Biology of a deep-water sea anemone (Anthozoa: Actiniidae) from eastern Canada: spawning, development, and growth

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Abstract

Knowledge of the general biology and reproductive ecology of deep-water species can help predict their resilience to environmental and anthropogenic disturbances. The present study centers on live specimens of a deep-water sea anemone which were collected at bathyal depths between 1100 and 1400 m and kept in a mesocosm for over 6 years. Morphology and DNA sequencing confirmed that the species belongs to the genus *Urticina*. Male and female (9-10 cm pedal disk diameter, 90 tentacles) spawned 4 years post collection, in early spring (March). Both sexes released gametes through the mouth. The negatively buoyant oocytes (550-600 μ m in diameter) quickly settled on the rocks and soft sediments surrounding the female. Lecithotrophic embryonic and larval development occurred on the substratum. Fully developed planula larvae were detected after 17-21 days. Planulae started to crawl and swim around but remained demersal. Metamorphosis and settlement occurred after 30-35 days, exclusively on hard substrata and preferentially on undersurfaces. Offspring grew slowly, developing 8 tentacles after 5 months and 24 tentacles after 12 months (3-4 mm pedal disk diameter). After 2.5 years of growth, the captive-born sea anemones reached 12-16 mm in pedal disk diameter and possessed 48-54 tentacles.

1. Introduction

Pelagic and benthic members of phylum Cnidaria have successfully colonized freshwater and saltwater environments all around the world (Rogers, 2009). They are particularly diversified and abundant in marine habitats, from coastal shores down to the deepest oceanic trenches. Studies of deep-sea cnidarians, including aspects of their distribution and biology, have largely

focused on corals (Cairns, 1982, 2007; Watling et al., 2011; Young, 2003) due to the fact that they create complex tri-dimensional habitats and play major roles in ecosystem functioning (Baillon et al., 2012; Buhl-Mortensen and Mortensen, 2005; Cerrano et al., 2010; Roberts et al., 2006). This focal interest has deflected attention from other very abundant cnidarians of the deep, including members of order Actiniaria (sea anemones). Sea anemones are reportedly abundant on rocky bottoms of bathyal and abyssal zones, and around hydrothermal vents (Aldred et al., 1979; Rodríguez and Daly, 2010); as an example, Aldred et al. (1979) indicated that the sea anemone *Actinoscyphia aurelia* could reach densities of up to 6 ind m⁻² at depths between 500 and 2000 m in the Northeast Atlantic.

Despite their abundance, deep-water sea anemones remain poorly studied, and the topic of a limited number of ecological or biological investigations (details below). From samples collected at bathyal depths in the Southern Ocean, the externally brooding *Epiactis georgiana* was determined to exhibit superfetation (several cohorts of offspring developing simultaneously) and seasonal release of embryos/larvae in the austral spring (Rodríguez et al., 2013). Van Praët and Duchateau (1984) and Van Praët (1990) revealed the existence of reproductive seasonality in correlation with the flux of organic matter at 2000 m in the sea anemone *Paracalliactis stephensoni* from the Bay of Biscay. Similarly, seasonal patterns of spawning in *Phelliactis hertwigi* and *P. robusta* from bathyal depths in the Porcupine Seabight (northeast Atlantic) were described by Van Praët et al. (1990), whereas Bronsdon et al. (1993) reported that *Amphianthus inornata* released gametes in the spring in the Porcupine Abyssal Plain (PAP). The sea anemone *Allantactis parasitica*, which lives on deep-sea gastropods in the Northwest Atlantic spawned twice a year in the laboratory, during the spring and fall peaks of phytodetritus abundance (Mercier and Hamel, 2009a). A few species were found not to display any detectable seasonal

breeding, including the sea anemone *Kadosactis commensalis*, living as commensal on holothurians in the PAP (Bronsdon et al., 1993), and two carpet anemones (order Zoanthidae) associated with hermit crabs, *Epizoanthus paguriphilus* (770-1065 m) and *E. abyssorum* (3749-4400 m) (Muirhead et al., 1986).

It is expected that most deep-water species will exhibit slow growth and great longevity (Gage and Tyler, 1991) compared with shallow-water counterparts, although data on growth rates of deep-sea cnidarians are scarce. Current knowledge is mostly centered on banding patterns and/or radiometric analysis of skeletal elements in corals (e.g. Andrews et al., 2002; Hamel et al., 2010; Mortensen and Rapp, 1998; Risk et al., 2002; Roark et al., 2009; Sherwood and Edinger, 2009). While these techniques cannot be used in sea anemones due to the absence of a hard skeleton, growth can also be estimated from the study of live specimens in the laboratory (Cordes et al., 2001; Hamel et al., 2010; Orejas et al., 2011) or in nature (Brooke and Young, 2009). To our knowledge, the only data available for deep-water actinarians were provided by Mercier and Hamel (2009a), who estimated that the sea anemone *A. parasitica* required 6-11 years to reach its maximum size, based on the growth of newly-settled juveniles to 15 months.

Sea anemones are ubiquitous in deep waters of the Northwest Atlantic. In some locations of the rocky continental slope of Newfoundland and Labrador (eastern Canada), densities between 5 and 9 ind m⁻² have been recorded (DFO, unpublished data). Genera whose members are commonly collected as trawl bycatch include *Urticina*, *Bolocera*, *Hormathia*, and *Actinostola*. Among these, individuals of the genus *Urticina* are the most frequently encountered. A wide range of life styles and reproductive habits have been reported so far in shallow-water species of this genus, reflecting both significant diversity within the group and raising questions

about its taxonomy (Fautin et al., 2005). Genetic studies (e.g. Solé-Cava et al., 1985; 1994) have failed to find differences among the North Atlantic species, but the circumscription of new species from within the previously global *Urticina crassicornis*, *U. eques*, and *U. felina* (Hand, 1955; Hauswaldt, 1999; Sanamyan and Sanamyan, 2006) have refined and limited the circumscription of the North Atlantic species.

Urticina (=Tealia) *crassicornis* was initially reported as a broadcast-spawner in Europe (Appellöf, 1900) and defined as a brooder on the west coast of the USA (Hand, 1955). Chia and Spaulding (1972) later confirmed that *U. crassicornis* in northwestern USA fits the description of Appellöf (1900), whereas it is viviparous in the Barents Sea (Kaliszewicz et al., 2012). Most species described so far exhibit seasonal breeding. Individuals of *U. lofotensis* along the coast of California (USA) were shown to reach full maturity and spawn at the end of December (Wedi and Fautin Dunn, 1983). Studies of the reproduction of *U. felina* off the coast of Newfoundland (eastern Canada) revealed that planulation peaked in August and that internal brooding could lead to chimerism (Mercier et al., 2011b; Sun et al., 2012). Larson et al. (2012) reported seasonal external brooding in the similar-looking, co-occurring species *U. fecunda*.

Limited knowledge is currently available on deep-water sea anemones and, to our knowledge, nothing is known of the biology of *Urticina* species that are common at bathyal depths. The purpose of the present work was to gain a better understanding of spawning, gamete behaviour, development, and growth of a deep-water sea anemone of the genus *Urticina* commonly collected along the continental slope of eastern Canada. Findings shed light on the traits and adaptations that make this species successful in heavily fished deep waters.

2. Materials and Methods

2.1 Collection and maintenance

Animals were obtained as bycatch from routine research surveys conducted by Fisheries and Oceans Canada (DFO) in December 2009 with the CCGS *Teleost* along the continental slope off Labrador (56°26'N, 57°36'W), at depths ranging between 1100 and 1400 m. Surveys followed a stratified random sampling design with a Campellen 1800 trawl towed for 15 minutes on ~1.4 km of seafloor (gear opened and closed at depth). A total of 7 large sea anemones (9-10 cm pedal disk diameter, ~90 tentacles), assumed to be of the same species based on morphology, were collected and kept onboard the ship in a large flow-through tank (5000 L). Live specimens were brought back to the laboratory, where they were kept in a mesocosm, consisting of a 600-L tank supplied with running ambient seawater (~300 L h⁻¹) under darkened conditions. Temperature in the tank ranged annually between -1 and 8°C, according to a temperature-light logger HOBO Pendant (UA-002-64). A chilling unit (Universal Marine Industries, 5 hp) was used for in-line cooling of seawater when ambient coastal temperatures were higher than those characteristic of the bathyal environment in eastern Canada (DFO, 2009). The bottom of the tank was filled with 15 cm of fine mud and scattered with a few boulders (15-20 cm in diameter) and deep-water coral skeletons to mimic the natural habitat. Sea anemones were fed periodically, generally once a month, either with complete live sea urchins (*Strongylocentrotus pallidus*), frozen deep-sea shrimps (*Eualus*, *Pandalus*, *Spirontocaris*) or shucked mussels (*Mytilus* sp.).

2.2 Taxonomic characterisation, DNA extraction and sequencing

Small tissue samples were taken from the pedal disk of three of the seven specimens collected and preserved in 100% ethanol. Total genomic DNA was extracted from the samples

with the DNeasy kit (QIAGEN). Using previously published primers (Supplementary Table 1), Template DNA, and illustra puReTaq Ready-To-Go PCR Beads the 12S, 16S, 18S, and partial 28S ribosomal genes were amplified following the thermalcycler profile: 4 min at 94° C, 30 cycles of 15 sec at 94° C, 1 min at 52° C, 1 min at 72° C, and 10 min at 72° C for the final extension step. PCR products were sequenced on the Applied Biosystems 3730xl DNA Analyzer by staff at Beckman Coulter Genomics (Danvers, MA). The forward and reverse sequences were assembled in the program Geneious version 7.1.7 (Kearse et al., 2012) and compared against the nucleotide database in GenBank (using BLAST searches) to determine whether the targeted loci were sequenced. The new sequences for *Urticina* sp. and *U. felina* have been deposited in Genbank (KT265262-KT265273). For our phylogenetic inference the newly acquired sequences of 12S, 16S, 18S, and 28S ribosomal genes were used in combination with previously characterized sequences (Daly et al., 2008; Rodríguez et al., 2014). For each ribosomal gene, the DNA sequences were aligned separately in MAFFT v7.017 (Katoh et al., 2002) using the search strategy L-INS-I and default parameters (scoring matrix: 200PAM/k=2; Gap opening penalty: 1.53; Offset value: 0.123). The concatenated alignments were then subjected to maximum likelihood analyses in RAxML v.7.2.8 (Stamatakis, 2006) using the GTR+ Γ option and allowing for the α parameter to be estimated for each marker independently. Clade support was determined using 1,000 bootstrap replicates (Fig. 1).

A whole specimen of *Urticina* sp. was deposited at the Canadian Museum of Nature (Ottawa, Canada) under the reference number CMNI 2015-0012.

2.3 Spawning and development

The mesocosm holding the sea anemones was monitored daily, and sometimes more often. When noted, the release/presence of gametes, including fertilized eggs (based on elevation

of the fertilization envelope) was documented. Propagules were regularly sampled to assess morphological changes and establish the development kinetic: once every hour during the first day, then roughly every day up to the planula stage, and once a week following settlement. A minimum of 10-15 individuals were examined on each occasion. They were described morphologically and photographed using a stereomicroscope (Nikon SMZ 1500) or a microscope (Nikon Eclipse 80i) coupled to a Nikon DXM1200F digital camera. Measures (either Feret diameter or maximum length of the elongated propagules) were taken using Simple PCI Imaging System®. A new stage of development was considered attained when ~50% of the individuals reached it.

Most of the fertilized eggs (~80%) were left in the mesocosm with the parents and the remaining propagules were transferred to 20-L flow-through culture vessels (~40 L h⁻¹) under similar environmental conditions. When propagules reached the planula larva stage, they were tested for substrate preference among the following: glass (microscope slide), plastic (tank bottom/walls), mud (0.2-5.0 nm particle diameter) and small rocks (3-5 cm). The larvae were exposed to a single substrate at a time (apart from the tank material itself).

2.4 Growth

The growth of 6 juveniles that had settled naturally on small rocks was monitored for 2 years. Measurements were taken every 30-60 days of the pedal disk diameter, the column height, and the number of tentacles. No food was provided to post-settled sea anemones apart from the natural occurrence of inorganic and organic particles and live plankton (including phytoplankton, copepods, and invertebrate larvae) present in the running seawater supply. Feeding of the juveniles on crustaceans (copepods) was confirmed visually.

Estimates of the age at full size were made using curve fitting with various models (e.g. linear or exponential growth, exponential rise to a maximum). Growth estimates were obtained from increments in pedal disk diameter measured in juveniles of *Urticina* sp., similar to the method used previously for sea anemones (Mercier and Hamel, 2009a) and other cnidarians (Cordes et al., 2001).

3. Results and Discussion

3.1 Species identification, habitat and density

The sea anemones under study were confirmed to belong to the genus *Urticina*, but did not fit the description of any known species of the genus, which are notoriously difficult to identify. The specimen examined for this description measured 9-10 cm in disk diameter and 10-11 cm in column height (when fully deployed) and possessed ~90 tentacles (7-9 cm long). The column is stout and cylindrical, without verrucae, and the whole individual including tentacles, oral disk and column is uniformly light pink in colour (Fig. 2). Darker pink lines run from the mouth to edge of the oral disk, between the tentacles.

The phylogenetic analysis placed both *Urticina* sp. specimens together; with high bootstrap support values supporting their position alongside the shallow-water *Urticina felina* and *Urticina coriacea* (Fig. 1). In phylogenetic tree, the species of *Urticina*, including the one under study, group together with strong support, but their affinity to other species or groups of species is unclear because the internal nodes in the tree are all short and lack support. However, the *Urticina* clade is not closely allied to the clade that includes the other deep-water species (*Bolocera* and *Liponema*), highlighting that the deep-water sea anemone fauna is diverse in terms of its evolutionary affiliations.

Urticina sp. is morphologically and genetically similar to *U. felina*, but in addition to occupying different depth strata, they exhibit different life styles and reproductive modes. In eastern Canada, *U. felina* broods its offspring to the planula stage, before releasing them in the water column (Mercier et al., 2011b), whereas *Urticina* sp. studied here is a broadcast spawner (details below). Attachment of the pedal disk can be used to distinguish live individuals: in *Urticina* sp., the pedal disk is the same diameter as the column (Fig. 2A), whereas in *U. felina* the pedal disk swells to form a larger base. In addition, the uniform light pink colour of *Urticina* sp. differs from the more patterned pigmentation exhibited by other *Urticina* species in eastern Canada (personal observations). Finally, the tentacle tips of *Urticina felina* are generally thinner and more pointed than those of *Urticina* sp., which are blunted.

Urticina sp. is a large sea anemone found exclusively on hard substrates (Fig. 2), including rocks and coarse gravel, like most species of the genus *Urticina* (Chia and Spaulding, 1972; Larson et al., 2012; Sun et al., 2012). Some of the specimens collected were still attached to small boulders (up to 15 cm in diameter) and occasionally to pieces of wood. *Urticina* sp. was absent from trawls conducted on muddy or sandy bottoms. In the mesocosm, which had both mud and rocks, *Urticina* sp. was almost always found attached to the latter and, occasionally, to the glass walls. Based on 23 research trawls conducted in November-December 2013, between 78 to 1782 individuals per trawl were counted, representing densities of 0.04 to 0.94 ind m⁻² (unpublished data). However, trawling is not an efficient method to collect firmly attached benthic species, and these numbers probably underestimate the density of *Urticina* sp. in the surveyed area.

3.2 Spawning

Urticina sp. is gonochoric and iteroparous like *U. felina* (Mercier et al., 2011b), *U. fecunda* (Larson et al., 2012), and *U. crassicornis* (Chia and Spaulding, 1972). Spawning of the female was only directly witnessed once, starting on the evening of March 28, 2013 and continuing until the next morning (Fig. 2A). Between 1000-1200 eggs were visually detected, either floating around the spawner or already laying on the substrate. Transit time from the site of release (mouth) to the substrate was up to 60 min under the study conditions. The male was seen to release faint wisps of sperm over 1-2 h on the morning of March 29 (Fig. 2B), roughly coinciding with the end of the female spawning. However, the eggs collected on the evening of March 28 (550-600 μ m in diameter) showed signs of development (elevation of the fertilization envelope) supporting the possibility that the male had started spawning earlier, in synchrony with the female (or even before), and that sperm release was too faint to be detected easily. It might be continuous or sporadic over the long 12-h spawning period. In some species, the release of sperm in the water column may trigger female spawning (Clark and Dewel, 1974; Nyholm, 1949; Siebert, 1974). In contrast, the deep-sea epibiotic sea anemone *Allantactis parasitica* living in association with deep-sea gastropods releases gametes in brief mass events, coincident with the breeding aggregations exhibited by its host (Mercier and Hamel, 2009a). Should a protracted gamete release at the individual level be confirmed in *Urticina* sp., it might offset the constraints of a sedentary lifestyle and relatively low densities, potentially maximizing fertilization success. Combined with negative oocyte buoyancy (see below for details), which would favour philopatry and the maintenance of reproductive densities, this spawning strategy could explain the success of this species in bathyal habitats of the Northwest Atlantic. Other adaptations suspected to favour fertilization across distant benthic conspecifics in deep-sea invertebrates include longer

spermatozoa mobility and competency, and retention of gametes close to the adults until fertilization in echinoids (Young 1994), as well as in the deep-sea coral *Flabellum angulare* (Mercier et al., 2011a).

Both the male and female of *Urticina* sp. released gametes through the mouth (Fig. 2A, b) while the column and tentacles were fully extended. The upper section of the gastrovascular cavity of the female was slightly visible during spawning (Fig. 2A) whereas the male oral cavity was forming a protruding conical shape (Fig. 2B). Newly-released sperm dispersed rapidly in the water column and oocytes initially floated in mid-water, about 10-12 cm from the bottom. Eventually (inside 1 h), the oocytes (or fertilized eggs) sank to layer the rock on which the female was attached and its immediate vicinity (Fig. 2C). Shick and Lamb (1977) described broadcasted oocytes that remained attached by mucus close to the base of the female in *Diadumene leucolena* along the Atlantic coast of North America. In *Urticina* sp., no oocytes were seen to adhere to the spawning female, and no mucus was apparent. None of the gametes were ingested, even though the tentacles were deployed and prey of similar sizes had previously been accepted by the same individual (e.g. copepods).

Urticina sp. spawned at the end of March, a period when most shallow-water species of benthic marine invertebrates reproduce along the eastern coast of Canada (Mercier and Hamel, 2010). This period coincided with a major increase in phytoplankton and zooplankton as well as slight increase in bottom temperature, including in deep waters (DFO, 2009). Spawning and larval release by many deep-sea cnidarians from the same geographic area are reportedly cued by phytodetritus (in press; Baillon et al., 2014; Mercier and Hamel, 2009a, 2011; Sun et al., 2010; Sun et al., 2011), and the coincident temperature cycle. Deposition of surface-derived particles at depth has been suggested to trigger lipogenesis during late gametogenesis in *Paracalliactis*

stephensoni (Van Praët and Duchateau, 1984), further supporting the role of marine snow in the reproductive cycle of deep-water sea anemones.

3.3 Development

Broadcasted oocytes were ~550-600 µm in diameter, orange in color, and negatively buoyant. Non-brooded negatively buoyant lecithotrophic oocytes are very rare and, as far as we know, have never before been reported in any sea anemone or deep-sea invertebrate before except perhaps in the deep-sea scleractian coral *Flabellum angulare* (Mercier et al., 2011a). Eggs that are released in the water column may exhibit different properties, in part through manipulation of their biochemical composition, as seen in sea stars that produce eggs of similar volumes but different buoyancies by altering the total amount of lipid present while leaving the protein levels unchanged (Prowse et al., 2008). Negative buoyancy of pelagic eggs likely favours recruitment in the natal habitat (philopatry), similar to brooding strategies, and could partly explain the patchy distribution of *Urticina* sp. in deep waters of the western Atlantic. All other deep-sea free-spawning lecithotrophic marine invertebrates from the same geographic area, including corals, echinoderms, and other sea anemones release pelagic oocytes that are positively buoyant (Mercier and Hamel, 2009b, a).

At the time of spawning and in the subsequent few hours, only the elevation of the fertilization envelope was visible, but there was no sign of early division (Fig. 3A). The first confirmed cleavages were at the 16-32-cell stage and occurred within 12 h (Fig. 3B), consistent with reports of meroblastic cleavage in *U. crassicornis* (Chia and Spaulding, 1972). Round blastulae emerged 2-3 days post spawning; these blastulae have poorly defined blastomeres, as also observed by Chia and Spaulding (1972). After about 4 days, the embryos reached the early gastrula stage (Fig. 3C, D). The formation of a yolk plug (Fig. 3E) occurred after 5-6 days. Chia

and Spaulding (1972) suggested that this yolk plug was formed by a process of epiboly. After 7-8 days, the yolk plug disappeared and the fully-developed gastrulae became slightly elongated with a visible blastopore (Fig. 3F). Early planulae appeared 8-15 days post spawning, and fully-developed elongated planulae after 17-21 days (Table 1, Fig. 3G). Both stages moved quickly but remained close to the substrate. No pelagic phase was ever noted in *Urticina* sp. A few planulae with visible tentacles buds (Fig. 3H, I) were observed to temporally attach to hard substrates between days 22-24. Metamorphosis to the polyp shape (Fig. 3J, K, L) occurred 30-35 days post spawning. The septum were then clearly visible along the transparent body wall (Fig. 3K, L).

Planulae settled close to each other (sometimes side by side) and occurred preferentially on rocks, generally on undersurfaces or inside crevasses. Some planulae were able to settle on artificial substrates (e.g. glass, plastic), but after a delay of 1-2 weeks relative to settlement on rocks. In addition, whenever planulae first settled on these sub-optimum substrates, they quickly relocated to rocks (inside a few hours) that were added a posteriori. It has been proposed that planulae of certain sea anemones, including *Urticina crassicornis*, may use a diffuse ectodermal sensory network to select a substrate for settlement (Shick, 1991). Moreover, post-settlement relocation to an optimal substrate has previously described in the temperate intertidal species *A. elegantissima* and *A. xanthogrammica* (Sebens, 1982).

3.4 Growth

Metamorphosis into the juvenile (polyp) was completed as the general body shape of the planulae flattened and the pedal disk enlarged (Fig. 3K, L; Fig. 4A) followed by the appearance of the first 8 tentacles (Fig 4B). At that stage, the juveniles of *Urticina* sp. were still not able to feed. After 5 months, the 8 tentacles elongated (Fig. 4C) and the mouth opened enabling feeding

in the juveniles which had reached ~1000 μm pedal disk diameter. Some individuals were observed to prey on copepods. The number of tentacles and the overall size of the juveniles increased over time to reach a maximum of 16 mm pedal disk diameter and 54 tentacles after 2.5 years (Table 1, Fig. 4). The juveniles are still growing in the mesocosm to this day.

The best curve fit between empirical data and the known adult size was obtained with exponential growth ($R^2 = 0.926$, $p < 0.001$): $S_t = e^{at}$ where S_t is the size reached after a certain time t based on a growth constant a estimated from the first 2.5 years of basal disk increments measured in the present study (Fig. 5). Exponential growth models are not uncommon in sea anemones (Balamurugan et al., 2014) and other animals (Sebens, 1987). The extrapolation revealed that growth to 90 mm in *Urticina* sp., which corresponds to the adult size measured here, would require ~4 years. Comparatively, the smaller sea anemone *Allantactis parasitica* (~35 mm pedal disk diameter) from the same geographic area and depth was estimated to require 6-7 years to reach full adult size (Mercier and Hamel, 2009a). To our knowledge, *Urticina* sp. represents only the second actiniarian species from the deep for which growth data are available. While valuable, growth rates measured in mesocosms remain approximations, as conditions in the laboratory do not perfectly mimic conditions occurring in situ (e.g. pressure and food quantity/quality).

Despite intensive bottom fisheries on the eastern coast of Canada (<http://www.dfo-mpo.gc.ca/Library/334918.pdf>), great biomasses of *Urticina* sp. and other large sea anemones continue to be recorded as bycatch of research surveys conducted along the continental slope of Newfoundland (DFO, unpublished data). The fact that most trawl-caught specimens observed during the research surveys were damaged (torn from the substrate) implies that trawl fisheries inflict significant damage to deep anemone communities. Nevertheless, the resilience of deep-

water sea anemones inferred by their prolonged survival in captivity, combined with the reproductive strategy determined here for *Urticina* sp., apparently contribute to offset disturbances to localised populations. However, if fishing pressure increases over the years to come, this scenario could change rapidly given the slow growth rate, limited fecundity and breeding periodicity of *Urticina* sp.

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Tables

Table 1. Development kinetic and propagule size of *Urticina* sp. from April 2013 to April 2015 at a temperature ranging from -1 to 8°C. A new stage was considered attained when ~50% of propagules reached it.

Stage	Time	Size	Image
Newly released oocytes	0	550-600 µm (diameter)	Fig. 3A
16 to 32 cells	6-12 h	600 µm (diameter)	Fig. 3B
Blastula	2-3 d	620-650 µm (diameter)	
Early gastrula	4 d	630-650 µm (diameter)	Fig. 3C, D
Gastrula (yolk plug)	5-6 d	630 µm (length)	Fig. 3E
Gastrula (fully formed)	7-8 d	670 µm (length)	Fig. 3F
Early planula	8-15 d	690 µm (length)	
Late planula	17-21 d	710 µm (length)	Fig. 3G
Planula (appearance of tentacle buds)	22-24 d	710 µm (length)	Fig. 3H, I
Metamorphosis and settlement (cone-shaped body with septa)	30-35 d	900 µm (diameter)	Fig. 3J, K, L; Fig. 4A
8 tentacles (non-feeding)	5 mo	950 µm (pedal disk)	Fig. 4B
8 tentacles (feeding)	5 mo	1000 µm (pedal disk)	Fig. 4C
12 tentacles	5-6 mo	1100 µm (pedal disk)	Fig. 4D, E
24 tentacles	9-10 mo	1500 µm (pedal disk)	Fig. 4F
24 tentacles	12 mo	3-4 mm (pedal disk)	Fig. 4G
48 tentacles	24 mo	9-11 mm (pedal disk)	Fig. 4H
54 tentacles	30 mo	16 mm (pedal disk)	

Figure Captions

Fig. 1. Best tree from maximum likelihood analysis of the combined dataset of 12S, 16S, 18S, and 28S ribosomal genes. Arrow points to species under study. Bootstrap values greater than 75% are shown. See supplemental Table S1 for accession numbers of included sequences.

Fig. 2. (A) Spawning female *Urticina* sp. showing the broadcasted oocytes (arrows) and the mouth with part of the gastrovascular cavity slightly everted, (B) Male spawning with sperm broadcasted (arrow) through the cone-shaped mouth, (C) General view of the female with eggs laying on the rock and mud around it (arrows). Scale bars: in A = 35 mm; in B = 20 mm; in C = 45 mm.

Fig. 3. (A) Newly-released oocytes/zygotes, (B) Early embryos 16-32 cells, (C-D) Early gastrula, (E) Embryo with yolk plug (yp), (F) Fully-developed gastrula showing the blastopore (b), (G) Late planula, (H-I) Planula showing the appearance of the tentacle buds (tb), (J-L) Planula during metamorphosis into cone shape with visible septum (s) and mouth area (m). Detail on the kinetics are provided in Table 1. Scale bars: in A = 420 μ m (also applies to B and D); in C = 640 μ m (also applies to E-L).

Fig. 4. (A) Newly-settled *Urticina* sp. attached to the substrate, with septum and mouth clearly visible (B) Appearance of tentacles (t) in non-feeding propagule, (C) 8-tentacle feeding juvenile, (D-E) 12-tentacle juvenile, 5-6 month old, (F) 24-tentacle juvenile, 9-10 month old, (G) 24-tentacle juvenile with elongating tentacles, 1 year post spawning, (H) 2-year old juvenile with 48 tentacles, measuring 9-11 mm pedal disk diameter. Detail on the

515 kinetics are provided in Table 1. Scale bar in A = 360 μm ; in A-F; in G =1.5 mm and in
516 H = 4.5 mm.

517 Fig. 5. Increment in basal disk diameter (mean $\pm$ SD) in *Urticina* sp. measured over the initial
518 2.5 years (solid line) and extrapolated growth (dashed line) to maximum size (90 mm).
519 Drop line/arrow shows time needed to reach the maximal size based on the extrapolated
520 growth curve.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5