

The manuscript #56492 "Embryo and larval biology of the deep octocoral species *Dentomuricea* aff. *meteor* under different temperature regimes" presents novel data on sexual reproduction of a deep octocoral species, as well as the effects of temperature narrow changes on reproductive success. There is very few information on the reproduction of deep-sea corals, although this knowledge is essential to understand the dispersal, connectivity and biogeographical patterns of benthic species. However, the current version of the manuscript presents some incongruent points in the results, some results are not discussed, and some scientific interpretation of the results must be clarified. Finally, in my opinion, the authors should go deeper in the discussion of their results.

General comments

1. In the current version of the manuscript, the concept of spawning event is not properly used. A spawning event is the breeding that occurs in a certain place during a particular interval of time, normally defined according to environmental conditions. For example, in the case of the species cited on the discussion:

1) *Plexaura homomalla*: the spawning occurs only at night (between 20:00-22:00 or 19:00-23:00) during 2-4 nights. Whereas in Virgin Islands only one spawning event occurs after 3-6 d of full moon on July (Wells et al. 2020), in Venezuela 2 spawning events occur after 6-7 days of full moon (one in August and the other in September) (Bastides et al. 2005).

2) *Eunicea flexuosa* (NOT *Plexaura flexuosa*): the spawning occurs only at night (between 20:00-21:30) during 2 or 5-9 nights. Two or 4 spawning events were recorded 6-7 days after full moon in August- September or June-September, respectively (Bastides et al. 2005; Beiring and Lasker 2000).

3) *Eunicella singularis* and *Corallium rubrum*: The breeding occurs in June-July and August, respectively. Both species only have one spawning event that can persist during several weeks.

So, since the spawning of *Dentomuricea* persists during approximately one month (line 241), the studied species have only one spawning event. Therefore, I guess what the authors want to say with "spawning event" is in reality what it is defined to as "spawning interval"? According to data presented, I am not sure. Please, clarify.

2. This is the first time that spawning of *Dentomuricea* is observed, and only few studies have been able to observe the spawning of deep octocoral species. Therefore, although obtained in aquaria, the authors have a very important dataset. In my opinion, the authors should go deeper in the analysis and interpretation of their data (and this will probably also help to solve the problem commented just above). I mean, does it exist a relationship of spawning with lunar phase? Or with hours of the day? Or between night and day? And so on... All this information, together with the data from the available literature, can be useful for further studies on deep-octocoral reproduction. Please, also note that in the current version of the

manuscript, the occurrence of spawning in fall and its possible relationship with environmental conditions, is not discussed. This should be properly addressed.

3. The authors should check some incongruent points. For example, in line 164-165 the authors say "day 15 after spawning, larvae displayed competency to settle", but in line 196 "after larvae presented competency (day 14), substrate was provided". Results are presented sometimes in day 14 and others in day 15. Finally, in the discussion the authors say "it was not possible to determine the exact onset of competency." So, it is completely unclear to me if authors were or were not able to determine the moment in which the larva display competency to settle. Please, clarify.
4. The authors state that larvae have non-selective settling behavior. However, in my opinion, the results exactly show the contrary. I mean, larvae present high selective settling behavior, since no larvae settle on the substrate provided, and those that settle do it on the plastic surface of the flasks. If the larvae are non-selective, why were they avoiding the basalt rock? Moreover, the percentage of larvae that started the metamorphosis without settling was higher than the settled larvae. As authors mentioned in line 413, pelagic metamorphosis might be an intermediate phase while waiting for the right settlement cue. But if larvae have a non-selective settling, why to wait?

Specific comments

L37 – In my opinion, not always. I mean, the species persistence depends on sexual reproduction, but also on asexual reproduction. However, in the long term (over several generations), asexual reproduction compromises the capability for adaptation to the environment due to the loss of genetic diversity introduced by sexual reproduction. Maybe, the authors should change "Species persistence" for "Species adaptation".

L102 – Is it possible to include sampling depth (aprox)?

L129 – The abbreviation SW has not been defined.

L133 – I understand that each culture flask was from a different batch. it is correct? Please, specify.

L143 – Please, include (n=5 in each water bath) after two water baths.

L144 – Please, include $\pm$ before 0.5.

L144 – How were embryos distributed in the two treatments? I mean, eggs spawned in one day were divided between treatments or all the eggs spawned during one day were placed in only one of the treatments? (This is also related to comment on L275).

L153 – In my opinion, to better clarify the procedure, this sentence could be moved after line 134. Moreover, what do the authors mean with "different spawning events"? Different days? Different hours? Can be really considered different events? See my first general comment.

L157 – At what time is the larval size estimated? Just after larval formation? Or according to larval age?

L182 – In my opinion, the sentence "some batches were spawned with 1-3 day difference" should be moved to the larval rearing section.

L189 – Why do the authors include the larval survival and PLD in the settlement and metamorphosis section? At this time, the larvae is still in the pelagic phase. In my opinion, the authors should include a specific section for larval survival and PLD.

L196 – In line 164, the author identify day 15 after spawning as the first day larvae displayed competency to settle. What is it correct? 15 or 14 days? Or what is the difference?

L196 – What are the characteristics of the substrate? I mean, is it CCA? Rock? Has it been previously conditioned (to allow growth of biofilm)? Please, add more details.

L206 – In my opinion, the correct citation is Kaplan & Meier 1958. Graham et al. 2008 apply and explain the same method that Kaplan and Meier developed.

Kaplan EL, Meier P (1958) Non-parametric estimation from incomplete observations. J Am Stat Assoc 53: 457–481

L241 – Only one time a day? Or how many times?

L246 – Authors should include here the corresponding letter of Fig. 1 (in this case 1b). Same comment for all the times Fig. 1 is cited.

L252 – Please include Fig. 2 also for the results under 13°C.

L253 – Early gastrula stage?

L266 – I agree that volume data is more correct, since the larva is very retractile. But, in all the previous literature, the size of larvae is expressed as maximum length. Do the authors also include this value? I would also like to point that this result is not discussed.

L269 – In Figure S1, the age of the larvae is expressed in hours, whereas in the text is expressed in days. Please use the same time unit (hours or days) for both.

L269 – The acronym LW is not previous defined. On the other hand, was the decreased of LW ratio from late gastrula embryos to planula larvae on day 4 significant? Again, these results are not discussed.

L273 – Please, include also Figure S1.

L275 - Do the authors related these differences with the spawning moment? Or due to the experimental design, was not possible this kind of comparison? In some coral species, for example, it has been demonstrated that the first eggs spawned are of lower quality compared with the subsequently spawned ones. On the other hand, why is this supplementary figure the number 5 if it is the second that the authors mention in the text? Should not be Fig. S2?

L278 – What do the authors mean with final survival rates? This value is after one month and in figure 3, the authors have values until 40 days (more or less). And please, add here the reference to Table 2.

L281 – The values presented in Table 2 are after 30 days. What is it correct (30 or 32 days)?

L289 – I do not understand. If in 52.1% of the recorded tracks larvae were directed upwards, and in 50.7% were directed downwards, the sum is larger than 100%. How is it possible? On the other hand, again these results are not discussed.

L293 – In Table 1, the authors presented the swimming speed on day 14, whereas in the text on day 15. What is it correct?

L294 – Fig. 4? This figure displays the proportion of larvae in different developmental stages under the two experimental treatments, and not the swimming speed. Do the authors mean Fig S3? But this Fig. only show the swimming speed and not upward and downward movements. Please, correct.

L297 – According to Table 1, $0.4 \pm 0.24 \text{ mm s}^{-1}$ is the swimming velocity for larvae under 13°C on day 14, and not under 15°C on day 4. Please check it and add "on day 14" at the end of the sentence.

L302 – In my opinion, the authors should check the order of this section. In the current version it is difficult to understand if the results are for 13 o 15°C, if the results are related to pelagic metamorphosis or not, or what are the PLD values under the two different temperatures. To better clarify, I suggest to following this order: 1) PLD 2) Settlement + metamorphosis, 3) pelagic settlement and 4) larval deformed.

L308 – Please, include the exact day when larval settlement started under 15°C.

L309 – What is the exact number of settled larvae?

L314 – At what temperature?

L319 – To my understanding, this sentence is an interpretation of the results, and should be consequently moved to the discussion section.

L320 – I understand that the results presented in the next two sentences are the median and maximum PLD. But, under what temperature? Why all the values related to PLD (median, maximum, minimum) are not presented together? Median and maximum PLD are presented at the end of the second paragraph, whereas the minimum PLD is in the first sentence of the section.

L326 – the sentence “as they appeared earlier in time under 13°C than at 15°C” is repetitive, since in the first line of the paragraph, the authors already say "Under 15°C, deformed larvae started appearing later in time".

L329 – Aha!! it is here!!! In my opinion this paragraph should be moved just after larval settlement.

L340 – To my understanding, according to previous results presented, the present study also includes larval size, PLD, settlement and metamorphosis.

L359 - How do the authors relate this with their results? I mean, do *D. aff. meteor* embryos present a superficial cleavage? It is not mentioned.

L362 – I do not understand why the temporal developmental profile of *D. aff. meteor* larva was comparable to octocoral broadcast of tropical species. The results presented showed the planula stage occurred within 3-4 days (2-3 days in tropical ones), the competency was 14 days after spawning (4-5 days in tropical ones), and the pelagic forms of metamorphosis could indicate the lack of the proper cues for settlement (Linares et al. 2008), and therefore, an high selectivity in settlement (low selectivity in tropical ones).

L374 – Please, correct to Martínez-Quintana et al. 2015.

L377 – Please, write extended. It is the first time *Lophelia pertusa* is mentioned.

L410 – Why the authors do not mention this high mortality of pelagic polyps in the results section? Why the results presented in Fig. 4 (metamorphosed) do not show this mortality?

L418 – Please, add more details about the values (or range) of dispersal potential, as well as appropriate references.

L419-432 – Be careful, the main difference among these species is that larvae of mollusks and *Lophelia* are planktotrophic, whereas larvae of the studied species are lecitotrophic. Planktotrophic larvae acquire external food sources during the pelagic phase, and therefore, it

is widely accepted that their PLD, and their dispersal capacity, is longer than for lecithotrophic ones.

L424 – Please, add the values of PLD.

L461 – What are these substantial effects on larval behavior, dispersal and success? Authors should be more specific. Please, explain these effects. On the other hand, both behaviour and behavior are correct, but one is British English and the other one is American. Please choose one and be consistent along the manuscript.

L486 – What about settlement and larvae deformed? Why are these points not included?

Figure 2: Gastrula =early gastrula?

Table 2:

What do the authors mean with median survival time?

How do the authors calculate minimum and maximum survival? In line 275, the authors say "under 13°C, a sharp decline in survival rates was observed during the first 48h". Therefore, how do the authors obtain a minimum survival time of 5 days?

Same comment for PLD. How do the authors obtain the minimum and maximum values of PLD? Since the competency was 14 days after spawning, how is it possible that the minimum PLD was 1 day?