Spawning of the grooved brain coral *Diploria labyrinthiformis* –

Follow-up on the Webinar Q & A session

by

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Questions #1 through 17 were answered during the webinar but are listed here in case they are of interest to those who could not attend and may want to watch the recording of the Q&A session. All questions that remained unanswered during the webinar are listed and addressed from # 18 through 41. Note that a full recording of this webinar along with slides is available on the CRC's website at http://crc.reefresilience.org/resources/webinar-series/

1. What is the name of the underwater photographer documenting spawning of many different species? The photographer´s name is Ellen Muller (see her photo gallery at https://www.pbase.com/imagine)

2. Is *Diploria labyrinthiformis* split spawning a partial spawning? In other words, is a partial spawning = some parts of the colony, or does the whole colony spawn three times (3 bundles released by a single polyp during the year)? Answered during webinar

3. What would be the correct way to cite the Caribbean-wide *Diploria labyrinthiformis* spawning monitoring information? Answered during webinar

4. How does full moon influence spawning? Answered during webinar

5. I remember you guys started thinking about an online database to collect spawning observation data. Are you still considering this option? Answered during webinar

6. *P. strigosa* and *P. clivosa* have been reported to spawn in Fall/Autumn. Have you seen these two species to spawn in the spring in other locations? Answered during webinar

7. Is there a minimum size that a *Diploria labyrinthiformis* colony needs to be to spawn? How healthy does a colony need to be to spawn i.e. percentage mortality or presence of disease? I don't have a lot of "healthy" colonies to survey Answered during webinar

8. Are there any morphometric and aggression studies to support genetic population evidence for the two cryptic spring/fall species? Are these two reproductive events independent or is it a case of split spawning? Answered during webinar

9. Can the larvae settle on any substrates? Are the larvae attracted to any specifically colored substrate? Answered during webinar

10. Given their rapid bundle breakdown, did you see any percentage of self-fertilization as it related to time? Answered during webinar

11. What is the recommended density of fertilized eggs from development to embryo in a culture? What is the tank volume used for the culture? Answered during webinar

12. What substrate is the best for larval settlement of *Diploria labyrinthiformis*? Answered during webinar

13. I have some *Diploria labyrinthiformis* histology data that could possibly be useful, who should I reach out to? Answered during webinar

14. Are there any data (that you know of) concerning *Diploria labyrinthiformis* spawning in a laboratory setting? Answered during webinar

15. What kind of collection devices are used to collect gamete bundles during spawning? Answered during webinar

16. Is it possible to induce symbiotic associations in the laboratory? Answered during webinar, but also see question #18

17. When monitoring *Diploria labyrinthiformis* spawning in the windward islands (e.g., Saba, St. Eustatius, St. Maarten), do we follow spawning predictions provided under "Dominican Republic"? Answered during webinar

18. How long after fertilization is it suggested to transfer the embryos into an in situ larval rearing pool? Do you have any results for recruits reared within in situ pools? If using one of SECORE's in situ pools or any similar ocean based larval rearing device, it is suggested to do the fertilization ex situ in smaller containers. Doing the fertilization directly in the pool will result in the dilution of sperm to very low concentration and may therefore result in much lower fertilization rates. Once fertilization was allowed for 30-45 min, the eggs should be rinsed at least once before they are poured in the in-situ rearing pool to remove most of the excess sperm. Settlement rates in the pools as well as recruit survival once outplanted to the reef are highly variable depending on, substrates, location and years. If referring specifically to the SECORE pools, please contact Dr. Margaret Miller (m.miller@secore.org) for a more detailed overview.

19. At what size does *Diploria labyrinthiformis* reach sexual maturity? Do colonies affected by diseases (e.g., SCTLD) still spawn? Answered during webinar

20. What is the spawning season of *Acropora palmata* and *A. cervicornis*? This varies per region and depends on the timing of the lunar cycle, but typically occurs in August. Spawning is however also possible late July and early September.

21. Is there any information on the sexual reproduction of these species? We are not sure which species you are referring to, but sexual reproduction information is

available for many coral species. See Szmant 1986 as a start for Caribbean species.

22. How can I set-up a coral spawning monitoring program when working as an independent researcher (i.e., not tied to any institution). Is there some way I can collaborate with researchers in the nearby region, such as Bonaire or Curaçao? We recommend forming a team of volunteer divers to monitor coral spawning at your location. You can for example reach out to dive operators to find experienced and enthusiastic divers. You can then plan the dives according to coral spawning calendars provided on the CRC's Larval Propagation Working Group webpage. If you need more information, please contact us directly via email (v.chamberland@secore.org, and banaszak@cmarl.unam.mx).

23. Do you have insights as to how many *Diploria labyrinthiformis* colonies we should aim to collect spawn from for successful fertilization and propagation? At least two but as many as you can collect from. It is better to have a little spawn from many colonies than lots of spawn from a few colonies. This helps to increase genetic diversity in your larval culture through sexual recombination, and further avoids fertilization failure in case of incompatibility issues between specific pairs of parents.

24. Did you ever observe successful spawning in aquaria? Yes. For *Diploria labyrinthiformis* specifically at the Florida Aquarium (led by Keri O'Neil) and at the Cape Eleuthera Institute (led by Valeria Pizarro). For a number of other species, for example at the Horniman Museum (led by Jamie Craggs), AIMS (led by Craig Humphrey), the California Academy of Sciences (led by Rebecca Albright), as well as the Florida Aquarium (led by Keri O'Neil).

25. How do I connect to a research group that is active in your research area? We suggest emailing the researcher in question and share your interest. Most researchers will respond.

26. Is it possible to artificially trigger spawning events in-lab settings using light or biochemical triggers? Yes, mostly by tweaking light cycles rather than biochemical triggers. See web pages from the Horniman Museum, AIMS, and the California Academy of Sciences. There is also a great Facebook page created by Jamie Craggs that is dedicated to captive coral spawning work (https://www.facebook.com/groups/420196901490017)

    Are you working with any Aquariums for coral larval propagation? Yes, staff from many aquaria are involved in sexual coral propagation.

27. ¿Que significa o que alcances tiene los resultados geneticos de la distincion de dos grupos bien diferenciados en DLAB? Very good afternoon, thank you. What is the significance or scope of the genetic results of the distinction of two distinct groups in *Diploria labyrinthiformis*? These could possibly be two distinct species,

despite the fact that they are occur in the same habitat, and that they morphologically look identical (at least to the naked eye).

28. Many Aquariums in the US are holding *Diploria labyrinthiformis* from the Florida Keys because of SCTLD and AZA-Florida Reef Tract Rescue Project. While intentionally trying to induce spawning in aquariums for this project is not suggested at this time, if spawning is observed would this information be helpful in your data set? Individuals can be tracked back to original location on the reef. Definitely! Such observations would be fantastic. Please share.

29. How do you "condition" settlement substrates with CCA? We normally leave substrates out on the reef for 1 to 2 months prior to spawning, and until CAAs are visible. Refer to the Caribbean Coral Spawning for Research and Restoration Webinar from October 2017 for more information.

30. Does the size of a colony affect spawning rates (fecundity)? Perhaps for smaller colonies and for very large colonies (which are potentially senescent). We currently do not have enough data on fecundity in relation to size for *Diploria labyrinthiformis*.

31. How do you provide symbionts to the recruits? If you are rearing recruits in a flow through system in which water is pumped directly from the reef, the most straightforward way is to not filter the water to finer mesh sizes than 50 µm to allow naturally occurring symbionts to infect the settlers/recruits. Another approach is to place fragments from an adult colony of the same species in your culture system to promote symbiont transfer between adults and recruits. You can also use fresh symbiont extractions from adult tissue or lab kept symbiont cultures.

32. Are there automation technologies for gamete separation, fertilization, and larval settlement in-lab? Not yet, as far as we know.

33. Our dive program has been working in collaboration with the Coral Restoration Foundation in the Caribbean and we currently are doing coral restoration work in the Philippines. I am very interested in learning the next steps and would love to volunteer with your groups. mromero32@mac.com (Mark Romero). Please contact us directly: v.chamberland@secore.org" v.chamberland@secore.org and banaszak@cmarl.unam.mx

34. Do you have any data on optimal light intensity for coral recruit growth? That is a great question. From our experience every species is different, but in general we recommend starting with very low light (~20 µmol photons m^{-2} s^{-1}) and to gradually increase irradiance as the corals grow. For *Acropora* species which typically grow in shallow habitats characterized by high irradiance, we increase light intensity more rapidly than for *Orbicella* species for example. If recruits start becoming pale it can indicate they are receiving too much light; reduce light

intensity using filters or screens. In contrast, if recruits are very darkly pigmented they are likely not receiving enough light.

35. Do you have any recommendations on methods to provide recruits with symbionts in the lab to improve post-planting survivorship? See answer to question #31.

36. We obtained gametes *ex situ* at Cape Eleuthera Institute, but only from one colony. Self-fertilization occurred, resulting in few larvae and settlers, which in then end all died. Discussed during webinar.

37. Is there any evidence that suggests SCTLD can be passed from parent colony to offspring? If this is the case, should we take this into consideration when selecting colonies to monitor and collect from? While not proven, it is likely that diseases can be transferred from parent to offspring. However, during spawning collecting dives, it is also possible to spread the disease via nets, diving equipment, etc. The precautions to take are to not use spawn collection nets on more than one colony during a collection dive, and to rinse all equipment and nets in chlorine after every dive. We further do recommend collecting spawn from healthy colonies when possible given conditions at your locality.

38. I am interested in the follow-up of settlers, in terms of survival monitoring after their release on the reef? What have been the data so far? Yes, we do monitor settler/recruit survival rates, as well as their growth. Soon, the CRC's Monitoring Working Group will release a Coral Restoration Monitoring Guide in which protocols are detailed, including for sexually propagated corals. But generally speaking, we map and follow a subset of outplanted substrates with recruits on them over time using permanent transects. Survival and growth rates currently are highly variable depending on species, sites and year, but generally speaking, are higher than natural recruitment rates for some of the species we are working with.

39. Can you make your manual on nets available to everyone? Yes, please email banaszak@cmarl.unam.mx. We will send you a manual which is in Spanish but with photos and very clear instructions. The English version will hopefully be available this year. There are also guidelines available in the appendix of the Coral Spawning for Research and Restoration Webinar from October 2017 available on the CRC's Larval Propagation Working Group webpage.

40. I need to learn more about larval rearing in laboratory for restoration. Please send some methods to jeraldreef@ gmail.com Thanks (Jerald Wilson). Please see the Coral Spawning for Research and Restoration Webinar from October 2017 available on the CRC's Larval Propagation Working Group webpage. If you need further information email either of us with more specific questions: v.chamberland@secore.org and banaszak@cmarl.unam.mx.

41. Are you working on the impact of increased temperatures or acidity on the development of coral larvae or the study of larvae from more resistant colonies? if so, can larval propagation be an effective response to climate change? Many research groups are currently investigating these questions, and there is a lot of published information available on these topics. It is indeed possible to produce larvae that are more resistant to thermal stress for example. However, it is important to recognize that temperature is not the only stressor leading to mass coral mortality. We currently are not able to produce larvae resistant to diseases and poor water quality for example. Generally, by simply producing larval cohorts issued from a good mix of parents allows to produce populations of larvae/recruits that are highly variable genetically, and therefore have greater chances of withstanding current and future threats altogether, as a population (in contrast to breeding programs aimed at selecting single traits at a time).

Thank you for your interest and all your great questions!

- *Val & Ania*