was observed on November 2nd, 14 dpf, on a piece of rubble. Metamorphosis from settled larva to primary polyp occurred at approximately 19 dpf, at which point food was offered, but no feeding was observed. Settled recruits were confirmed to feed at 22 dpf. By 38 days after the spawning event, there were 19 total recruits, many that had settled on the underside of tiles. As of 48 dpf, all 19 recruits remained

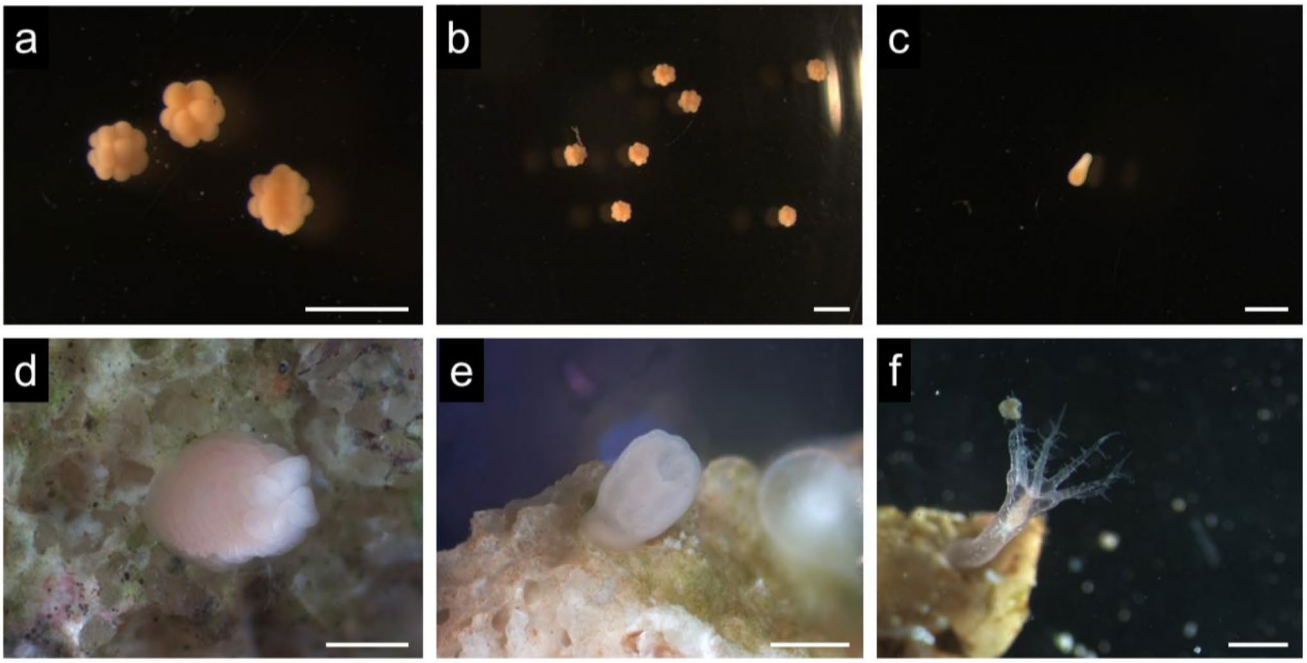

Fig. 6 Development of *S. exserta* from 4-hour-old embryos to primary polyps. (**a**) Oocytes and embryos four hours after spawning. Smooth oocytes, 8-cell, and 16-cell embryos were all present and distinguishable at this point. (**b**) Embryos one day after spawning, all appear at 16-cell stage and beyond. (**c**) Planula larva of *S. exserta* one week after spawning. (**d**) Newly settled *S. exserta* larva imaged on November 2, 2021, two weeks after spawning. (**e**) Recently settled *S. exserta* larva imaged on November 4, 2021, 16 days after spawning. (**f**) Primary polyp with pinnate tentacles, sclerites, and a brine shrimp visible in its gastrovascular cavity, imaged on November 10th, 21 days after spawning. All scale bars are 1 mm

hours of fertilization (Fig. 6a, b), with signs of gastrulation at two dps (Fig. 6c). Embryos prior to gastrulation ranged from approximately 550–700 µm in diameter, and after becoming larvae were larger and elongated, approximately 750–900 µm from end to end. The larvae were highly mobile swimmers, initially spending most of their time near the surface, but sinking over the following days to either crawl or appear to rest on the bottom of their containers. Unsettled larvae did not appear to have a mouth, and were presumed, therefore, to be lecithotrophic. Substrate was introduced in the form of rubble from the husbandry aquaria systems holding adult fragments or CCA-conditioned tiles

(Online Resource 3). The first settlement was observed in 2021 at WARC at 14 dps, on a piece of rubble. In the following days, more larvae settled (Fig. 6d, e), commonly on CCA-conditioned tiles. Newly settled, the larvae measured approximately 1–1.5 mm from base to apical surface. Settled recruits were confirmed to feed at 22 dps, and in some settlers (Fig. 6f), feeding began as quickly as three days after attachment to the substrate. Despite this progress by settled recruits, some larvae were still swimming. As of 48 dps, 19 recruits had settled and were being fed periodically. During this period, the larvae that had not settled were lost to mortality and appeared to rapidly disintegrate. Larvae spawned in 2022 also appeared to follow the timeline observed in 2021, where the 16-cell stage was reached within 4–6 h of spawning, gastrulation occurred within 48 h of spawning, and planulation had proceeded by five dps. In 2022, settlement was observed at 10 dps ($n = 204$ at WARC and 193 at SEFSC).

Discussion

This description of *Swiftia exserta* spawning and larval development is the first for the species and is among the first for mesophotic octocorals (but see Grinyo et al. 2018 and Liberman et al. 2018 for descriptions of spawning and larval development in *Paramuricea macrospina* and *Rhytisma fulvum*, respectively). To date, most detailed accounts of natural spawning and larval development have concentrated on shallow-water (0–30 m depth) coral species (reviewed in Coelho and Lasker 2016b; Wangensteen et al. 2017), and while efforts are underway to expand this focus to include mesophotic coral species (30–150 m depth), the majority of studies dedicated to that goal have considered scleractinian species (reviewed by Shlesinger and Loya 2019). From work on shallow octocorals and other cnidarians, a general pattern of development has been established, but many knowledge gaps remain, including temporal aspects of larval formation and settlement (Coelho and Lasker 2016b; Shlesinger and Loya 2019; Waller et al. 2023). Basic observations such as those in this study represent major steps toward a more complete understanding of these taxa.

Insights into *Swiftia exserta* reproductive ecology

Based on our results, *S. exserta* appears to be a gonochoric species, with separate male and female colonies that broadcast spawn their gametes for external fertilization in the water column. As in other octocorals, histological analysis or observed gamete release is the only way to conclusively determine the sex of a given colony, and in these samples the observed sex ratio was strongly female-skewed (8.2: 3.3: 1, female: male: non-reproductive specimens). Given the immature status of most of the male fragments (very early-stage spermatocysts) and the timing of collection of the fragments used to determine this ratio, it is possible that some or all of the individuals deemed "non-reproductive" were actually males collected prior to the start of gametogenesis. As the indicators for sex are the gametes themselves, males with no spermatocysts present at the time of collection would be misidentified as non-reproductive. If all non-reproductive specimens are recharacterized as males, the sex ratio becomes 1.9: 1, female: male, presenting a female-biased sex ratio as seen in other octocoral species (reviewed in Kahng et al. 2011). In their review of octocoral reproduction, Kahng et al. (2011) found that a 1:1 sex ratio was only reported for approximately half of the octocoral species for which a sex ratio was available. In most of the other cases, the sex ratio was female-biased (Kahng et al. 2011), as reported here.

The alternative to this hypothesis is that fragments with no apparent gametes are, in fact, non-reproductive, either due to immaturity or some other factor. In other octocorals, sexual maturity, (i.e., onset of reproduction), is correlated with the colony or polyp reaching a minimum size (Harrison 2011; Kahng et al. 2011). For example, *Pseudopterogorgia elisabethae* fragments are not reproductive until they reach 18–20 cm in height (Gutiérrez-Rodríguez and Lasker 2004); *Paramuricea clavata* fragments become sexually mature at an average of 20 cm tall (Coma et al. 1995); *Leptogorgia sarmentosa* male fragments are not reproductive at sizes less than 21 cm in height (Rossi and Gili 2009); and *Corallium rubrum* colonies require sizes of 4–6 cm in length before attaining 100% fertility (Tsounis et al. 2006). The smallest source colony for an *S. exserta* fragment in this experiment that went on to spawn in captivity and was observed releasing gametes was 38.29 cm tall, but gametes were observed in a colony that measured 29.49 cm tall. Based on these observations, colonies as small as 29.49 cm in height appear to be reproductively mature.

Further histological analysis to compare *S. exserta* fragments collected from different times of year, aggregations, and locations across a range of colony sizes would bolster the reported sex ratio, and enable estimates of fecundity, reproductive periodicity, and synchrony across geographic scales. Recording morphometrics of oocytes and spermatocytes from fragments collected at different times could also help resolve outstanding questions about seasonal patterns of reproduction in *S. exserta*.

Spawning observations

In 2021 and 2022, spawning of *S. exserta* in captivity was synchronized across aquaria with spawning behavior

observed at multiple federal labs often within hours of each other, if not concurrently. Observations from 2022 demonstrated that spawning is repeated and ephemeral, lasting no more than a few hours. An extended spawning period of 29 days occurred, spanning the month of October, as opposed to the discrete one- and two-day events observed in 2021. A multi-week spawning period that includes regular, repeated spawning has been reported for other octocoral species, including *Eunicella singularis* and *C. rubrum* (Viladrich et al. 2022). Some fragments of *S. exserta* collected in 2021 and 2022 were split upon collection, with subsamples of the same specimens distributed to more than one lab for husbandry. Subsamples then spawned simultaneously in both locations, suggesting conserved endogenous timing for spawning within a single source colony. For example, after collection from Elvers Bank in the FGBNMS in September 2022, some individual *S. exserta* specimens were split and shared between SEFSC and WARC. Direct comparisons between spawning volume and timing between fragments show a tight coupling, with initial spawning on the same day at both labs, and a second substantial oocyte release at SEFSC occurring within two days of a similar oocyte release at WARC (Fig. 4c, Online Resource 2). Not all specimens were split and distributed across multiple federal labs. Instead, fragments of some colonies were housed at one lab only, and fragments of other colonies, sometimes collected on the same mission, were housed at another. Again, spawning proceeded concurrently at multiple locations, providing further evidence for endogenous control of reproduction among fragments from the sampled *S. exserta* populations.

Although not every spawn was matched across multiple labs, the first spawns of the six-week spawning period in 2022 proceeded within three hours of each other, with SEFSC-held corals spawning first and WARC-held fragments following shortly thereafter. On many of the days that spawning was observed at one lab, it was also observed at another facility. These observations suggest that spawning may be seasonal or periodic, with some degree of endogenous control or programming, as the participating fragments had been held in captivity for up to five months previously with no exposure to the seasonal environmental fluctuations that can often control spawning timing in the wild. However, the observations of spawning in captivity do not provide evidence for the tight synchronicity seen during the "mass spawning" events characteristic of other coral species. In these cases, discrete spawning events are synchronized, in some cases to the minute, across populations of a given species spanning vast geographic areas (Harrison 2011; Kahng et al. 2011; Wolstenholme et al. 2018). In the spawns described in this study, spawn timing, volume, and participation seemed to vary between fragments without clear patterns or trends to suggest differences based on collection time, collection location, depth, or lab facility. For example, it was common for only one or two individuals to spawn on a given day, with other individuals spawning later, earlier, or not at all (Online Resource 2). It is, however, possible that *S. exserta*, in order to achieve high synchronization, relies on specific cues in the wild (light, temperature, or some other variable) that were not provided in captivity, as other corals have been shown to depend on a hierarchy of cues to precisely time their mass spawning events, which range in scale from solar irradiance to locally released pheromones (Fogarty and Marhaver 2019). Thus, further investigation to identify contributing factors may reveal new aspects of *S. exserta* spawning synchrony and timing.

One potential environmental cue is the lunar cycle, especially as the 2021 spawning events were observed within 24 h of the full moon in October. Other shallow-water corals and some deep-sea octocorals are known to spawn predictably, cued to lunar rhythms (Szmant 1986; Wyers et al. 1991; van Veghel 1993; Lasker et al. 1996; Sun et al. 2010a, b; Marhaver et al. 2015; Rakka et al. 2021), lending support to a lunar component for the timing of spawning in *S. exserta*. The observation of only two events in 2021, tightly coupled to the full moon, and the replication of those observations across two lab facilities initially seemed to indicate that spawning in this species could be highly synchronized and potentially tuned to the lunar cycle. However, this correlation was not repeated in 2022. The October full moon in 2022 was on October 9th, 10 days after the first spawning events at WARC and the SEFSC, and 30 and 21 days before the last spawns were observed at WARC and SEFSC, respectively. The one and two-day events of 2021 that happened to be observed near the full moon were more likely part of an extended spawning period as reported in 2022 but with other events that went overlooked.

Another environmental cue that contributes to spawning timing in other coral species and may be at play in *S. exserta* is temperature (Kahng et al. 2011; Gomez et al. 2018). Data from temperature loggers deployed at a collection site in PT&DSR to 65-m revealed that between June 2022 and May 2023, the warmest month was August, when temperatures averaged 22.63 °C. However, it is possible that the impact of Hurricane Ian (September 23–30) disrupted or obscured a seasonal temperature trend toward even warmer temperatures in September and October, indicating that *S. exserta* typically spawns during the warmest months of the year. Alternatively, it may be the case that *S. exserta* spawning takes place in the weeks following the warmest water temperatures. Offsets from peak temperatures in spawning have been reported in other octocoral species: *Leptogorgia alba* spawns two months after the coldest temperatures of the year, while *Muricea austera* and *Pacifigorgia ferruginea* spawn one month prior to the warmest temperatures

(Gomez et al. 2018). Long-term monitoring of seawater temperature at a FGBNMS collection site revealed September and October to be the warmest months of 2020, 2021, and on average, 1990 to 2015 (Johnston et al. 2022). The correspondence between the warmest months of the year and spawning activity may indicate a role for temperature in spawning periodicity and synchronization.

Observations from fall 2022 also suggest a potential link between ambient light and spawning. The onset of spawning at WARC ranged from one hour and 45 min to three hours and 15 min after the lights came on in the facility. Photoperiod in the laboratory settings were not modulated to match seasonal variation, and SEFSC and WARC, the two facilities with substantial spawning in 2022, took different approaches to modulating light exposure. At SEFSC, the tanks were shielded with plastic sheeting, such that the corals experienced PAR readings of 0–4 when the room lights were on, six days of the week, and complete darkness on the seventh day. At WARC, the tanks were exposed to ambient light on an automated schedule, so the corals experienced 5–10 PAR for 10 h of the day, all days of the week. Both facilities saw successful spawning, and both saw a consistent interval between initial exposure to light and the onset of spawning. The possible connection between light and spawning events in *S. exserta* warrants exploration, as dynamics and characteristics of available light, including, for example, solar insolation (Penland et al. 2004), daily light cycles (Boch et al. 2011), and spectral shifts between sunset and moonrise (Boch et al. 2011; Sweeney et al. 2011; Brady et al. 2016; Kaniewska et al. 2015), have been established as influences on spawning timing in other coral species. In order to characterize the role of light on spawning in *S. exserta*, dedicated trials are needed to disentangle the effect of light availability from that of coincident variables such as seasonal temperature changes (Keith et al. 2016) or tidal conditions (Wolstenholme et al. 2018) that the corals would experience in a natural environment (Craggs et al. 2017).

A key outstanding question is the impact of long-term husbandry on reproductive condition. With only two years of spawning data to suggest that spawning in *S. exserta* is an annual event, it remains to be seen if fragments held in aquaria for the full duration of gametogenesis can be relied upon to spawn repeatedly in captivity. In 2022, none of the fragments collected in 2021 spawned, regardless of whether they had participated in spawning in October 2021. To date, successful spawning has been demonstrated by fragments held in captivity for up to five months. Learning more about how to maintain adult fragments in lab aquaria could facilitate the culturing of "broodstock" and reduce dependency on collection from natural populations to support restoration efforts. Additionally, while successful sexual reproduction in captivity could be a critical tool for species restoration, there are several environmental variables, as described, in the laboratory facilities that were not tightly matched to in situ conditions, so care should be taken to apply the results presented here to wild populations with caution. Some aspects, such as the onset of spawning in the fall and the pacing of larval development, are more likely to be shared by wild populations, than others, such as, perhaps, the duration of spawning season, which may be longer or shorter in situ.

Larval development

Embryos from the *S. exserta* spawning events became buoyant, active-swimming larvae within 2–3 days. This is a similar developmental pace to other broadcast spawning octocorals, including the Caribbean species, *Plexaura homomalla* (Tonra et al. 2021)d *kuna* (Lasker and Kim 1996), the Mediterranean gorgonian *Paramuricea clavata* (Linares et al. 2008), and the deep-sea plexaurid *Dentomuricea* aff. *meteor*, despite the *S. exserta* embryos experiencing temperatures more than 10 °C warmer than *D.* aff. *meteor* (Rakka et al. 2021). After spending a minimum of several days swimming near the surface, *S. exserta* larvae sank to the bottom of their container and began to search for a suitable settlement location. This biphasic larval behavior before settlement was also documented in *Antillogorgia americana* (Coelho and Lasker 2016a). In *A. americana*, although larvae were apparently capable of settlement as soon as four days after spawning, they usually remained in the water column for longer than one month (Coelho and Lasker 2016a). Other shallow-water octocorals develop more quickly, such as *D. hemprichi*, which was seen to begin metamorphosis as soon as two days after spawning (Dahan and Benayahu 1998), and *P. homomalla*, whose larvae are competent to settle within four days of spawning (Tonra et al. 2021). The *S. exserta* larvae studied here started to settle within two weeks of spawning, with larvae becoming feeding primary polyps at approximately four weeks after spawning. The relatively long pre-competency period observed in the current work compared with other octocoral species might be attributed to differences between shallow and mesophotic octocoral life histories, or it could indicate that the husbandry conditions presented were not ideal for settlement, resulting in a delayed settlement by the studied *S. exserta* larvae.

Future larval behavioral observations and settlement choice trials could help provide a more detailed picture of larval behavior prior to settlement and inform the type of substrate to present. These results might then, in turn, reveal knowledge gaps that could be addressed with further trials during future spawning events, such as the impact

of substrate on pre-competency period or settlement success. Fixed specimens from larval behavioral trials could also support future work to correlate form and function, for example, using scanning electron microscopy to resolve structures such as cilia and comparing the timing of their development with observations of swimming. Of particular interest would be the characteristics and activity of larvae before they settle, such as larval position in the water column, swimming speed, time to settlement, and substrate preference, as these may influence larval dispersal and are useful for the construction of models of larval dispersal.

Conclusions

These preliminary observations provide key pieces of missing information necessary for the successful restoration of one species of octocoral, *S. exserta*. To our knowledge, this is the first record of spawning, or indeed any aspect of sexual reproduction, in this species. The results presented here, including reports of reproductive periodicity, spawning behavior, and larval development, are fundamental components of a complete understanding of population maintenance in these valuable mesophotic coral communities. Histological analysis and observations of spawning revealed a female-skewed sex ratio, as is reported in other octocoral species. The observations of spawning in captivity at multiple federal lab facilities even when controlling for environmental factors in aquaria suggest that October may be an important month for *S. exserta* reproduction, that spawning activity may be linked to temperature or light levels, and that spawning timing is likely controlled by a hierarchy of cues. Additionally, the observations of developing larvae reveal patterns that are consistent with other mesophotic octocorals, including the pace of development and the time to settlement. Work to discern relationships between spawning effort or timing and exogenous or endogenous factors such as environmental or chemical cues can help further our understanding of the reproductive ecology of these species, and, ultimately, how they can be best supported by restoration efforts.

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Declarations

All applicable guidelines for sampling, care, and experimental use of organisms were followed, but as this work was conducted with an unregulated invertebrate species, no approvals from research ethics committees were required. This work was supported by the Coral Propagation Technique Development project. Funding for the Mesophotic and Deep Benthic Communities Coral Propagation Technique Development Project was provided by the Open Ocean Trustee Implementation Group to restore natural resources injured by the 2010 *Deepwater Horizon* oil spill in the Gulf of Mexico. The authors have no relevant financial or non-financial interests to disclose. All authors contributed to the study conception, design, and manuscript preparation. Julia Johnstone prepared the histological material, drafted the manuscript, compiled the figures (except for the map, which was provided by E. Salgado), and analyzed the data. Will Jenkins, Mackenzy Jankeiwicz, Jonathan Quigley, Janessy Frometa, Enrique Salgado, and Ben Higgins collected the data across the two years of spawning and provided edits and images during the drafting of the manuscript. Amanda Demopoulos, Chris Gardner, Peter Etnoyer, and Kris Benson supported the work as federal project managers or lab leads. All authors read and approved the final manuscript. The datasets generated and/or analyzed during the current study are included as online supplementary information. The scientific results and conclusions, as well as any views or opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA, the Department of Commerce, USGS, or the Department of the Interior. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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