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Reproduction and early life stages of the poecilosclerid sponge *Crella incrustans*

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Abstract

Despite their important ecological roles in marine ecosystems, reproduction and early life stages of the majority of marine sponges remain undescribed. Here we characterize the mode of reproduction and the early life stages of an abundant sponge in temperate Pacific waters, *Crella incrustans*. Through histology, we characterized the production of gametes and the sequential steps of larval ontogeny. Using *in vivo* observations, we described larval release, settlement, and metamorphosis. Specimens of *C. incrustans* presented spermatocytes, oocytes, and several developmental stages in the sponge mesohyl during the Australasian summer (from January to March 2020), demonstrating this sponge to be a simultaneous hermaphrodite with internal fertilization, asynchronous development, and brooded embryos. As in other viviparous demosponges, mature embryos were released during the Australasian summer as free-swimming non-tufted parenchymella larvae. Under laboratory conditions, 94.3% of larvae settled within 2 days and metamorphosed into functional settlers within a week. Gametogenesis, embryonic development, larval characteristics, settlement, and metamorphosis of *C. incrustans* are consistent with the reproductive features common to the majority of poecilosclerid sponges. Overall, our study provides important information on the early life stages of this temperate model species for future ecophysiological experiments.

KEYWORDS
life cycle, metamorphosis, ontogeny, parenchymella, Porifera

1 | INTRODUCTION

The majority of benthic invertebrates are characterized by complex life cycles that include one or more free-swimming larval stages (Eckman, 1996; Pechenik, 1999; Strathmann, 1974). Planktonic stages are important for determining adult distribution patterns and promoting genetic connectivity among populations (Cowen et al., 2000; Eckman, 1996; Uthicke et al., 2009).

Sponges are considered the most ancient and simplest metazoans on Earth (Brien, 1973; Müller, 1998, 2003; Nielsen, 2008; Petralia et al., 2014). They are currently represented by more than 8500 described species (Van Soest et al., 2012) and are important members of tropical, temperate, and polar benthic ecosystems because of the large number of ecological functions they perform (Bell, 2008; Maldonado et al., 2017). Despite their importance, the pre- and post-larval development, phenology, and reproductive ecology of the majority of sponge species remain undescribed (Bergquist, 1978; Maldonado & Riesgo, 2009a).

Sponges lack true organs, including gonads and a predetermined cell lineage for gamete development, and their body plan is based

on a series of water canals that, together with the activity of pluripotent cell types, support their physiological functions, including reproduction (Bergquist, 1978; Ereskovsky, 2018; Gaino et al., 1995; Maldonado, 2014). Reproduction in sponges may be either asexual, by the processes of fragmentation, gemmulation, or budding, which are mainly thought to be involved in population maintenance (Cardone et al., 2010; Zilberberg et al., 2006), or sexual, with the production of planktonic larvae that not only promote population maintenance but also enhance genetic connectivity within and among populations (Whalan, de Nys, et al., 2008).

Sponges may be gonochoristic or hermaphroditic, and during the process of gametogenesis, sperm cells and oocytes are formed from several types of somatic cells by transdifferentiation (Ereskovsky, 2010; Leys & Degnan, 2001; Maldonado & Riesgo, 2009a). Sponge fertilization and larval development can occur inside the sponge or in the water column, and sponges can be categorized as viviparous, ovoviviparous, or oviparous (Ereskovsky, 2018; Leys & Ereskovsky, 2006). Depending on their phylogenetic position, sponges show different developmental pathways: the majority of Demospongiae are characterized by the parenchymella larva, but may also have a disphaerula (Family Halisarcidae), clavablastula (Order Hadromerida), or hoplitomella (Family Alectonidae) larva. Sponges belonging to the class Homoscleromorpha are characterized by a cinctoblastula larva. Hexactinellids have a trichimella larva, whereas calciblastula and amphiblastula larvae are typical in Calcinea and Calcaronea subclasses (Class Calcarea), respectively (Maldonado & Bergquist, 2002). Sponges belonging to the order Spirophorina (Class Demospongiae) generally show direct development (Ereskovsky, 2010). Larval morphology and behavior are fundamental traits to consider when trying to understand changes in sponge abundance and distribution (Mariani et al., 2006; Wahab et al., 2014). In particular, the duration of the larval phase and photobehavior are important in larval dispersal and habitat selection during settlement (Bergquist & Sinclair, 1968; Leys & Degnan, 2001; Maldonado & Uriz, 1998; Maldonado & Young, 1996; Maldonado et al., 2003; Whalan, Ettinger-Epstein, et al., 2008).

Order Poecilosclerida is characterized by the highest diversity, containing 2350 described species, with high morphological diversity (Hooper & Van Soest, 2002; Vacelet & Boury-Esnault, 1995; WoRMS, 2020). Poecilosclerid sponges are known to be viviparous and hermaphroditic (Riesgo et al., 2014). Within this family, *Crella incrustans* (Carter 1885) is distributed in subtidal habitats of temperate Pacific regions, including the East China Sea, and temperate Australian and New Zealand waters (Cook, 2010; Hooper & Wiedenmayer, 1994; Kelly et al., 2009; Kim & Sim, 2001). This sponge occurs across a wide range of environments, from harbors to exposed rocky shores (Berman & Bell, 2010; Cook, 2010), often living associated with habitat-forming species (Cárdenas et al., 2016), and it is known for the production of antifouling allelochemicals and sterols (Butler et al., 1996; Davis et al., 1991; Ragini et al., 2017). Moreover, a number of ecophysiological experiments have shown this species to be resistant to ocean acidification and high levels of suspended sedimentation (Bates & Bell, 2018; Cummings et al., 2020).

Despite the potential of this sponge to be a model species for ecological and experimental work, its reproductive features and early life stages remain undescribed. In this study, we coupled *in vivo* observations and histological analysis to characterize the mode of reproduction, larval ontogeny, and settlement and metamorphosis of *C. incrustans*, and compare these features with the known reproductive ecology of other poecilosclerid sponges.

2 | METHODS

2.1 | Sponge sampling and identification

To assess possible release of larvae, five samples of *C. incrustans* were collected every month from September 2019 to August 2020, with the exception of April 2020. Sponges were collected from subtidal rocks by SCUBA divers at 5 m depth from Mahanga Bay in Wellington Harbour, New Zealand (41°17′32.0″S, 174°50′00.8″E). Species identification was validated by histological analysis (Bergquist & Fromont, 1988). Sponges were maintained in separate containers with 9 L of filtered seawater (10 μm), and larval release was visually monitored for 1 week.

2.2 | *In vivo* observations of early stages

To characterize larval behavior settlement and metamorphosis in *C. incrustans* under laboratory conditions (January–March 2020), 10 samples (at least 5 m apart) were haphazardly collected in the field once a month. All samples were similar in size (mean buoyant weight 118 ± 26 mg, n = 10). A scalpel was used to detach the basal layer of the sponge from the substrate, because the reproductive structures are mostly present in this region of poecilosclerid encrusting sponges (Bergquist, 1978). Sponges started to release larvae after 4 h in laboratory conditions. After the first larva was released, all larvae released for each sponge were collected with Pasteur pipettes every 12 h for 1 week and maintained in Petri dishes (60 mm in diameter) with 15 ml of filtered seawater (10 μm). To facilitate water changes and ontogenetic observations, a maximum of 10 larvae were maintained in each Petri dish. From January to March 2020, larvae and settlers were maintained at a constant temperature of 16°C, which corresponds to the mean sea surface temperature in Wellington Harbour during summer. After larval collection, water in Petri dishes was partially replaced once a day to prevent disturbing the larvae or settlers. Larval settlement was observed under a dissecting microscope (Olympus SZ61) daily for 8 days between January and March 2020. During February 2020, larval metamorphosis was monitored and settlers photographed (Canon EOS 70D digital camera) daily for the first 4 days after settlement; a final measurement of the settlers was taken at the end of metamorphosis, 7 days after settlement. To measure larval length and the area of settlers during the settlement assay, 30 larvae and 15

settlers were selected haphazardly and photographed every 24 h, and pictures were analyzed with ImageJ (version 1.51j8, Rasband, National Institute of Health). *In vivo* images of the larvae were also obtained with a compound light microscope (Leica Microsystems DM LB) combined with a Canon EOS 70D digital camera. Underwater pictures were obtained with a Sony a7 II digital camera.

Possible photosensitivity of sponge larvae was tested: 20 sponge larvae (12–24 h old) were collected with Pasteur pipettes and positioned in a 100 mm × 15 mm Petri dish with 15 ml of filtered seawater (10 μm). A microscope light (2300 lm of light intensity) was placed on one side of the Petri dish, and larval swimming behavior was monitored for 20 min.

2.3 | Histological characterization of reproductive structures

To characterize larval development, 10 samples of *C. incrustans* were fixed once a month for histological analyses, from January to March 2020. Samples were fixed in Davidson's solution (formalin 37%, two parts; absolute ethanol, three parts; glacial acetic acid, one part; filtered seawater, three parts; eosin solution 0.2%, one part) for 12 h at room temperature and then stored in 70% ethanol at 4°C. A fragment of ~0.5 cm^3 was cut from each sample (including spicules) and dehydrated sequentially in ethanol (70%, 80%, 90%, 95%, and 100%), washed in xylene (50% xylene in ethanol and 100% xylene) and embedded in paraffin wax under vacuum with an automated tissue processor (Leica Biosystems TP1020). Samples were embedded in paraffin wax using an embedding station (EG1160). Sections (5 μm) were manually cut with a rotary microtome (Leica Biosystems RM2235), stained with hematoxylin and eosin, and mounted on microscope slides with DePeX-Gurr mounting medium. To characterize different developmental stages and reproductive structures, sections were observed and photographed under a compound light microscope (Leica Microsystems DM LB) combined with a Canon EOS 70D digital camera. To calculate the area of reproductive structures, pictures were analyzed with ImageJ. To estimate the mean density of embryos in the sponge tissue during the 3 months of study, 10 histological sections for each of the 10 sponges sampled each month were randomly selected and photographed under a compound light microscope (Leica Microsystems DM LB) combined with a Canon EOS 70D digital camera. From the resulting pictures, the area of the histological sections was calculated by ImageJ. The histological sections were then analyzed under a compound light microscope (Leica Microsystems DM LB), and the embryos were counted.

3 | RESULTS

3.1 | Reproductive features and larval ontogeny

We found that *C. incrustans* is a simultaneous hermaphrodite and viviparous species. Individuals developed spermatic cysts and oocytes, with consecutive developmental stages in the mesohyl of the same sponge. Early spermatic cysts were found in just one of the sampled sponges (collected in January 2020) and were bounded by follicular cells and filled by large primary spermatocytes (Figure 1A,B). Of the sampled sponges, 57% (n = 30) presented embryos at different developmental stages; among these, 47% presented oocytes. Oocytes had a mean (±*SD*) diameter of 38.85 ± 4.8 μm and occupied a mean mesohyl area of 867.9 ± 246 μm^2. Oocytes were either round or oval in shape and were characterized by a large nucleus (Figure 1C,D).

Some sections revealed the presence of oocytes undergoing vitellogenesis; that is, oocytes were comparatively large and characterized by the engulfed nurse cells, of which nuclei were still evident (Figure 1E,F). Several embryonic stages were found in the mesohyl of the specimens of *C. incrustans* (i.e., morulae at different phases of cell division, early and differentiating embryos) and all of them were enclosed in follicles (Figure 1G,M; Table 1). The mean density (±*SD*) of embryos per square millimeter of sponge tissue was 0.45 ± 0.21, 0.09 ± 0.05, 0.12 ± 0.07 during January, February, and March 2020, respectively. Before being released into the external environment, the pre-larval stage located in the sponge mesohyl was still surrounded by follicular cells and was characterized by an external layer of ciliated cells covering the whole surface, with the exception of the posterior pole, where internal spicules were present (Figure 1N). Along with reproductive structures, we found bacterial aggregations in the mesohyl of specimens of *C. incrustans* (Figure 1O,P).

3.2 | Larval production, settlement, and metamorphosis

Specimens of *C. incrustans* released larvae from the beginning of January to the end of March 2020 (Figure 2), corresponding with the Australasian summer (mean sea surface temperature 16°C in Wellington Harbour). Larval release was not observed during the rest of the year (Figure 2). We found that 73.4% of the sponges sampled between January and March 2020 (n = 30) released a mean of 10.03 ± 2.75 larvae during 1 week of observations (159, 76, and 66 larvae were observed during January, February, and March 2020, respectively). Larvae were mostly released during late afternoon under laboratory conditions. Larvae of *C. incrustans* were oval in shape (mean length 270 ± 34 μm) and brick red in color, which is similar to the adult sponge (Figure 3A,B). Larvae were entirely covered by short beating cilia except for the whitish and swollen posterior pole, which was characterized by the presence of internal spicules (Figures 3B and 4A). Upon release, larvae showed no preferred swimming direction (i.e., neither towards nor away from the water surface). During swimming, larvae of *C. incrustans* showed a clockwise rotatory movement around the anterior–posterior axis, but did not respond to a light stimulus (larvae were 12–24 h old; light source was 2300 lm). Twelve hours before settlement, larvae started to slowly swim laterally over the bottom of the Petri dish and became more stationary;

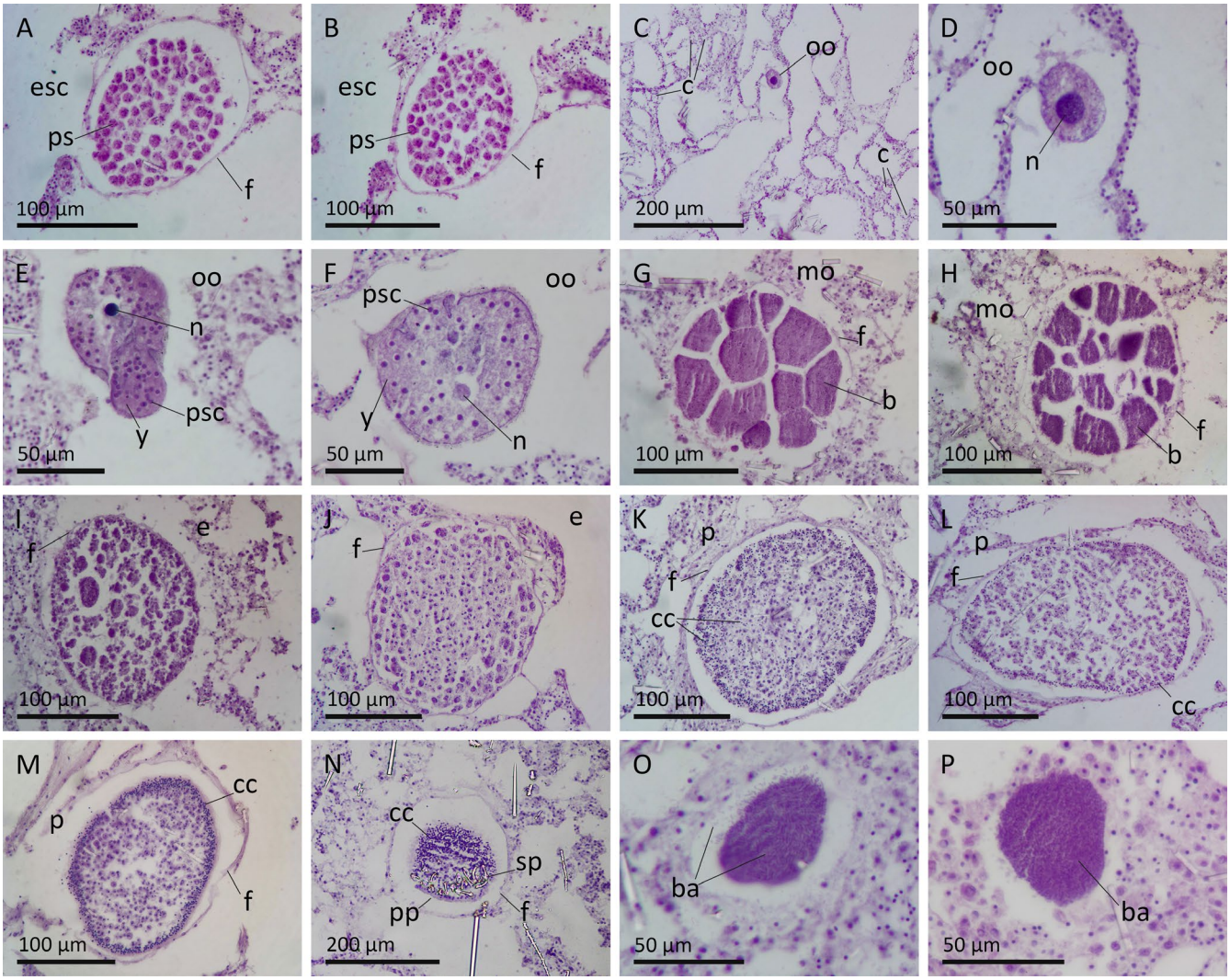

FIGURE 1 Histological sections (5 µm) of the reproductive structures of *Crella incrustans*. **A,B**. Consecutive sections of an early stage spermatic cyst with large primary spermatocytes surrounded by a follicle. **C,D**. Oocyte at different magnifications. **E,F**. Oocytes during vitellogenesis engulfing nurse cells. **G,H**. Morulae at the beginning of delamination characterized by a few large blastomeres. **I,J**. Early embryo with internal differentiating cells. **K**. Pre-larval cell differentiation stage. **L**. Pre-larva stage. **M**. Pre-larva, with external ciliated cells and internal spicules, surrounded by a follicle. **N**. Section of the posterior pole of the pre-larva stage. **O,P**. Bacterial aggregations. b, blastomeres; ba, bacterial aggregation; c, choanocyte chamber; cc, ciliated cells; e, embryo; esc, early spermatic cyst; f, follicle; mo, morula; n, nucleus; oo, oocyte; p, parenchymella larva; pp, posterior pole; ps, primary spermatocytes; psc, phagocyted somatic-nurse cells; sp, spicules; y, yolk

when resting, larvae laid vertically motionless, with the posterior pole in contact with the bottom of the Petri dish. The dimensions of the larvae remained unchanged during the 3 months of larval production.

Metamorphosis was complete within 7 days through five main stages: (a) 24 h after settlement, larval components, including spicules, cells, and an orange extracellular matrix, appeared coated on the substrate (Figure 4B); (b) 2 days after settlement, the internal area of the settlers appeared more homogeneous and dense, with a distinctive pale orange color, together with the formation of new spicules and with the formation of a few choanocyte chambers (Figure 4C); (c) 3 days after settlement, internal channels developed mostly in the central area of the settler, the number of choanocyte chambers increased on one side of the settler, and further spicules were produced (Figure 4D); (d) 5 days after settlement, choanocyte chambers were formed uniformly within the entire settler, a primordium of the first osculum became visible, interconnected channels established the aquiferous system, and more spicules were formed (Figure 4E); (e) 6 days after settlement, a first finger-like oscular tube opened and the settlers became functional sponges (Figure 4F). None of the settlers died during metamorphosis.

Of the total number of settlers (n = 284), 36% and 47% were settled 24 and 48 h after larval release, respectively (Figure 5A). A third peak in settlement occurred after 120 h, accounting for 14% of total settlement. After 144 h, all competent larvae had settled, with 5.7% of larvae dying before settlement. Once settled, the mean area of settlers increased linearly as they metamorphosed to juvenile sponges at a constant temperature of 16°C (Figure 5B).

TABLE 1 Overview of the reproductive ecology of the order Poecilosclerida

Species	Months of larval release	Type of development	Primary oocyte diameter (μm)	Embryo diameter (μm)	Larva dimensions (μm)	Larva type	Predominant color	Reaction to light	Duration of planktonic stage (h)	Duration of metamorphosis (h)	References
Acarnidae											
Iophon piceus	(n) Aug.–Oct.	A	?	∽100	260 L; 200 D	ntP	?	?	?	?	Ereskovsky (2010)
Iophon radiatus	?	?	?	3000	?	ntP	?	?	?	?	Burton (1931)
Chondropsidae											
Chondropsis sp.	(s) May–July	?	?	?	1600 D	dl	Yellow	?	48–96	?	Ayling (1980)
Cladorhizidae											
Asbestopluma formosa	(n) June	?	?	300	?	?	?	?	?	?	Vacelet (2006)
Asbestopluma occidentalis	(n) Sep.–Nov.	?	?	?	∽250 L; 138 D	ntP	?	?	>10	?	Chu and Reiswig (2014)
Lollipocladia tiburoni	(n) Aug.	?	?	∽315	∽300 D	?	?	?	?	?	Vacelet (2008)
Crambeidae											
Crambe crambe	(n) July–Sep.	A	?	?	1159 L; 467 D	ntP	?	(–)	24–72	?	Uriz et al. (1998)
Crambe crambe	?	?	?	?	1200 L; 600 D	ntP	Dark-orange	0	∽48	168	Uriz et al. (2001)
Crambe crambe	(n) July–Sep.	?	?	?	1200 n/s	ntP	Orange-red	0	?	?	Mariani et al. (2005)
Crellidae											
Crella elegans	(n) Sep.–Oct.	A	?	309.9	270.4 L	ntP	?	?	?	?	Pérez-Porro et al. (2012)
Crella incrustans	(s) Jan.–Mar.	A	43.7	200	304 L	ntP	Brick-red	0	∽48	120	Present study
Esperiopsidae											
Esperiopsis koltuni	(n) Oct.	A	28	?	500 L; 380 D	P	?	?	?	?	Ereskovsky and Willenz (2007)
Ulosa sp.	?	?	?	?	?	P	?	?	?	168	Bergquist and Green (1977)
Desmacididae											
Desmapsamma anchorata	(s) all year	A	?	?	?	?	?	?	?	?	Lanna et al. (2018)

(Continues)

TABLE 1 (Continued)

Species	Months of larval release	Type of development	Primary oocyte diameter (μm)	Embryo diameter (μm)	Larva dimensions (μm)	Larva type	Predominant color	Reaction to light	Duration of planktonic stage (h)	Duration of metamorphosis (h)	References
Hymedesmiidae											
Anchinoe sp.	(s) Feb.–May	?	?	?	179 L; 146 D	ntP	Pale-yellow	?	48–96	?	Ayling (1980)
Hamigera hamigera	(n) June–July	?	?	?	500 n/s	ntP	Dark-red	(+)	∽72	?	Boury-Esnault (1976)
Hemimycale arabica	(n)May–Oct.	A	?	400	362 n/s	ntP	Bright-orange	?	?	?	Ilan et al. (2004)
Hemimycale columella	(n) Sep.–Oct.	A	30.8	349.3	405 D	ntP	?	?	?	?	Pérez-Porro et al. (2011)
Hymedesmia sp.	(n) June–Jan.	?	?	?	400 n/s	ntP	Bright-yellow	?	?	?	Mariani et al. (2005)
Phorbas tenacior	(n) Aug.–Dec.	?	?	?	350 L; 200 D	ntP	Greyish-blue	(–)	?	?	Mariani et al. (2005)
Stylopus sp.1	(s) Dec.–Feb.	?	?	?	407 L; 392 D	ntP	Bright-orange	?	48–96	?	Ayling (1980)
Stylopus sp.2	(s) Dec.–Feb.	?	?	?	240 L; 210 D	ntP	Pink	?	48–96	?	Ayling (1980)
Latrunculiidae											
Latrunculia magnifica	(n) Dec.–Feb.	?	50	484	1200 L	P	Red	?	?	?	Ilan (1995)
Microcionidae											
Microciona coccinea	?	?	?	?	444 L; 300 D	ntP	Bright-red	?	∽4	48	Bergquist and Sinclair (1968)
Microciona prolifera	(n) July–Aug.	A	50	250	300 D	ntP	Red-orange	0	<51	?	Simpson (1968)
Microciona rubens	?	?	?	?	?	ntP	?	?	?	108	Bergquist and Green (1977)
Ophlitaspongia seriata	?	?	?	?	429 L; 384 D	ntP	Bright-orange	?	∽4	48	Bergquist and Sinclair (1968)
Rhaphidophlus jolicoeuri	(n) Sep.–Dec.	?	?	?	500 D	ntP	Dark-orange	0	?	?	Mariani et al. (2005)
Mycalidae											
Mycale acerata	?	?	?	600	?	?	?	?	?	?	Riesgo et al. (2015)
Mycale contarenii	(n) Mar.–May	A	50	520	800 L; 500 D	P	?	?	?	?	Corriero et al. (1998)

(Continues)

TABLE 1 (Continued)

Species	Months of larval release	Type of development	Primary oocyte diameter (μm)	Embryo diameter (μm)	Larva dimensions (μm)	Larva type	Predominant color	Reaction to light	Duration of planktonic stage (h)	Duration of metamorphosis (h)	References
Mycale fistulifera	(n) all year	A	?	∽420	456 n/s	ntP*	Bright-red	?	∽30	10	Meroz and Ilan (1995)
Mycale laevis	(n) July–Sep.	A	?	?	752 L; 589 D	P	Green	?	∽12	?	Loh and Pawlik (2012)
Mycale laevis	(n) Mar.	?	?	500	700 D	ntP	Green	?	?	?	Riesgo et al. (2015)
Mycale macilenta	(n) July–Aug.	?	?	?	∽350 D	ntP	Bright-orange	0	?	?	Mariani et al. (2005)
Mycale macilenta	?	?	?	?	527 L; 429 D	ntP	Dark-orange	(–)	?	?	Bergquist and Sinclair (1968)
Mycale micracanthoxea	(n) Aug.–Oct.	?	?	?	360 n/s	ntP	Yellow	?	?	?	Wapstra and Van Soest (1987)
Mycale phyllophila	(n) Aug.–Oct.	A	50	∽350	∽450 D	ntP	Red	?	?	?	Huang et al. (2016)
Mycale rotalis	(n) May–June	?	?	?	∽350 D	ntP	Bright-orange	0	?	?	Mariani et al. (2005)
Myxillidae											
Myxilla incrustans	(n) July	A	∽25	∽101	?	P	?	?	?	?	Ereskovsky (2010)
Tedaniidae											
Tedania anhelans	(n) July–Nov.	?	?	?	∽800 n/s	ntP	Orange	?	?	?	Mariani et al. (2005)
Tedania anhelans	(n) May–Oct.	A	43	250	920 L	ntP	?	?	?	?	Di Camillo et al. (2011)
Tedania gurjanovae	(n) July	?	?	?	576 L; 433 D	ntP	Cream	0	∽7	?	Bakus (1964)
Tedania ignis	(n) Apr.–June	?	?	?	836 L; 594 D	ntP	Orange-red	?	?	?	Jaekle (1995)
Tedania ignis	(n) Apr.–Aug.	A	?	?	869 L	ntP	Red-orange	(–)	>96	?	Maldonado and Young (1996)
Tedania ignis	(s) all year	A	?	?	?	?	?	?	?	?	Lanna et al. (2018)

All sizes are maximum reported values.

(s), Southern Hemisphere; (n), Northern Hemisphere; A, asynchronous; D, diameter; L, length; n/s, larval dimension not specified; ntP, non-tufted parenchymella; dl, dispherula-like; P, parenchymella; ntP*, non-tufted parenchymella with nonciliated anterior pole; (+), positive phototaxis; (–), negative phototaxis; 0, absence of phototaxis;?, unknown or missing information.

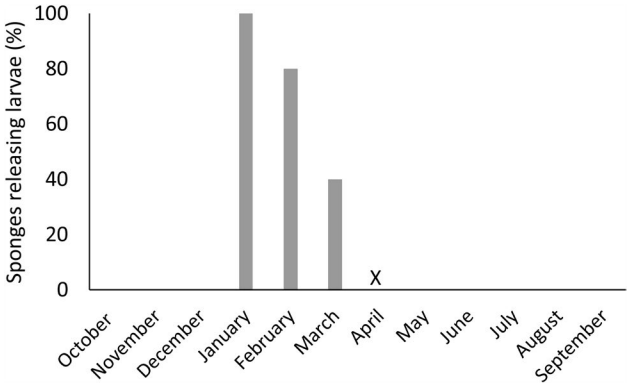

FIGURE 2 Sponges producing larvae from October 2019 to September 2020, with the exception of April 2020

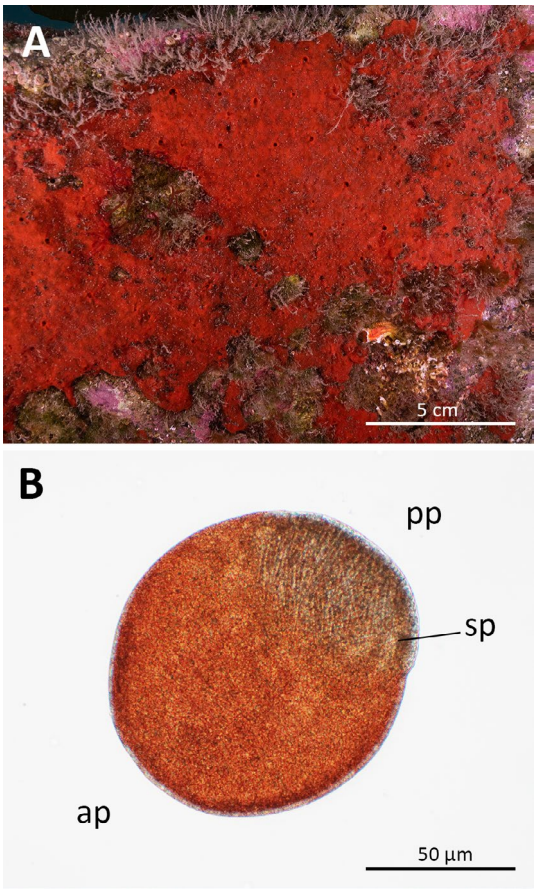

FIGURE 3 *In vivo* photographs of the adult and larva of *C. incrustans*. **A**. Adult of *Crella incrustans* in the subtidal habitat, at 5 m depth in Wellington Harbour. **B**. Non-tufted parenchymella larva with internal spicules concentrated at the posterior pole. ap, anterior pole; pp, posterior pole; sp, spicules

4 | DISCUSSION

This study aimed to describe the mode of reproduction, larval ontogeny, settlement, and metamorphosis of *C. incrustans*, and to characterize the morphological and functional features of its early life stages. Specimens of *C. incrustans* produced larvae during the Australasian summer, although from our approach we could not determine how long gametic production extends before or after the summer period. The fact that swimming larvae were seen in early January means that fertilization probably happened at least 1 month before (December of the previous year) and that intense gametogenic activity took place in at least November. Although larval release for the majority of poecilosclerid sponges occurs during summer months (see Di Camillo et al., 2011; Ereskovsky, 2000; Huang et al., 2016; Pérez-Porro et al., 2011), in tropical regions it can also take place all year round, with temperature considered to be one of the main factors driving these reproductive patterns (Lanna et al., 2018; Meroz & Ilan, 1995). However, in some instances, phenological events have not been directly correlated with seawater temperature (Corriero et al., 1998). For example, Ayling (1980) reported the production of larvae from other common poecilosclerid sponges in New Zealand coastal waters, with the production of larvae in *Stylopus* sp., *Anchinoe* sp., and *Chondropsis* sp. during summer, late summer, and Australasian winter, respectively (Table 1). Consistent with our results for *C. incrustans*, the duration of larval release in the majority of poecilosclerid sponges occurs over a 1–3-month period (Table 1). Despite this, *Hemimycale arabica*, *Hymedesmia* sp., *Phorbas tenacior*, and *Tedania anhelans* produce larvae for 5–8 months of the year (Di Camillo et al., 2011; Ilan et al., 2004; Mariani et al., 2005), and *Desmapsamma anchorata*, *Tedania ignis*, and *Mycale fistulifera* produce larvae all year round (Lanna et al., 2018; Meroz & Ilan, 1995). Further studies may clarify the possible relationship between gametogenesis, fertilization, and larval release with temperature or other environmental factors in *C. incrustans*.

Only 1 of 30 sampled specimens of *C. incrustans* presented spermatic cysts and developing embryos simultaneously. For this reason, we propose that *C. incrustans* is a viviparous simultaneous hermaphrodite, which is similar to the majority of poecilosclerid sponges (Bergquist, 1978; Ereskovsky, 2010; Maldonado & Riesgo, 2009a; Riesgo et al., 2014). The rarity of spermatic cysts in *C. incrustans* may be the result of the timing of gametogenesis in Poecilosclerida, in which the formation of spermatic cysts may predate (by several weeks) the period of larval production (Ereskovsky, 2010). Moreover, in some cases sponge spermatogenesis may be a very rapid process resulting in only a short period where spermatic cysts are found in the sponge tissue; this could easily be missed during sampling (Maldonado & Riesgo, 2009b; Shaffer et al., 2020). In specimens of *C. incrustans* different developmental stages occurred concurrently in the same sponge, indicating asynchronous development, which can only be derived from asynchronous gamete development and maturation. Reproductive asynchrony and viviparity are both characteristics of the Poecilosclerida (Table 1), whereas marked synchronization of gametogenesis and spawning events are more characteristic of oviparous sponges belonging to other sponge orders (Fromont & Bergquist, 1994; Hoppe, 1988). Importantly, developmental asynchrony at the individual and population level results in an extended period of larval production, reducing the risk of larval mortality over the reproductive season (Maldonado & Young, 1996). Additional interannual histological analyses may allow the

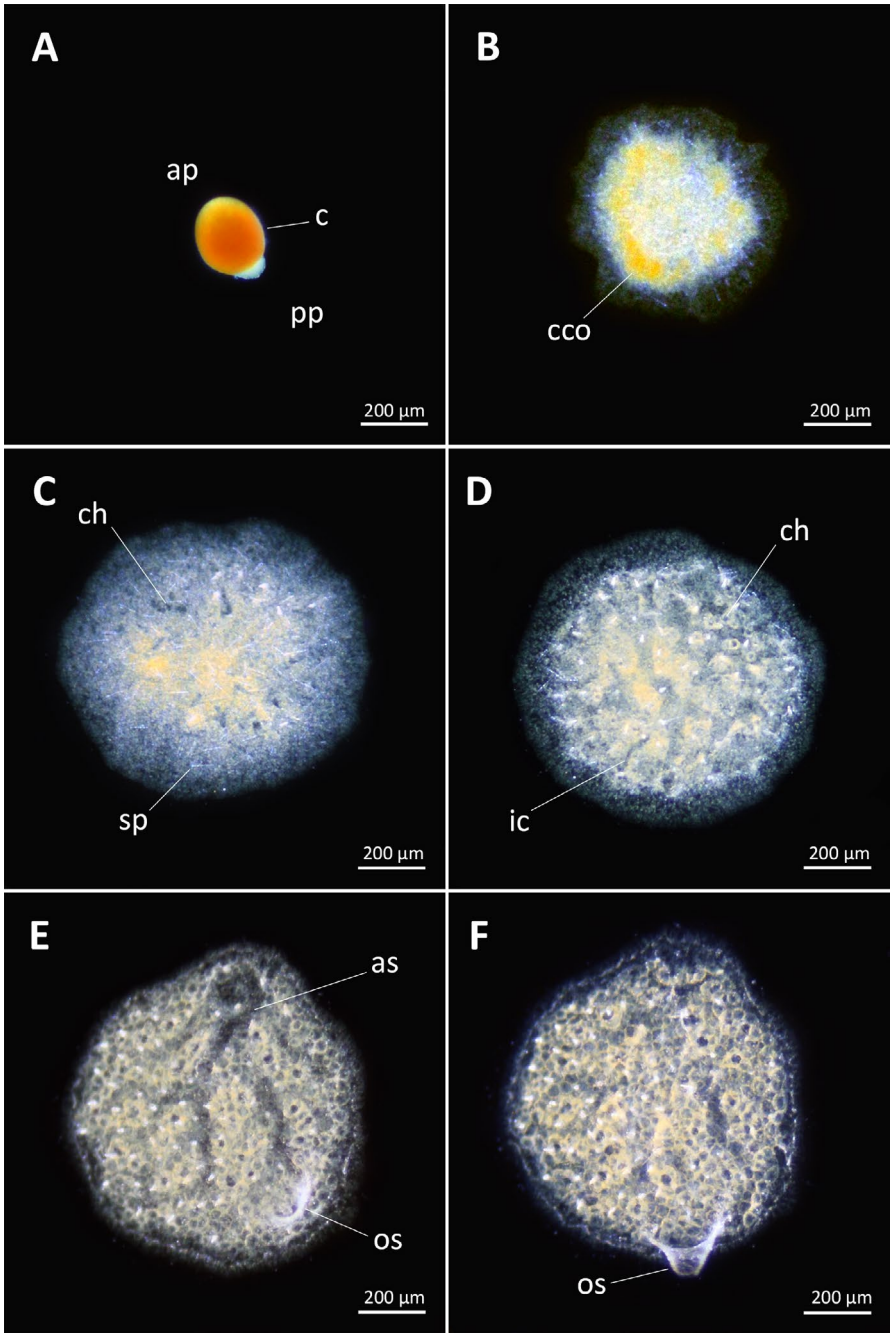

FIGURE 4 Larval settlement and metamorphosis. **A**. Non-tufted parenchymella larva with cilia covering its entire surface, except for the posterior pole. **B**. Early settler, 24 h after settlement. **C**. The internal area of the settler is at this stage characterized by few new spicules (48 h after settlement); some choanocyte chambers and canals are already visible. **D**. Internal channels are present in the central area of the settler 72 h after settlement. **E**. Formation of the aquiferous system 120 h after settlement. **F**. Functional sponge characterized by the first open osculum, 144 h after settlement. ap, anterior pole; as, aquiferous system; c, cilia; cco, larval cellular components; ch, choanocyte chambers; ic, internal channels; os, osculum; pp, posterior pole; sp, spicules

determination of the timing of spermatogenesis and the characterization of the phenology of *C. incrustans*.

In our histological analyses, the nucleus of the primary oocyte of *C. incrustans* was overstained by the hematoxylin, masking the presence of the nucleolus that was not visible in our histological preparation (Figure 1C,D). Despite this, primary oocytes of *C. incrustans* showed size and characteristics similar to other poecilosclerid sponge oocytes (Table 1). Embryogenesis in *C. incrustans* is consistent with the developmental pathway common to poecilosclerid sponges, characterized by total and chaotic cleavage, resulting in the development of the morula (stereoblastula), cell differentiation, and segregation through the process of delamination, and the formation of the parenchymella larva (Leys & Ereskovsky, 2006).

Larvae of *C. incrustans* were uniformly covered by beating cilia, with the exception of the posterior pole. This larval form corresponds to the non-tufted parenchymella type (Maldonado & Bergquist, 2002) and is typical of poecilosclerid sponges (Bergquist, 1979). Moreover, the morphological features of the non-tufted parenchymella of *C. incrustans* remained constant during the whole period of larval release, whereas in some other poecilosclerid species larval dimensions may change based on the timing of larval production (Uriz et al., 2001). Temperature represents an important variable influencing sponge larval duration (Whalan, Ettinger-Epstein, et al., 2008). Despite other laboratory conditions differing from natural environment, we maintained sponge larvae at 16°C, which represents the mean sea surface

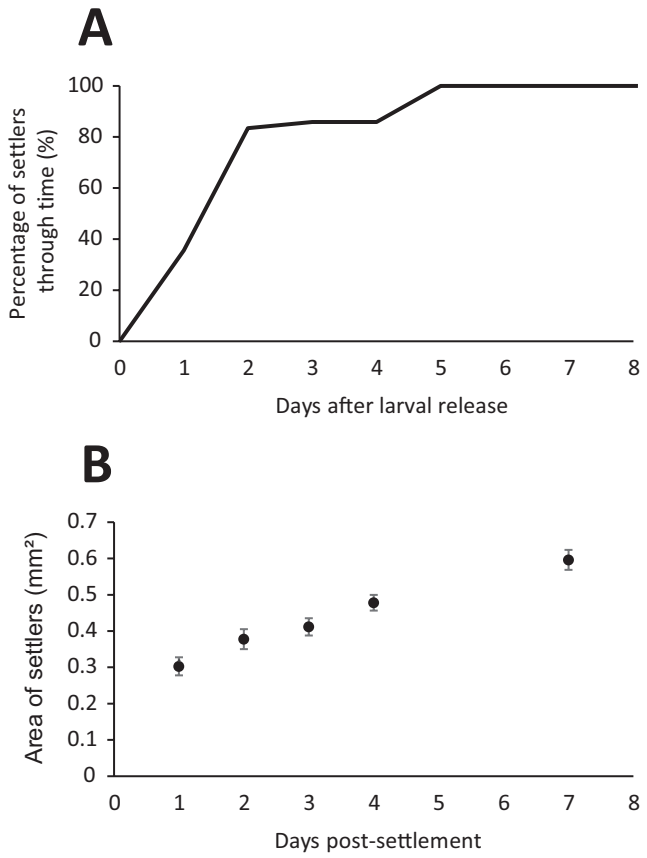

FIGURE 5 Larval settlement and surface growth of settlers at 16°C. **A**. Percentage of larvae of *C. incrustans* (0–12 h old) undergoing metamorphosis (n = 284) from January to March 2020. **B**. Mean area of settlers (n = 15) undergoing metamorphosis in February 2020. Error bars indicate standard errors

temperature in Wellington Harbour between January and March. Under those thermal conditions, 83% of larvae settled within the first 48 h after larval release.

Larvae of *C. incrustans* showed evidence of directional swimming with a constant rotation along the anterior–posterior axis, a behavior which has been commonly described in other sponge larvae during their planktonic phase (Bergquist & Sinclair, 1968; Maldonado, 2006), but they did not respond to light. Larval age and light intensity may represent important variables in phototactic behavior of sponge larvae (Leys & Degnan, 2001; Maldonado et al., 2003), but in some cases larvae of poeciloscelrid sponges may not show sensitivity to light (Table 1). The non-tufted parenchymellae of *C. incrustans*, between 12 and 24 h old, showed no evidence of photosensitivity when exposed to a light source of 2300 lm. In tropical waters light cues are thought to have an important role in release and settlement of larvae (Nada et al., 2020) and also in regulating larval chemoreception (Say & Degnan, 2020). The natural habitat of *C. incrustans* is characterized by turbid waters and low light conditions (daylight mean intensity 1891 ± 354 lm/m^2 and maximum light intensity 15,155 lm/m^2; see Figure S1), and possibly other physical or chemical cues may have a major role in the pre-settlement behavior of this sponge species.

The posterior pole of the larva of *C. incrustans* was characterized by an internal aggregation of spicules. Interestingly, when larvae became competent, they became vertically orientated, with just the posterior pole in contact with the bottom of the Petri dish. This is an atypical behavioral pattern compared with the larva of other demosponges. The parenchymella of many sponge species is known to have internal spicules (Bergquist & Sinclair, 1973), typically located toward to the posterior larval pole. This skeletal accumulation at the posterior pole is thought to create an unequal distribution of mass, which may have a role in both sensing gravity while rotating and depth regulation before settlement (Maldonado et al., 1997). Phototactic sponge larvae may react to light by showing positive phototaxis, which often occurs immediately after larval release and enhances dispersal, or negative phototaxis, which typically takes place at the end of the larval phase and determines the selection of microhabitats suitable for settlement (Maldonado et al., 2003; Wahab et al., 2014). The progressive accumulation of internal spicules at the posterior pole of larvae of *C. incrustans* appears to assist the larvae in finding the bottom in the absence of photosensitivity, and facilitates larvae in maintaining a vertical position prior to attachment to the substratum. Larvae of *C. incrustans* metamorphosed completely within 7 days through sequential stages that are common in other poecilosclerid sponges (Bergquist & Green, 1977; Delage, 1892; Ereskovsky, 1999). During metamorphosis, the aquiferous system asymmetrically developed inside the settlers (Figure 4D,E). The *Wnt* signaling gene pathway is known to be important for lateralization during metazoan development (Richter & King, 2013) and it is also involved in the polarization of sponge aquiferous system during metamorphosis (Reid et al., 2018). Further studies may clarify the role of this ontogenetic pathway in the development of the aquiferous system in *C. incrustans*.

Along with reproductive structures, histological sections revealed the presence of bacterial aggregations in the mesohyl of *C. incrustans* (Figure 1O,P). Vertical transmission of bacteria through gametes and larvae has been reported in several sponge species (Maldonado, 2007). The bacterial aggregations dispersed in the mesohyl of *C. incrustans* could be involved in vertical transmission during larval development, and further analysis may clarify this important aspect of the life history of this sponge species.

As a result of its abundance and wide ecological distribution, *C. incrustans* is increasingly considered a suitable model organism for experimentally assessing the impacts of natural and anthropogenic ecological changes in marine benthic communities (Bates & Bell, 2018; Cummings et al., 2020). Wellington Harbour is a semi-enclosed basin subjected to several anthropogenic pressures (Anderlini, 1992), and our study provides information on the early life stages of this temperate model species that will be important in further ecophysiological experiments.

In summary, developmental asynchrony coupled with viviparity, and the release of highly specialized non-tufted parenchymella larvae appear to be common features of poecilosclerid sponges. Further ecological studies that take into account early life stages may identify possible adaptive features underpinning not only the high

abundance and wide distribution of *C. incrustans* in temperate Pacific waters (Berman & Bell, 2010; Cook, 2010; Hooper & Wiedenmayer, 1994; Kelly et al., 2009; Kyung & Chung, 2001), but also the evolutionary success of the order Poecilosclerida globally (Hooper & Van Soest, 2002; Vacelet & Boury-Esnault, 1995; WoRMS, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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