Embryo and larval biology of the deep octocoral species *Dentomuricea* aff. *meteor* under different temperature regimes (#56492)

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Embryo and larval biology of the deep octocoral species *Dentomuricea* aff. *meteor* under different temperature regimes

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Deep-sea octocorals are common habitat-formers in deep-sea ecosystems, however our knowledge on their early life history stages is extremely limited. The objective of this study was to describe the embryo and larval biology of the deep-sea octocoral *Dentomuricea* aff. *meteor*, a common habitat-forming species in the Azores, under two temperature regimes, corresponding to the minimum and maximum temperatures in their natural environment during the spawning season. Under temperature of 13 ± 0.5°C, embryos of the species reached the planula stage after 96h and displayed Pelagic Larval Duration (PLD) of 24 days. Planula larvae displayed swimming only after stimulation, larval swimming speed was 0.24 ± 0.16 mm s^{-1} and seemed to increase slightly but significantly with time. Under a higher temperature (15°C ± 0.5°C) embryos reached the planula stage 24h earlier (after 72h), displayed PLD of 27 days and had significantly higher swimming speed (0.3 ± 0.27 mm s^{-1}). Survival was not affected significantly by temperature, however our results highlight how small changes in temperature can affect larval characteristics with potential cascading effects in larval success and dispersal. In both temperatures, larvae displayed unselective settlement behaviour and metamorphosis occurred even without settlement. Such information is rarely available for deep-sea corals, although it is essential to achieve a better understanding of dispersal, connectivity and biogeographical patterns of benthic species.

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Abstract

Deep-sea octocorals are common habitat-formers in deep-sea ecosystems, however our knowledge on their early life history stages is extremely limited. The objective of this study was to describe the embryo and larval biology of the deep-sea octocoral *Dentomuricea* aff. *meteor*, a common ~~habitat-forming~~ species in the Azores, under two temperature regimes~~,~~ corresponding to the minimum and maximum temperatures in their natural environment during the spawning season. ~~Under~~ temperature of 13 ± 0.5°C, embryos of the species reached the planula stage after 96h and displayed Pelagic Larval Duration (PLD) of 24 days. Planula larvae displayed swimming only after stimulation, ~~larval~~ swimming speed ~~was~~ 0.24 ± 0.16 mm s^{-1} ~~and seemed to~~ increase slightly but significantly with time. ~~Under~~ a higher temperature (15°C ± 0.5°C) embryos reached the planula stage 24h earlier (after 72h), displayed PLD of 27 days and had significantly higher swimming speed (0.3 ± 0.27 mm s^{-1}). Survival was not affected significantly by temperature, however, our results highlight how small changes in temperature can affect larval characteristics with potential cascading effects in larval success and dispersal. In both temperatures, larvae displayed unselective settlement behaviour and metamorphosis occurred even without settlement. Such information is rarely available for deep-sea corals, although ~~it is~~ essential to achieve a better understanding of dispersal, connectivity and biogeographical patterns of benthic species.

Introduction

Species persistence requires the successful completion of a life cycle against biotic and abiotic odds, starting with survival at early life history stages. These stages are key for sessile species, such as benthic marine invertebrates, which upon reaching the adult stage cannot escape

unfavorable conditions. For these organisms, early life events such as larval survival and settlement determine the fate of the adult phase and are therefore extremely important (Marshal and Morgan, 2011; Byrne, 2012). Moreover, larval stages ~~consist~~ the only pelagic phase that ensures dispersal and connectivity among populations (Cowen and Sponaugle, 2009). In deep-sea communities ~~which are~~ dominated by benthic marine invertebrates, knowledge on early life stages is therefore key in understanding species distributions, biogeographical patterns, population and metapopulation dynamics (Treml et al., 2015), ~~consisting~~ an essential tool for management (Hilario et al., 2015).

Deep-sea octocorals are major habitat-formers in the deep-sea, usually occurring in complex geological settings such as continental shelves and margins (Yesson et al., 2008, Taylor et al., 2013), underwater canyons (Brooke et al., 2017), and seamounts (Tempera et al., 2012; Braga-Henriques et al., 2013). Due to the habitat requirements of some octocoral species, ~~–~~including hard substrates for settlement and strong currents which optimize food delivery, ~~–~~their distribution can be quite ~~fragmented~~ (Bryan and Metaxas, 2006; Tong et al., 2012), as observed for other deep-sea benthic species (Miller and Gulasekera, 2017). Anthropogenic disturbance and global climate change are likely to increase habitat fragmentation even more, by altering habitat characteristics (Sweetman et al., 2017; Levin et al., 2019) and causing a decrease in ~~the~~ available suitable habitat ~~of~~ some species (Morato et al., 2020). Under these circumstances, obtaining a solid understanding of larval biology and population connectivity is essential to understand community dynamics and the potential of deep-sea octocoral populations to recover from disturbance (Cowen et al., 2007).

So far, our knowledge on larval biology of deep-sea octocorals ~~is~~ limited to a few brooding species (Cordes et al., 2001; Sun et al., 2010; 2011; Mercier and Hamel, 2011). In most of these

cases, larvae settled within 2-5 days after ~~larvae~~ release and shortly metamorphosed into primary polyps, e.g. *Anthomastus ritteri* (Cordes et al., 2001), *Gersemia fruticosa* and *Duva florida* (Sun et al., 2011). They also displayed short competency periods ~~with~~ limited swimming behaviour (Sun et al., 2010). However, many deep-sea octocorals are broadcast-spawners and are therefore expected to display different larval characteristics and dispersal capabilities (Harrison and Wallace, 1990; Nishikawa et al., 2003). To our knowledge, so far no information exists on the larval biology of broadcast spawning deep-sea octocorals.

Larvae from broadcast spawning species undergo early development in the water column, where they are mostly transported as passive particles until they reach the planula stage. During transportation, embryos can be exposed to variable environmental conditions which may affect their development (Melzner et al., 2009). This phenomenon can be even more pronounced in deep-sea species, in which embryos and larvae can often display upward swimming, crossing water masses with very different physicochemical characteristics (Young et al., 1996, 2012; Arellano et al., 2014; Strömberg and Larsson, 2017). One of the most important factors influencing early development in marine organisms is temperature (Hoegh-Guldberg and Pearse, 1995; Przeslawski et al., 2015). Despite its importance and the increasing interest for the effect of global warming on larvae of shallow scleractinian species (Randall et al., 2009; Figueiredo et al. 2014), so far limited attention has been ~~brought on temperature effects~~ on the embryonic development of deep-sea coral species, with some information existing only for the scleractinian *Lophelia pertusa* (Stromberg and Larsson, 2017).

The aim of this study is to provide a detailed description of the larval biology of the deep-sea broadcast spawning species *Dentomuricea* aff. *meteor*, a common habitat-forming deep-sea octocoral in the Azores. To achieve our goal, we employed an experimental approach with

assisted fertilization and larvae rearing in aquaria. We also report on the embryonic development and larval characteristics of *D.* aff. *meteor* under two temperature regimes, representing the minimum and maximum temperatures experienced by the species in its natural habitat.

Materials and Methods

Target species and specimen collection

The Azores Archipelago, located above the Mid-Atlantic Ridge, is a biodiversity hotspot for deep-sea octocorals (Sampaio et al., 2019). Coral gardens (OSPAR, 2010) formed by deep-sea octocorals are among the most prominent deep-sea communities on regional seamounts and island slopes (Braga-Henriques et al., 2013). *Dentomuricea* aff. *meteor* is an octocoral species of the family Plexauridae, so far only recorded on the seamounts of the North Mid-Atlantic Ridge. It is common in regional seamounts between 200-600 meters (Braga-Henriques et al., 2013), where it forms dense populations, often in combination with other octocoral species such as *Viminella flagellum* and *Callogorgia verticillate*. The species is gonochoristic and presents gametes all year round, with seasonal peaks of gamete maturation and spawning usually occurring in autumn (Rakka et al., unpubl. data).

A total of 11 colonies of ~~the species *Dentomuricea*~~ aff. *meteor* were collected from Condor Seamount in September and October 2019, as by-catch from experimental long-line fisheries on board RV Archipelago (ARQDAÇO monitoring programme). Colonies were divided in large fragments (20-30 cm height) and were kept at the DeepSeaLab aquaria facilities (Orejas et al., 2019), in six 33L aquaria positioned in a thermo-regulated room at 14°C. Aquaria were supplied continuously with seawater pumped from 5m depth, previously treated with UV light (P10

UVsystem & Vecton 600 TMC™) and passed through 50 μm and 1 μm mesh filters. Circulation within the aquaria was maintained by pumps. Seawater temperature was kept between 13-14°C with the aid of chillers while colonies were fed twice per day with a mixture of frozen zooplankton and microplankton which was frequently enriched with live microalgae (*Chaetoceros calcitrans* and *Nannochloropsis gaditana*) and live rotifers.

Larvae rearing

Larvae were obtained by maintaining reproductively active female and male colonies in the same aquaria to achieve natural spawning and fertilization. Coral fragments were allowed to acclimatize in the above aquaria conditions for approximately a month. Subsequently, colonies with mature gametes were identified by dissecting two branchlets (3-5 cm height) from each colony and observing their tissue under a dissecting microscope. Reproductively immature colonies and fragments in poor condition were excluded from further analysis. This procedure resulted in selection of six female and three male colonies. Coral fragments from the female colonies were distributed in two aquaria, referred to as spawning aquaria. Subsequently the fertile male colony with the higher number of available fragments was selected and four of its fragments were distributed in each of the two spawning aquaria. The remaining male colonies were not used to avoid polyspermy (Levitan et al., 2007).

To increase the potential of spawning, we enriched the aquaria water with free mature sperm, obtained from the selected male colony. This was achieved by dissecting mature spermatocysts from coral tissue, which were subsequently concentrated in 50 ml flasks with filtered (0.2 μm) SW, ~~homogenized~~ by gently shaking and redistributed to the aquaria. Water ~~inflow~~ was stopped

and aquaria pumps were substituted with aeration to ensure water circulation without losing or harming potentially spawned gametes. Upon gamete ~~spawning~~, which happened in batches, all gametes/fertilized eggs were collected from the water column and distributed in ten 750 ml-culture flasks (20-100 fertilized eggs per flask) that were filled with filtered SW (mesh size: 0.2 µm).

Temperature experiments

In order to chose appropriate temperature regimes for larval rearing, we utilized temperature data collected during annual CTD surveys, under the framework of the projects CONDOR (EEA Grants PT-0040) and SMaRT (SRECC- Azores Regional Government M.2.1.2/029/2011). Data were collected between 2010 and 2012, above the coral garden where specimen collection took place. Subsequently, we utilized the minimum and maximum recorded values (13.4°C and 15.12°C respectively) during the spawning season of the target species (October-November) to define the target rearing temperatures (13 ± 0.5°C and 15 ± 0.5°C). Upon collection of fertilized eggs/embryos, culture flasks were distributed in two water baths, maintaining temperature within 0.5 °C of the target temperatures, with the aid of an aquaria chiller and a heater respectively. Culture flasks were equipped with glass pipettes connected to an aquaria air pump, achieving continuous light circulation, while water in the flasks was exchanged daily.

Embryonic and larval development

Embryos were monitored every 3-4 hours during the first 48 hours and subsequently once a day until reaching the planula stage, to study their early development. ~~In~~ every monitoring event, all embryos were counted to estimate survival, while 10-15 embryos were randomly removed from the flask, photographed with a digital camera (DIGICAM 5M~~EG~~ LCMOS MAC) attached to a

microscope (10x) and returned to the flasks. Images were later processed to record the developmental stage and size of each embryo. Due to repetitive and frequent spawning within the aquaria and continuous collection of embryos, oocytes from different spawning events were frequently mixed and therefore the timing of embryonic development is approximate. Moreover, since it was not possible to define the moment of fertilization, embryo development is presented in respect to spawning time. To estimate size, we measured width and length (mm) of each larva using the open software Fiji/Image J (Schindelin et al., 2012). The data were subsequently used to estimate volume (mm^3) assuming larvae had the shape of a prolate spheroid (Larsson et al., 2014). The ratio of length to width was used as a proxy of sphericity.

Swimming behaviour

Data on swimming speed and behaviour were collected by video recording and analysis. Videos were recorded with a Canon EOS 600D digital camera, equipped with a regular 22-55mm lens, on day 4 and day 15 after spawning, which corresponded to the first day larvae reached the mature planula stage and the first day larvae displayed competency to settle, respectively. To avoid larva~~e~~ handling, swimming behaviour was recorded in the same culture flasks used for larva~~e~~ rearing. Videos were captured in the dark, using lateral led lights for illumination (Stromberg and Larsson, 2017). Flasks were positioned in front of a black slide with a calibrated grid that was used as background and a 2-minute waiting period was implemented to ensure no water movement was interfering with larva~~e~~ swimming. Subsequently, three videos (duration: 1 min) were recorded ~~between~~ intervals of three minutes.

Videos were converted to ~~video~~ frames and were analyzed by an automatic particle tracking method, using the open software Fiji/Image J (Schindelin et al., 2012) and the plugin TrackMate

(Tinevez et al., 2017) to record data on vertical swimming behaviour, namely swimming direction (up/down), displacement and swimming speed. Estimates of swimming speed only considered tracks with displacement higher than 2 mm, to exclude data from larvae that did not move or moved minimally.

Settlement and metamorphosis

After reaching the planula stage, larvae were counted every 2-3 days until day 39 to estimate survival. Moreover, during counting, each larva was assigned to one of four stages: planula, settled, pelagically metamorphosed and deformed. Because counts were made simultaneously for all flasks and each flask contained a batch of different age, e.g. some batches were spawned with 1-3 day difference, when average counts were estimated these were sometimes heavily influenced by the available count for that day. To be able to estimate robust mean counts for each monitoring day, missing values were regenerated for each flask by using linear interpolation between existing data points. Lastly, on days 4 and 14 after spawning, five planulae were removed from each flask (total n=25 for each temperature regime) and photographed with a digital microscope camera to estimate their size.

Pelagic Larval Duration (PLD) was inferred from the proportion of larvae abandoning the planula state, either by settling or deceasing, thus taking survival and larvae development into account. We report the time point at which 50% of the initial number of larvae left the pelagic stage as median PLD, as well as the minimum and maximum observed in individual batches. Metamorphosed larvae that did not settle were assumed to continue in a pelagic phase. Since larvae did not display clear bottom probing behaviour, the onset of competency was defined by the first settling larvae.

After larvae presented competency (day 14), substrate was provided to the culture flasks in order to monitor settlement behaviour. Three flasks from each temperature regime were randomly selected and three pieces (approximate diameter: 0.5 cm) of basalt rock attached to a plastic slide (1 cm x 8 cm) were offered as potential substrate in each flask. Settled larvae were observed and photographed every 2-3 days to assess and describe settlement and metamorphosis, during a period of approximately two weeks. After metamorphosis was observed, a mixture of live microalgae (*Nannochloropsis gaditana* and *Chaetoceros calcitrans*) and rotifers was provided weekly.

Statistical analysis

Survival analysis was performed using the Kaplan-Meier method, following Graham et al. (2008). Since monitoring was done in time intervals and the exact time of death for each larva was not known (interval-censored data), we assumed that time of death was the moment at which each larva was observed for the last time. However, for each batch, the remaining larvae at the last monitoring event were considered alive (censored data). As the Kaplan-Meier method does not allow for incorporation of replicate information into the analysis, we performed the analysis by pooling data from all batches together, for each rearing temperature. Subsequently the analysis was repeated separately for each batch, to provide information about the variability among batches (Graham et al., 2008). A log-rank test was performed to compare the survival curves between larvae reared under 13°C and 15°C. Survival analysis was performed by using the packages survival (Therneau and Grambsch, 2017) and survminer (Kassambara et al., 2019) in R 3.5.0 (R Core Team, 2018).

For the rest of the dependent variables, we firstly performed exploratory analysis (Zuur et al., 2010) to select the most appropriate modeling method. The effect of each independent variable was subsequently tested with linear models (LMs), by adding the independent variables progressively to the respective model and using maximum likelihood ratio (MLR) tests and the Akaike Information Criterion (AIC). Data collected from monitoring larvae stages (proportions) were modeled by means of Generalized Linear Models (GLMs) with a binomial distribution. Summarized results of the MLR test for each variable in question are provided in Table 1, while the results from each selected model are provided graphically as supplementary material (Fig. S1-S4). Statistical analysis was performed in R (R Core Team, 2019).

Results

Spawning

The first spawning event occurred on the 27th of November. Oocytes were encountered within 15 minutes from enrichment with free live sperm, in both aquaria. During spawning, oocytes and early-stage embryos floated in the water column, however, spawning was not coordinated among colonies, neither among polyps of the same colony. Despite careful observation, it was not possible to directly observe polyps releasing sperm or oocytes and determine whether one or more colonies participated in each spawning event. Similarly, it was not possible to directly observe if fertilization was internal or external. All collected oocytes were fertilized, therefore fertilization was either internal or external with very high fertilization rates. Oocytes were spherical, they had no visible germinal vesicle and were released in batches of 10-80 at a time. They were mostly negatively buoyant, however, they remained in suspension for several hours

240 due to water movement within the aquaria. Average oocyte diameter was 365.4 ± 24.2 µm.
241 Frequent spawning events, i.e. every 2-3 hours, continued for a week, while spawning continued
242 with lower frequency for approximately a month.

243 Embryonic and larval development

244 Cell division was always equal but cleavage varied highly among stages and embryos. It was not
245 possible to determine the ~~time~~ of the first division after spawning. Cytokinesis was never visible
246 for the 2-cell stage, in which cleavage seemed to be always superficial (Fig. 1). During the
247 following stages, cleavage varied from radial to pseudospiral and in some cases superficial,
248 leading to embryos with substantial differences in shape. However, development always led to a
249 hollow blastula (Fig. 1) followed by gastrulation and the formation of planula larvae without
250 visible oral ~~track~~ (Fig. 1). Cleavage and cell division did not differ between the two rearing
251 temperatures.

252 ~~Under~~ 13°C, all ~~the~~ embryos reached the blastula stage within 10h ~~from~~ spawning, and ~~the~~
253 gastrula stage within 48h. After 72h all embryos reached ~~the~~ late gastrula stage and could
254 perform slow, mainly rotating movements by cilia, while fully competent, swimming planulae
255 ~~larvae~~ were formed after 96h (4 days). During their development, embryos were negatively
256 buoyant and accumulated at the bottom of the flasks. In the first batch this resulted in the
257 formation of embryo aggregations and abnormal embryo development, however this issue was
258 solved by adding slight aeration that ensured water and oxygen circulation within the flasks.
259 ~~Under~~ 15°C, during the first 10 hours cleavage seemed to be occurring at similar intervals until
260 reaching the blastula stage (Fig. 2), however embryos reached the gastrula and subsequently the
261 planula stage approximately 24h (after 72h) earlier than embryos reared ~~under~~ 13°C (Fig. 2).

Embryos between the 2-cell and 32-cell stage obtained variable shapes (Fig. 1) and their volume was on average 0.03 ± 0.0073 mm^3. Subsequently, during the 64-cell ~~stage~~ and blastula they turned more spherical but had similar volume range (0.03 ± 0.005 mm^3). After reaching the planula stage, embryos increased significantly in size (Table 1) with planula ~~larvae~~ reaching 0.28 ± 0.1 mm^3 on day 4 and 0.67 ± 0.28 mm^3 on day 14. ~~However~~ on day 14 a substantial variability in larva~~e~~ volume was observed. Mature planula larvae displayed the capacity to change their shape between spherical and elongated, and more elongated larvae were observed on day 14 compared to day 4 (Figure S1). This was also confirmed from the LW ratio which decreased from late gastrula embryos (1.49 ± 0.17 mm^3) to planula ~~larvae~~ on day 4 (1.22 ± 0.29 mm^3) but increased significantly (Table 1) on day 14 (2.02 ± 0.45 mm^3). Embryo sizes were not statistically different between the two temperatures (Table 1). However, planula ~~larvae~~ on day 4 had significantly higher LW ratios under 15°C (LW=1.59 ±0.39; Table 1), showing a tendency to maintain a more elongated shape than under 13°C.

Under 13°C, survival differed substantially among batches (Fig. S5). In most batches, a sharp decline in survival rates was observed during the first 48 hours, followed by a decrease in mortality until day 10 after which a more moderate mortality rate was established (Fig. 3). Overall, median survival time, i.e. time when mortality reached 50%, was 11 days while final survival rate after one month was 16.4%. Under 15 °C, the average mortality rate seemed to be more constant (Fig. 3). Median survival time was 5 days ~~higher~~ than under 13°C (16 days, Table 2), however, final survival rate after 32 days was slightly lower (11.5%, Table 2). Overall, these differences were not statistically significant according to the log-rank test (p=0.07; Fig. 3).

Swimming behaviour

Planula larvae remained mostly at the bottom of the culture flasks, where they displayed slight rotating and unidirectional movements, however, they rarely became waterborne without the aid of water movement. Once in the water column, larvae did not show a specific swimming pattern but followed random trajectories. Overall, for larvae reared under 13°C, 51.2 ± 14.2 % of the recorded larval tracks were directed upwards while 50.7 ± 6.33 % were directed downwards. It was not clear if downward movement involved swimming or just sinking. The proportion of upward/downward swimming larvae did not change significantly with time (Table 1). Larvae displayed an average swimming speed of 0.24 ± 0.16 mm s^{-1} on day 4 and 0.36 ± 0.21 mm s^{-1} on day 15. Swimming speed did not differ significantly between upward and downward movements (Table 1; Fig. 4), however it was significantly higher on day 15 compared to day 4 (Table 1; Fig. 4).

Swimming velocity for larvae reared under 15 °C was similar between upward and downward swimming (Table 1) and increased slightly but significantly with time (Table 1), from 0.4 ± 0.24 mm s^{-1} on day 4 to 0.44 ± 0.23 mm s^{-1}. Overall, 52.7% of the recorded tracks were directed downwards and the proportion of upward/downward swimming tracks did not differ significantly between dates (Table 1). Larvae swimming velocity was significantly higher under 15 °C compared to 13 °C, (Fig. 4) both on day 4 and day 15 (Table 1).

Settlement and metamorphosis

In both rearing temperatures, most larvae settled on the flask walls and plastic slides whereas no larvae attached to the provided basalt rock. Planula larvae under 13°C started settling on day 14 and the proportion of settled larvae increased significantly with time, reaching 23.96 ± 24.1 % on day 36 (Fig. 4). There was high variance associated with the estimates of the average proportion

of settled larvae (Fig. 4), however, this was due to a single batch in which very few larvae settled throughout the study period. Larvae under 15°C started settling later (Table 1) and overall, a lower proportion of larvae settled throughout the study. Moreover, under 15°C a significantly higher proportion (Table 1) remained in the planula stage for longer time (Fig. 4), leading to a slightly ~~higher~~ PLD. However, in both temperatures, on day 36 only a minimal proportion of planula ~~larvae~~ remained (Fig. 4).

After day 14, an increasing proportion of larvae initiated metamorphosis without settling (Fig. 4), reaching 57.31 ± 6.75 % on day 36. This form of pelagic metamorphosis started with planula larvae obtaining a pear shape (Fig. 5) and continued with formation of mouth, mesenteries, tentacles, and finally sclerites (Fig. 5). Metamorphosis from planula ~~larva~~ to primary polyp took approximately 2-3 days. None of the larvae that displayed pelagic metamorphosis settled during the course of the study. This pelagic form of metamorphosis resulted in a high percentage of deformed larvae (Fig. 5). Metamorphosed larvae were still able to get transported by water movements, however, they displayed limited swimming ability. By day 28, 50% of the planula ~~larvae~~ had settled, metamorphosed or deceased. The last free swimming planula ~~larva~~ was observed on day 39.

Under 15°C, metamorphosed and deformed larvae started appearing later in time (Fig. 4), however, in the last 5 days a significantly higher proportion of metamorphosed larvae were observed compared to 13°C (Table 1). The pattern for deformed larvae between the two temperatures was also significantly different (Table 1), as they appeared earlier in time under 13°C than at 15°C. Under 15°C, the number of deformed larvae increased only after day 32 but within the last two days it reached higher values compared to 13°C (Fig. 4).

For settled larvae, metamorphosis followed a similar pattern to pelagic metamorphosis. Larvae firstly obtained a pear-like shape and subsequently became more round, gradually forming a polyp base, mouth and mesenteries (Fig. 6A). Fully developed primary polyps were formed within approximately 2-3 days, after the formation of tentacles, sclerites, and tentacle pinnules (Fig. 6B).

Discussion

So far, studies on the biology and ecology of deep-sea octocorals have focused mainly on the adult stage (Watling et al., 2011), with very few studies tackling early life history stages (Cordes et al., 2001, Sun et al., 2010; 2011). To our knowledge, the present study is the first to provide a detailed insight to the larval biology of a deep-sea octocoral species including embryo development, larval survival and swimming behaviour, which are essential variables to understand dispersal and connectivity in the deep-sea (Gary et al., 2020).

In our study, it was not clear if spawning was actually induced, assisted, or just coincided with sperm enrichment, due to the small time interval between sperm enrichment and the first spawning event. Repetitive spawning within a specific period is common for tropical broadcast spawning octocorals within the family Plexauridae such as *Plexaura homomalla* (Wells et al., 2020) and *Plexaura flexuosa* (Pakes and Woolakott, 2008). Repetitive planulation has also been recorded for some brooding octocorals, including temperate (e.g. *Corallium rubrum*, Martínez Quintana, 2015 and *Eunicella singularis*, Weinberg and Weinberg, 1979) and deep-sea species (e.g. *Gersemia fruticosa*, Sun et al., 2011). It has been suggested that repetitive gamete release can increase the probability of embryo and larva development under optimal conditions (Kahng

et al., 2008). In species that display this behaviour, studying the effects of different environmental variables on embryo development is crucial, as embryos of different cohorts are likely to be released in different environmental conditions including temperature, salinity, pH and food availability.

Embryo and larval development of *D.* aff. *meteor* had many similar characteristics with shallow-water, tropical octocorals. Unequal cleavage that ranges from radial to pseudospiral is common among cnidarians (Fritzenwanker et al., 2007), including tropical brooding (Benayahu and Loya, 1983; Dahan and Benayahu, 1998), and broadcast spawning (Mandelberg-Aharon and Benayahu, 2015) octocorals. Superficial cleavage is frequently encountered in embryos with high amounts of yolk reserves (Scriba, 2015). Similarly to other octocoral species (Mandelberg-Aharon and Benayahu, 2015; Wells et al., 2020), larvae of *D.* aff. *meteor* appeared to be lecithotrophic since no mouth or oral opening was observed before metamorphosis. Moreover, the temporal developmental profile of *D.* aff. *meteor* was comparable to that of broadcast spawning tropical species from the same family which reached the planula stage within 2-3 days and had relatively quick onset of competency (4-5 days), short longevity, and low selectivity in settlement (Lasker and Kim, 1996; Coelho and Lasker, 2016; Wells et al., 2020). Overall, these findings suggest that some reproductive and larval characteristics might be conserved among taxonomically related groups, despite local adaptations due to depth and other habitat limitations.

For most deep-sea octocorals studied so far, planulae appeared to have low mobility, with negative buoyancy and crawling (e.g. *Drifa glomerata*, Sun et al., 2010; *Duva florida*, Sun et al., 2011) or limited swimming capacity generated by body contractions (e.g. *Drifa sp.*, Sun et al., 2010). Larvae of *D.* aff. *meteor* were active swimmers, however, they initiated swimming only after stimulation. Similar behaviour has been reported for the octocoral *Corallium rubrum* which

displayed increased motility after stimulation (Martínez-Quintana, 2015). Swimming speed was also similar to that of *C. rubrum* (0.45-0.55 mm s^{-1}), however larvae in that study were maintained under higher temperature (19-20 °C). When compared to other deep-sea broadcast spawners, such as the scleractinian *L. pertusa*, *D.* aff. *meteor* had lower swimming capabilities, especially since *L. pertusa* displayed intense, negatively geotactic behaviour and higher swimming velocity (Larsson et al., 2014).

Larvae planktonic period can be divided in two phases, an obligatory phase that lasts until the onset of developmental competence (the ability to respond to settlement cues) and a facultative phase that depends on settlement behaviour in response to the existence of certain chemical and physical properties of benthic substrate (Elkin and Marshal, 2007). In this study it was not possible to determine the exact onset of competency since larvae did not display any specific geotactic or bottom probing behaviour. Instead, larvae displayed three types of behaviour: non-selective settling, pelagic metamorphosis and continuation in the pelagic phase as planula larvae. All larvae that settled presented non-selective settling behavior, settling on the flask walls and plastic slides after 14 days of larvae development, whereas no larvae attached to the provided basalt rock. This might have been a result of the absence of settlement cues or adequate settlement surface in the provided substrate. Non-selective settlement behavior in the absence of proper substrate has been observed before in coral larvae, including octocorals (Lasker and Kim, 1996: Freire et al., 2019). This phenomenon has been tentatively explained by the desperate larvae hypothesis (Gibson, 1995; Marshal and Keough, 2003), which states that larvae should accept less preferred habitats as time progresses, because metamorphosis is energetically demanding and non-feeding larvae can only delay metamorphosis until reaching a specific reserve level (Elkin and Marshall, 2007). The hypothesis is particularly pertinent for non-feeding

lecithotrophic larvae, since the duration of the planktonic phase is likely determined by the availability of energetic reserves (Wendt, 2000). However, there is considerable interspecific variation in the ability of larva to delay competence, and this is not always correlated to larval size or maternal provision, suggesting the existence of additional factors, such as developmental and phylogenetic constrains (Bishop et al., 2006).

A high proportion of non-settled planulae initiated metamorphosis after 14 days of development, resulting in a high proportion of pelagic polyps after 36 days of larvae development. Pelagic metamorphosis of planulae into polyps has been reported for tropical (Zaslow and Benayahu, 1996; Lasker and Kim, 1996) and temperate (Linares et al., 2008) octocorals, for the deep-sea octocoral *Gersemia fruticosa* (Sun et al., 2011) and for some scleractinian species (Vermeij 2009; Mizrahi et al., 2014), suggesting that this behaviour is not uncommon among corals. In some scleractinians, pelagic polyps can display high survival and dispersal potential (Mizrahi et al., 2014), and in some cases pelagic polyps have been observed feeding (Zazlow and Benayahu, 1996; Linares et al., 2008). In our study, pelagic polyps had high mortality but this could be due to the absence of sufficient or adequate food sources. Nevertheless, pelagic metamorphosis might be another response to potential shortage of energy reserves, as it provides a way to acquire feeding structures and allows the acquisition of energy while waiting for the right settlement cue. In the case of *D.* aff. *meteor*, this behaviour, along with the non-selective settling, enhance the hypothesis that larvae had limited energy reserves and possibly reached their maximum larval duration during the experiment.

Collectively, the embryonic and larval characteristics of *D.* aff. *meteor* suggest a higher dispersal potential than most deep-sea octocorals studied so far. However, when compared to other deep-sea species, the dispersal capacity of *D.* aff. *meteor* appears to be limited due to its short PLD,

short competency period, weak swimming (Table 2), and unselective settlement behaviour. For example, *L. pertusa* displayed onset of competency within 3-5 weeks ~~from~~ spawning, active upward swimming and survival without settlement for a year (Larsson et al., 2014). Similarly, other deep-sea species such as the bivalve *Bathymodiolus childressi* and the gastropod *Bathynerita naticoidea* display longer PLDs which indicate much higher dispersal potential than *D.* aff. *meteor* (Arellano and Young, 2009). The three deep-sea species, *L. pertusa*, *B. childressi and B. naticoidea,* have been shown to perform ontogenetic migrations to the surface (Arellano et al., 2014; Larsson et al., 2014) which does not seem likely for *D.* aff. *meteor*, based on the characteristics described herein. Nevertheless, the dispersal capacities of these species ~~corroborate~~ with their distributions, with *L. pertusa* and *B. childressi* displaying strong dispersal potential which is consistent with their wide distributions, and *D.* aff. *meteor* displaying lower dispersal capacities, ~~corroborating~~ with its narrow regional distribution in the Mid-Atlantic Ridge.

Temperature is considered one of the main factors affecting larva~~e~~ biology, with higher temperatures usually resulting in ~~higher~~ developmental rates (Hoegh-Guldberg and Pearse, 1995). Our results were consistent with this premise, with larvae reaching the planula stage 24 hours earlier ~~under~~ 15°C ~~when~~ compared to 13 °C. These results also ~~corroborate~~ with studies on other deep-sea species, such as *L. pertusa* (Stromberg and Larsson, 2017) and *B. childressi* (Arellano and Young, 2009) which displayed faster development with increased temperature. However, both studies tested larger temperature differences and concern species which perform long vertical migrations to the surface and therefore display high tolerance to different temperature and salinity (Arellano and Young, 2011; Stromberg and Larsson, 2017). The higher developmental rate observed ~~under~~ 15 °C is expected to be accompanied by earlier competency,

443 shorter pelagic duration, and higher settlement rates (O'Connor et al., 2007; Heyward and Negri,
444 2010), with further consequences for larval transport and dispersal (Metaxas and Saunders, 2009).
445 However, ~~under~~ higher temperature, larvae of *D.* aff. *meteor* displayed lower settlement rate and
446 higher proportion of deformed larvae. Faster developmental rates, accompanied by decreased
447 ~~settlement under~~ increased temperature (+ 3°C) has been reported for the tropical octocoral
448 *Heliopora coerulea* (Conaco et al., 2020), while abnormal metamorphosis and lower survival has
449 been recorded in larvae of the temperate octocoral *P. clavata* that were transferred to higher
450 temperature (+ 5°C) after reaching the planula stage (Kipson et al., 2012). It is possible that these
451 results are related to temperature-induced changes in aspects that were not evaluated in our
452 study, including developmental and physiological mechanisms. For example, it is possible that
453 faster development under higher temperature was accompanied by faster metabolic rates
454 (O'Connor et al., 2007), and resulted in faster consumption of reserves, leading to high mortality
455 under the absence of proper settlement cues. Ontogeny depends on certain developmental
456 processes and their timing, and while developmental rate can be plastic, changes in timing are
457 likely to have consequences on structure and function, ultimately affecting individual
458 performance (Kováč, 2002).

459 Since the two temperature regimes used in this study are likely to be experienced by embryos of
460 the target species during spawning, our results highlight how small changes in temperature can
461 affect an array of larval characteristics with substantial effects on larval behaviour, dispersal and
462 ultimately, success. Similar results have been reported for tropical scleractinian species (e.g.
463 Randall and Szmant, 2009; Heyward and Negri, 2010). In the deep-sea, refined embryo and
464 larval responses under a narrow temperature range (2-3°C) have even allowed species to disperse
465 in specific water masses and expand their range to greater depths, e.g. the Antarctic echinoderm

Sterechinus neumayeri (Tyler et al., 2000). Larvae dispersal and success are important features not only from an ecological but also from an evolutionary perspective, as they can define the selection of reproductive strategies, including reproductive timing. Reproductive timing can be the result of complex mechanisms that are difficult to unravel. In deep-sea corals it has been discussed in relation to adult physiology and its seasonal constraints, or to environmental cues that induce gametogenesis and spawning (e.g. Orejas et al., 2002; Waller et al., 2014). ~~However,~~ the adaptive significance of reproductive timing can also be defined by processes that affect larval survival and success (Olive, 1992). The connection between ~~the~~ optimal time of spawning and larval success has been addressed in other ectotherms such as fish (Asoh and Yoshikawa, 2002), crustaceans (Morgan and Christy, 1995), and tropical scleractinian corals (Crowder et al. 2014; Fan et al., 2017), however, these aspects have not been addressed yet for deep-sea corals. Further studies on the effect of temperature on larval development, physiology, and behaviour are therefore essential to obtain a holistic view of reproductive timing and the potential impacts of climate change on deep-sea corals.

Conclusions

In our study, we provided a detailed description of embryo and larval characteristics of the species *D.* aff. *meteor*. Our results suggest that *D.* aff. *meteor* larvae are lecithotrophic with development similar to other octocorals, and low dispersal capacity compared to other deep-sea species. This ~~corroborates~~ with its limited geographical distribution. ~~Temperature changes~~ did not affect survival, however, significant effects were detected on the rate of embryo development and swimming speed, which in the field can potentially alter larval dispersal and ultimately success. Deep-sea octocorals are receiving increasing attention as a growing number of studies focus on the habitat requirements and environmental conditions shaping deep-sea communities

(Radice et al., 2016; Barbosa et al., 2020; Morato et al., 2020). ~~However,~~ species distribution is a result of complex interactions between factors ~~in~~ various ecological levels, with early life history biology and dispersal playing a key role in the successful occupation of available suitable habitat (Schurr et al., 2007; Robinson et al., 2011). ~~As biophysical dispersal modelling attempts~~ are increasing in the deep-sea (Hilario et al., 2015; Ross et al., 2016), further research on embryo and larval biology are essential to obtain ~~a better understanding of deep-sea ecosystems.~~

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Figure 1

Stages of embryo development of the octocoral species *Dentomuricea* aff. *meteor*

(a) fertilized oocyte, (b) 2-cell, (c) 4-cell , (d) 8-cell, (e) 16-cell, (f) 64-cell, (g) hollow blastula, (g) gastrula, (i) planula.

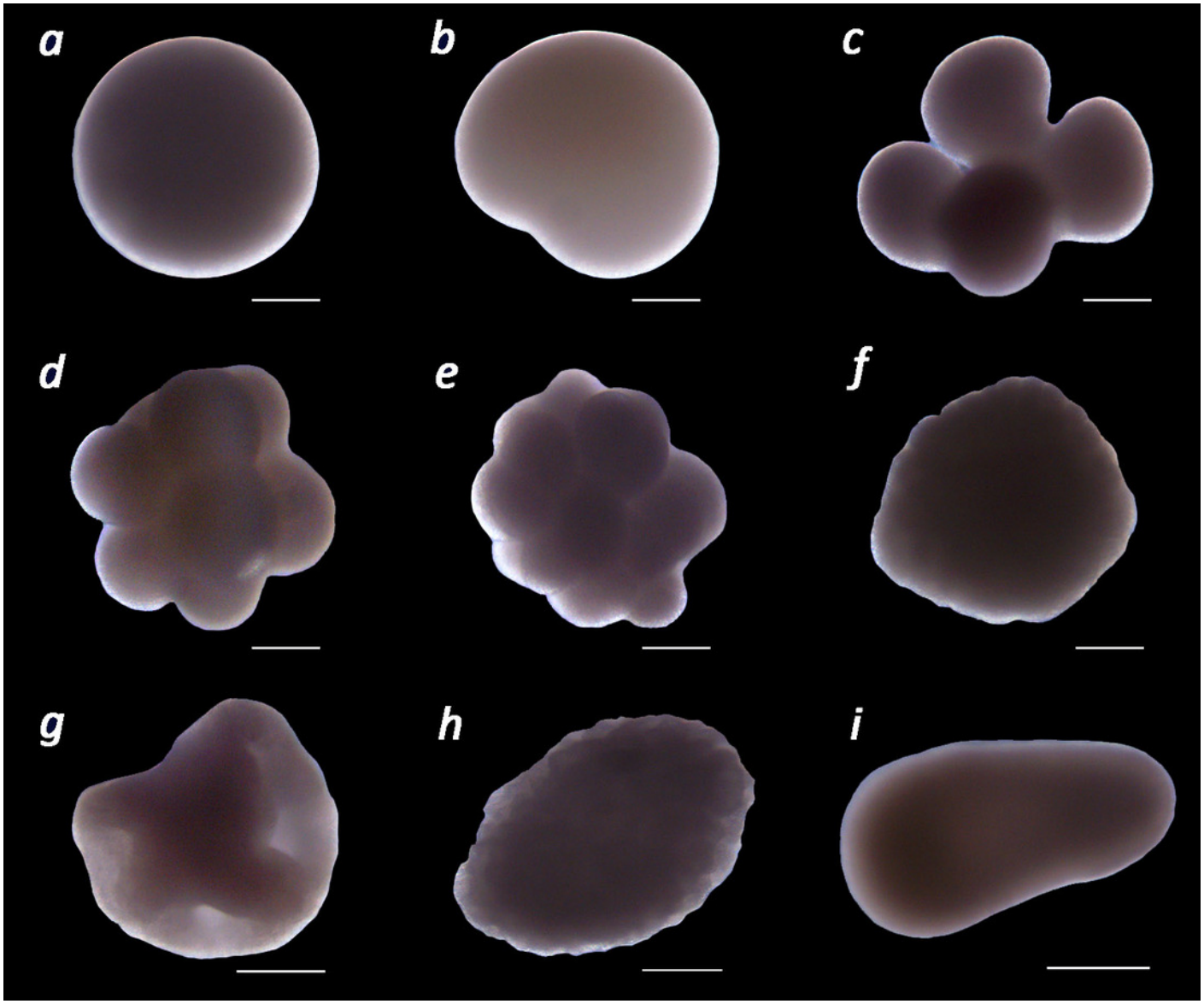

Figure 2

Early development of embryos of the octocoral ~~species~~ *Dentomuricea* aff. *meteor* reared ~~under~~ 13°C and 15 °C.

Bars display the proportion of embryos in each developmental stage over ~~a~~ course of 96 hours after spawning.

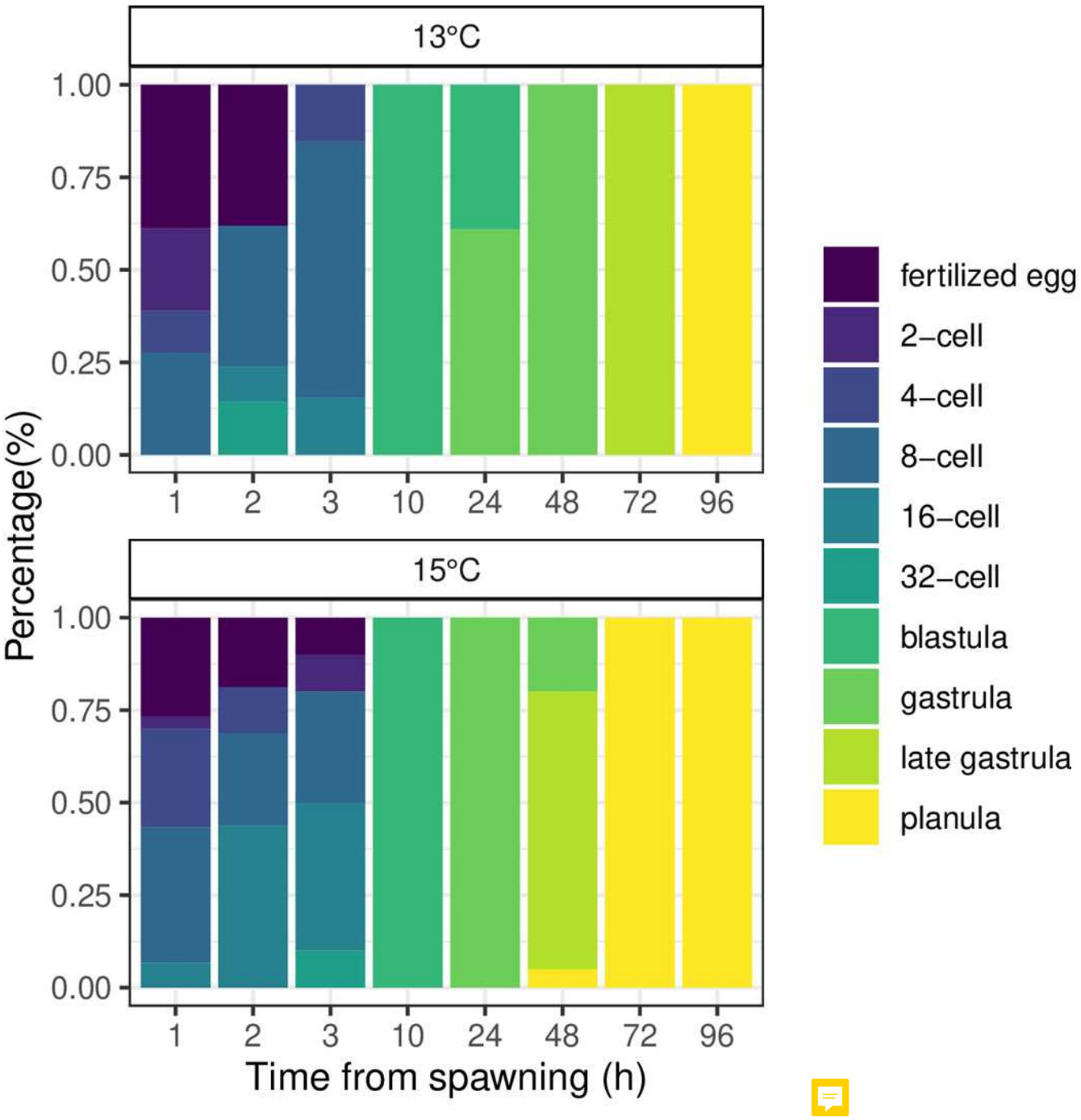
13°C
15°C
Percentage(%)
1.00
0.75
0.50
0.25
0.00
1
2
3
10
24
48
72
96
Time from spawning (h)
fertilized egg
2–cell
4–cell
8–cell
16–cell
32–cell
blastula
gastrula
late gastrula
planula

Figure 3(on next page)

Comparison of Kaplan-Meier estimates of larvae survival of ~~the species~~ *Dentomuricea* aff. *meteor* under two temperature regimes.

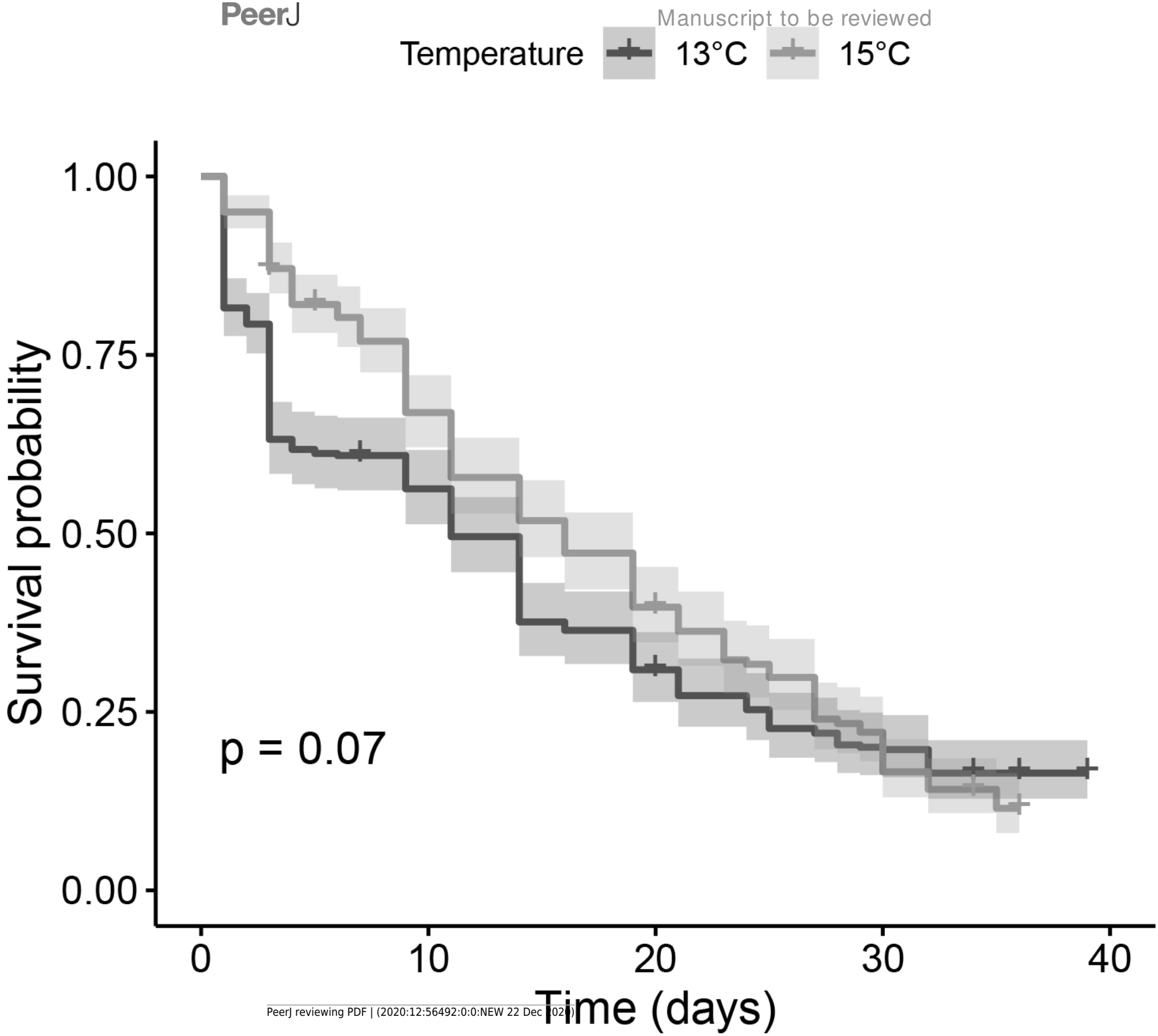
Temperature
13°C
15°C
1.00
0.75
0.50
0.25
0.00
Survival probability
p = 0.07
0
10
20
30
40
Time (days)

Figure 4

Larval stages of the octocoral ~~species~~ *Dentomuricea* aff. *meteor.*

The graph displays the proportion of larvae in different developmental stages (planula, metamorphosed but not settled, settled, deformed) under two experimental rearing temperatures.

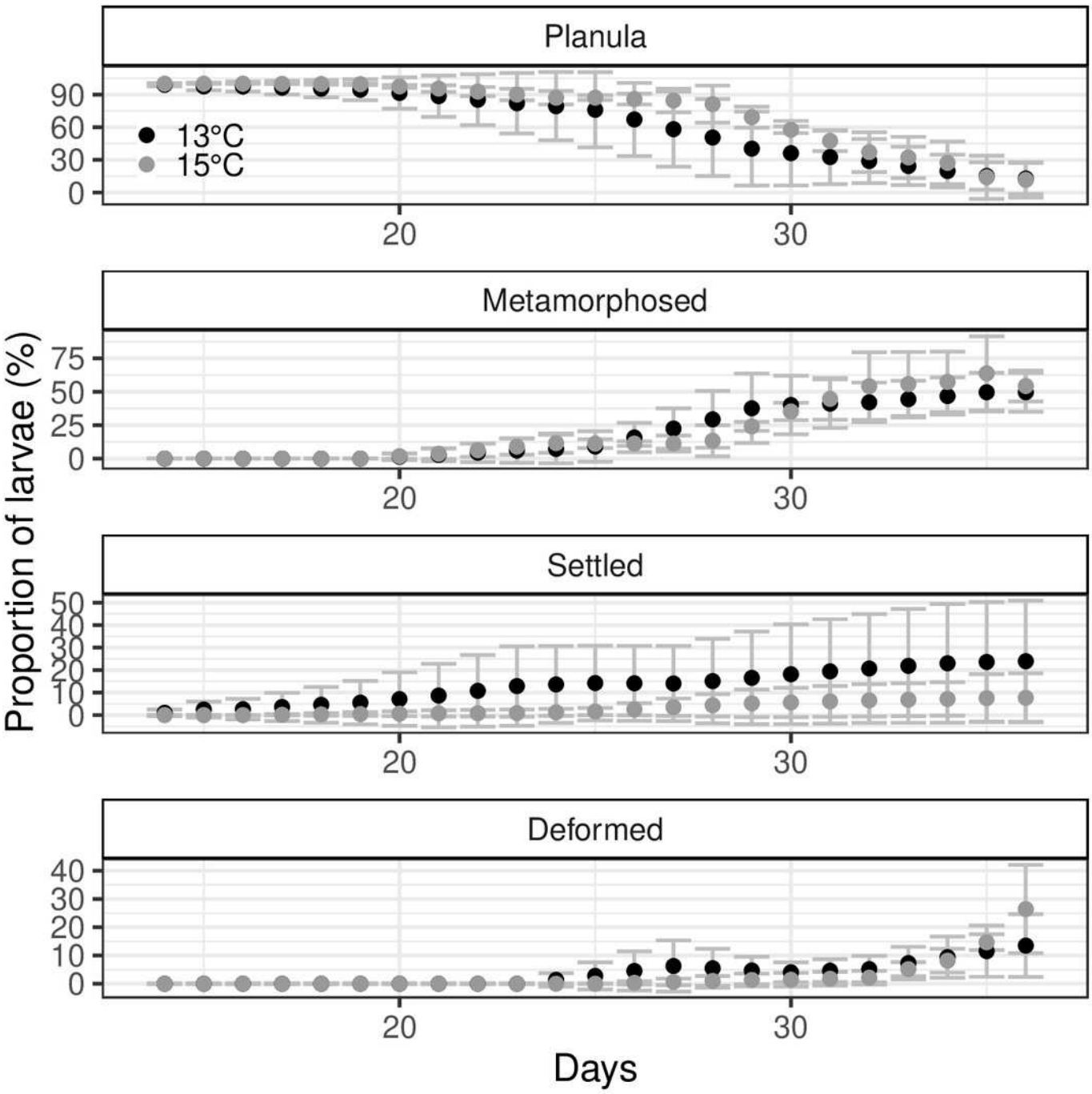
Planula
Metamorphosed
Settled
Deformed
13°C
15°C
Proportion of larvae (%)
Days
20
30

Figure 5

Pelagic metamorphosis of larvae of the octocoral ~~species~~ *Dentomuricea* aff. *meteor.*

A: pear shaped larva with formed mouth ~~in~~ the oral ~~side~~ (o) and closed aboral ~~side~~ (ab); B: tentacle formation on the oral ~~side;~~ C: Fully formed tentacles, mesenteries and sclerites (sc); D: deformed larva with abnormal mesentery and tentacle formation.

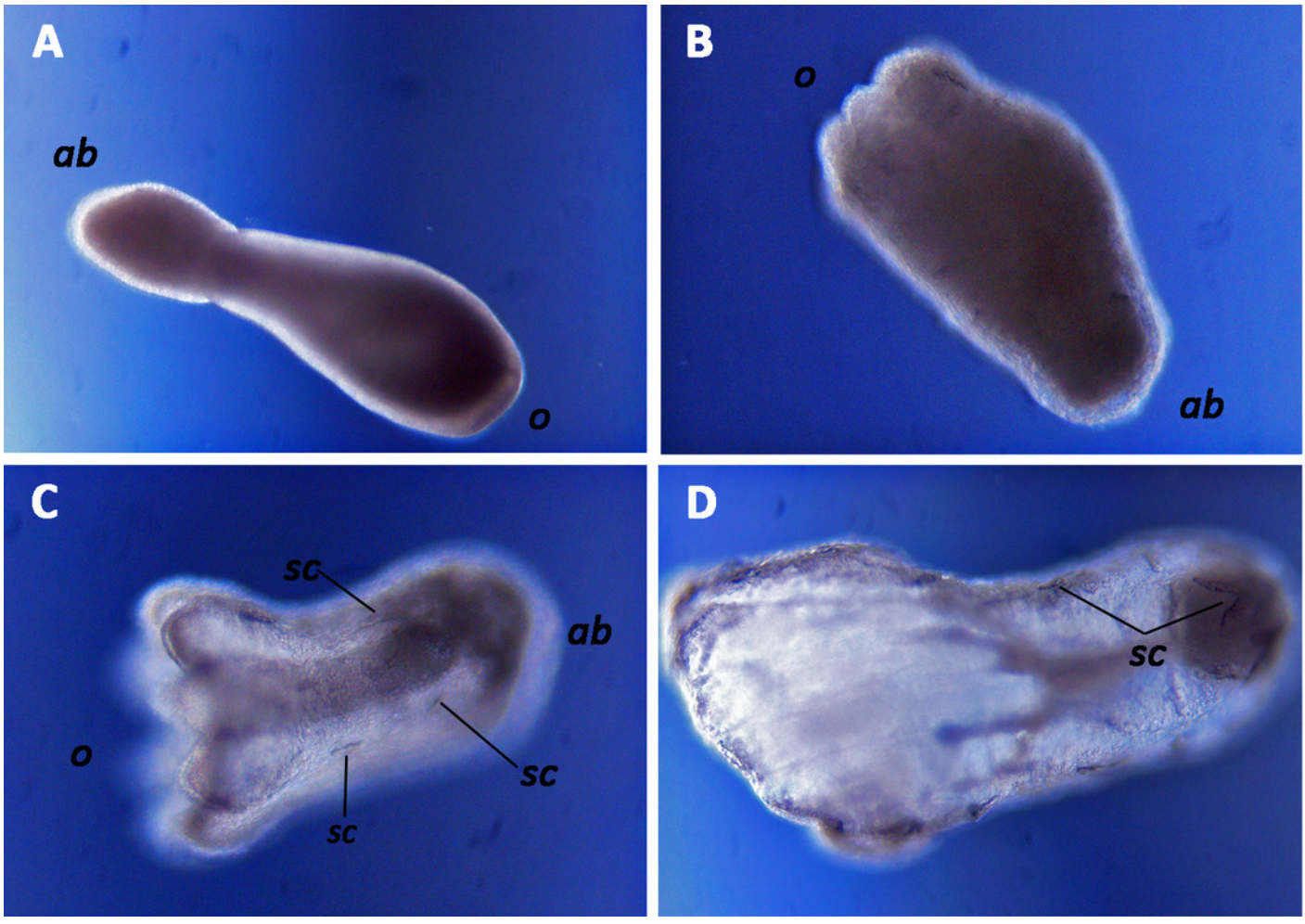

Figure 6

Formation of primary polyps from planula larvae of the octocoral *Dentomuricea* aff. *meteor*.

A: Recently settled primary polyp with a polyp base (b) and formation of eight mesenteries (m); B: final primary polyp with sclerites (s), tentacles and tentacle pinnules (p). Scale bar: 500 µm

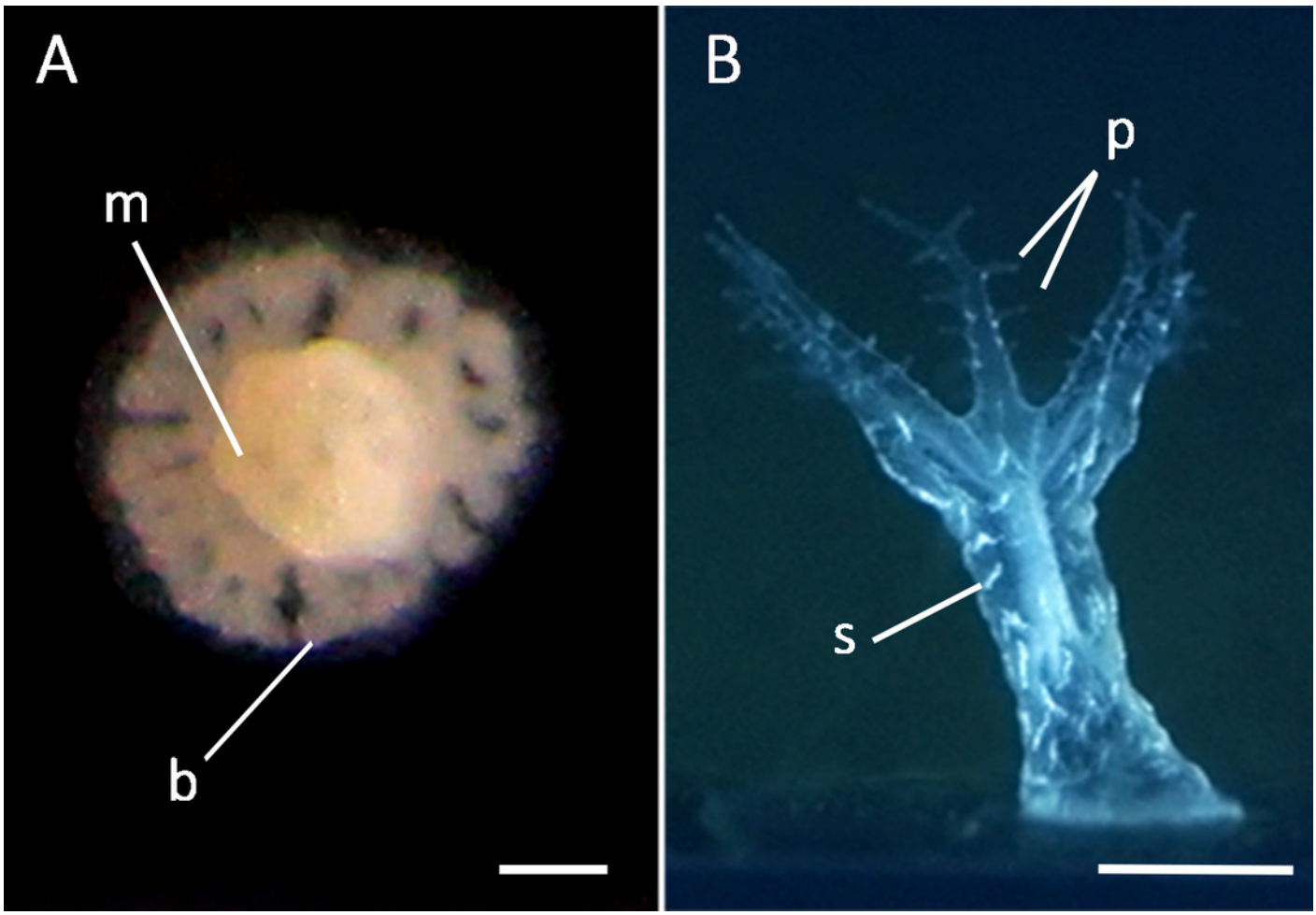

Table 1(on next page)

Model selection results.

The results of Maximum Likelihood Ratio (MLR) tests, reveal significant effects of the independent variables in question. Selected models are highlighted in grey.

Table 1: Results of Maximum Likelihood Ratio (MLR) tests, revealing significant effects of the independent variables in question. Selected models are highlighted in grey.

Dependent variable	Model type	Model	AIC	X^2	df	p
Size	LM	Null	-186.35			
		Stage	-725.87	8.63	13	2.20×10^{-16}
		Stage + Temperature	-724.69	0.004	12	0.37
		Stage x Temperature	-708.56	0.03	11	0.90
Length/width ratio	LM	Null	184.68			
		Stage	-132.57	21.66	13	2.20×10^{-16}
		Stage + Temperature	-130.58	0.0004	12	0.91
		Stage x Temperature	-159.44	1.66	11	4.78×10^{-7}
Proportion of planula	Binomial GLM	Null	2770.58		31	
		Time	633.32	2137.27	30	2.20×10^{-16}
		Time + Temperature	567.36	67.95	29	2.20×10^{-16}
		Time x Temperature	558.94	10.42	28	0.0012
Proportion of metamorphosed	Binomial GLM	Null	1676.96		31	
		Time	482.96	1196.60	30	2.20×10^{-16}
		Time + Temperature	484.12	0.19	29	0.65
		Time x Temperature	475.91	10.22	28	0.001
Proportion of settled	Binomial GLM	Null	744.30		31	
		Time	484.34	261.90	30	2.20×10^{-16}
		Time + Temperature	398.76	87.57	29	2.20×10^{-16}
		Time x Temperature	387.71	13.05	28	0.0003
Proportion of deformed	Binomial GLM	Null	466.04		31	
		Time	201.97	266	30	2.20×10^{-16}
		Time + Temperature	203.65	0.32	29	0.56
		Time x	160.93	44.7	28	$2.27 \times$

		Temperature				10^{-11}
Swimming speed (13°C)	LM	Null	-141.32			
		Time	-170.05	1.24	1	1.95 x 10^{-8}
		Time + Direction	-168.18	0.005	1	0.71
		Time x Direction	-166.18	0.00001	1	0.99
Swimming speed (15°C)	LM	Null	77.12			
		Time	63.7	1.02	1	7.91 x 10^{-5}
		Time + Direction	64.11	0.10	1	0.20
		Time x Direction	65.75	0.23	1	0.54
Swimming speed	LM	Null	42.04			
		Time	-24.12	4.00	1	2.20 x 10^{-16}
		Time + Temperature	-79.80	3.17	1	1.41 x 10^{-14}
		Time x Temperature	-77.89	0.04	1	0.767
Swimming direction (13°C)	LM	Null	80.31			
		Time	82.31	0	1	1
		Time + Direction	84.15	5.35	1	0.70
		Time x Direction	82.90	95.15	1	0.11
Swimming direction (15°C)	LM	Null	89.65			
		Time	91.65	0.00	1	1
		Time + Direction	92.38	88.60	1	0.34
		Time x Direction	94.38	0.09	1	0.97

3

4

Table 2 (on next page)

Summary of embryo and larval characteristics of the deep-sea octocoral ~~species~~ *Dentomuricea* aff. *meteor* reared under two temperature regimes.

Data include estimated (average ± ~~mean~~) survival time, survival rate, Pelagic Larval Duration (PLD), and swimming speed. The min and max values correspond to minimum and maximum estimates observed in individual batches, whereas median takes into account all batches pooled together. Swimming speed data were collected on day 4 and day 14.

Table 2: Summary of embryo and larval characteristics of the deep-sea octocoral species Dentomuricea aff. meteor reared under two temperature regimes, including estimated (average ± ~~mean)~~ survival time, survival rate, Pelagic Larval Duration (PLD) and swimming speed. The min and max values correspond to minimum and maximum estimates observed in individual batches, whereas median takes into account all batches pooled together. Swimming speed was calculated on day 4 and day 14.

	13 °C			**15 °C**		
	Survival time (days)	**Survival rate after 30 days (%)**	**PLD**	**Median survival time (days)**	**Survival rate after 30 days (%)**	**PLD**
Min	5	17.1 ± 4.5	1	6	0	1
Max	28	34 ± 6.91	35	23	21 ± 3.47	35
Median	11	16.4 ± 2.07	24	16	11.5 ± 2.09	27
Swimming speed$_{day4}$ (mm s^{-1})	0.24 ± 0.16			0.36 ± 0.21		
Swimming speed$_{day14}$ (mm s^{-1})	0.4 ± 0.24			0.44 ± 0.23		