These Q & A followed the webinar presented by the Genetics Working Group of the Coral Restoration Consortium, and hosted by the Reef Resilience Network on 1 April 2020. The title of the webinar was “Maximizing the Adaptive Potential of Restored Coral Populations”, which presented a summary of the working groups' published guidelines (Baums et al., 2019 Ecol Appl 29:e01978. doi:10.1002/eap.1978).

The Working Group recognizes that restoration goals vary across programs and that some of the recommendations detailed in the webinar and this associated Q&A document may not be appropriate and/or feasible in every program. However, there are some basic guidelines outlined in the webinar and paper that are universal and suggested to be followed by all programs to help ensure that our restoration work is supporting the natural recovery of the species we are working with and not having unintended genetic consequences.

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1. Until the legal framework is in place to allow movement and crossing of corals from more distantly located populations, is there any concern regarding inbreeding depression for crossing and propagating locally derived stock within current restoration gene pools? If so, can this be addressed primarily with genetic relatedness analyses?

Inbreeding depression is the result of *repeated* rounds of mating between close relatives that carry lethal or deleterious recessive alleles. Some brooding coral species naturally mate with close relatives (i.e. they have inbred populations) but may not show inbreeding depression. Others, like most of the Caribbean broadcast spawning species, rarely mate with close relatives and have low levels of inbreeding in natural populations. Inbreeding depression is more likely to occur in these naturally outbred populations if and when they continue breeding at greatly depressed population sizes or when they are bred in captivity over multiple generations.

At this time, only one generation of mating has been completed in nursery settings so this is not a concern yet. Relatedness analyses that rely solely on genomic markers have become more accurate but whenever possible, should be supplemented by direct information about parentage. In programs that contemplate repeated rounds and generations of *ex situ* mating from a single spawning stock, it would be prudent to set up coral “stud books” to keep track of pedigrees. This means that batch culturing should be avoided and instead two-parent crosses performed to make the book keeping easier. That said, we sometimes observe reduced fertilization rates in two-parent crosses in some coral species - for example, in *Acropora palmata* in the Caribbean, but not in *Acropora millepora* from the Great Barrier Reef. Hence, depending on the species, we have to weigh the benefits of easier pedigree analyses in two-parent crosses versus the benefits of higher larval production in multi-parent crosses.
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In other programs focused on restoring in situ coral populations, two-parent crosses are not scalable enough and instead multi-parent crosses should be performed. Outplanting of these sibs or half-sibs next to each other at the same outplanting site may not increase chances of inbreeding depression much. This is because inbreeding depression would require several things: continued successful production of sexually derived larvae from surviving genets, recruitment of these larvae back to their natal reef and settlement in close proximity to their parents, maturation of the recruits to reproductive age and then successful fertilization of their gametes with their parent's gametes. For Caribbean acroporids in Florida, we do not have evidence of successful sexual reproduction at this point so concerns about inbreeding depression in natural populations are low on the list of things that worry us. The risks might be higher for other coral species that are still successfully recruiting, for example *Siderastrea siderea* or *Montastrea cavernosa*.

Since we are talking about this: it appears that in some coral species inbred groups can emerge within otherwise panmictic populations naturally, possibly through co-recruitment of relatives; we have observed this in *Acropora hyacinthus* from Micronesia: https://www.biorxiv.org/content/10.1101/2020.02.26.956680v1.abstract

2. What coral propagation practices would you recommend for mitigating the spread of stony coral tissue loss disease? Please speak about what can be done to avoid the spread of disease during coral propagation activities as well as what can be done using coral propagation to help affected areas to be restored.

The FL Disease Advisory Committee working Group, the Restoration Trials Team (RTT), led by Erinn Muller (Mote) and Kristi Kerrigan (DEP) have conducted a webinar on this very topic.

https://reefresilience.org/restoration-in-the-age-of-disease/

Briefly, diseased corals should be removed immediately from nursery stock - this is probably the most we can do genetically to maximize the chance that more tolerant individuals are propagated in the nursery. This would be another instance when tracking genets would be helpful! Additionally, there should be no movement of coral from SCTLD locations/nurseries to SCTLD-free locations. Finally, the RTT has published an Action Plan online. One goal of the Action Plan includes assessing the survival rates of SCTLD-susceptible species outplanted within the endemic region of the Florida Reef Tract. The proposal is currently in prep and will hopefully be implemented within the next 12 months.

RTT Action Plan:

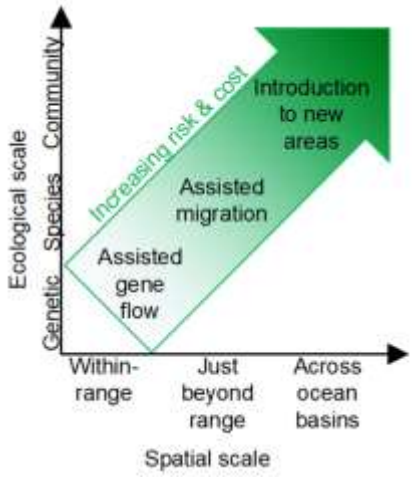
https://floridadep.gov/rcp/coral/documents/restoration-trials-team-action-plan-stony-coral-tissue-loss-disease-november

Another tactic could be to focus on the non-susceptible acroporids right now while the disease is playing out in the western Atlantic. The RTT also recently released a report titled "Are acroporid corals a potential vector of stony coral tissue loss disease?", which provides some evidence to

the contrary. Preliminary studies suggest that asymptomatic acroprids do not transmit the disease to other more susceptible species, but additional work is needed. See https://floridadep.gov/rcp/coral/documents/are-acroporid-corals-potential-vector-stony-coral-tissue-loss-disease

3. Is there a danger of introducing new pathogens with corals brought in from even relatively close areas? Is there a risk of introducing novel pathogens and how might one reduce this?

Yes, there is always a risk of introducing new pathogens or parasites, but from nearby areas, this same risk is likely already posed by waterborne transmission. We know little about these dangers at this point but we are proposing that the use of gametes, rather than whole organisms, to implement Assisted Gene Flow (AGF) is the safest option. Cryopreservation of coral sperm is now possible for many coral species, and might be the best way to introduce genetic diversity from other regions to local populations while minimizing the risk of pathogen/parasite transmission. Also, the risk of unwanted introductions probably scales with the distance (either geographic or effective), suggesting that small-scale translocations are likely to minimize these risks. This figure from the National Academies report (NAS 2019;https://www.nap.edu/catalog/25424/a-decision-framework-for-interventions-to-increase-the-persistence-and-resilience-of-coral-reefs) illustrates that point.



4. Are disease transmission experiments effective to evaluate the suitability of fragments for restoration?

We recommend ex situ disease transmission and bleaching experiments be conducted on nursery stock to screen genets for desirable phenotypes (Baums et al. 2019 Ecol Appl 29:e01978. doi:10.1002/eap.1978.). Groups that are conducting disease transmission experiments to guide restoration practices, such as Mote Marine Lab, are also field testing genets to assess whether lab experiments and exposure in the field provide similar results. Coupling experiments with downstream analyses can help characterize why some genets are

more/less disease susceptible and hopefully utilize that information to guide future research and restoration activities.

5. Is there a role for sperm freezing and storage in this?

Absolutely! See answer to 3. Cryopreserving sperm is the best means to allow hybridization of corals from entirely different reef areas while minimizing risk of transferring unwanted environmental features during AGF.

6. Is there an ability to transfer gametes between locales rather than individuals or fragments for reproduction in the nursery setting?

See above - this is exactly what we are proposing. We removed this from the presentation in the interest of time...

7. Have you observed strong correlation in the performance of individuals (in terms of enumerated biometrics) with the type/ conditions of the environment that they were obtained?

This varies by species and by the particular trait examined. For *A. cervicornis*, *A. millepora*, *A. hyacinthus* and *P. lobata* evidence is increasing. For *A. palmata* the situation is more complicated: here ramets of the same genet diverge in the assessed phenotypes (Devlin-Durante et al. 2019 doi:10.1111/mec.15140). It also depends on the metric you are using for phenotype. Many coral species can change their growth and colony morphology when introduced to a newenvironment, e.g., *Montipora capitata* in Hawaii will start growing into a plating morphology when you move a branched colony from a shallow, well-lit spot to a deeper, low-light habitat. Also linear extension rate and skeletal density are traits that are plastic in *A. cervicornis*, but calcification rate (mass gained per unit time) was more conserved within genet (see https://doi.org/10.1007/s00338-017-1560-2). In addition, thermal tolerance is associated with corals from warmer environments (work by Coles and Jokiel in the 1970s) and more variable environments (see work by Palumbi and Kenkel).

8. Given that it is becoming more common to source genets from coral that has not bleached or has not become diseased when others have, is there any research that shows that corals propagated from such sources survive better than other corals?

There is mixed evidence on this point. A paper about *Acropora millepora*, showing that a "winner" for any tolerance trait like the ones you mention is likely to be a "winner" in all other aspects: https://matzlab.weebly.com/uploads/7/6/2/2/76229469/wright19_globalchangebio_winnerslosers.pdf. Andrew Ross in his thesis (2012. The decline and restoration of *Acropora cervicornis* in Montego Bay: Exploring the Anthozoics and Anthozoculture of *A. cervicornis*. Ph.D. dissertation, UWI Mona) also found that "strong" genets are always strong, in *A. cervicornis*. This may not be universally so, however. In contrast, a study on *A. cervicornis* found few genets were both

infectious- disease resistant and temperature resistant (Muller et al (2018) elife 7:e35066. doi:10.7554/eLife.35066.001.)

So this might be the case for some species or some traits. Indeed, the major stress events that have occurred in the last several years (bleaching and disease) have been selective events, so a coral sourced from the wild today may be more valuable than those that were collected 20 years ago and brought into a less challenging environment. Luckily (or not?), in-situ nurseries have not escaped most of these selective events. This is important to keep in mind as corals are rescued from the field in front of the SCTLD disease line--- a specific goal of the Florida Coral Rescue effort is the potential use of these pre-exposure corals as broodstock to cross with individuals from within the endemic zone that are putatively disease-resistant.

9. Is there any negative impact to capturing more than just 3 samples/local area? ((obvious you want to limit wild collections)) From the standpoint of having diversity represented is there such thing as too much sampled? Also- the sample number of 3 captures the majority/common allelic diversity- but how do you capture what is rare? and should that be a consideration?

This is really a “minimum recommendation” for genetic considerations. The concern would be opportunity cost and impacts to the local population (which as you point out might be a consideration for rare species, e.g. *D. cylindrus*). Generally, few genets sourced from multiple habitats is preferable to more genets sampled from fewer habitats. Otherwise, we don’t see a downside of collecting more if you can manage it and not impact the wild population too much. More would be better!

10. You mention that older genotypes might not be best to source from, but if they remain today and are visually healthy, wouldn't it make sense that they have adapted successfully through the years?

I think we answered this one during Q&A, but it is perhaps worth making this point again. The coral genotype does not change as it grows (except for some somatic mutations), so even after 500 years of growth it is basically the same genotype as in the larva that successfully recruited 500 years ago. Now, the thing is, surviving the first couple of years on the reef is by far the greatest challenge for a coral, as it becomes progressively more and more resilient as it grows bigger. An old coral faced and overcame the “young-survival” challenge 500 years ago, and although it can appear robust in current circumstances, that is not a guarantee it would be able to recruit under present-day environment, with its potentially outdated 500-year old genotype.

11. Is it fair to say that the really old corals have greater genetic plasticity that has enabled them to survive varying environmental conditions over multiple decades and even multiple centuries and thus make the case for using such survivors as source genets?

I think whoever asked this question probably meant phenotypic plasticity (unless they’re thinking about somatic mutations, the role of which remains highly contentious in corals).

As far as phenotypic plasticity goes, it is possible that very old genets on reefs today have managed to survive because their genetic makeup confers a greater level of plasticity or allows them to acclimate to novel conditions well. On the other hand, a very old genet could have simply benefited from decades, or even centuries, of growing in a more benign environment. As noted above, since small corals are far more likely to die than larger colonies, a colony that grew very large (or had lots of opportunities for genetically identical ramets to be scattered across different habitats along the reef) would simply have a higher probability of surviving than more recent recruits to the same area. The fact that we're seeing consistent decline in many of these very old genotypes suggests the latter. That doesn't mean they shouldn't be included at all as a nursery genotype, but it also doesn't mean a genotype is well adapted for today's reefs simply by virtue of being old.

12. To Margaret. I found huge elkhorn corals (2.5 - 3m) and we thought to bring these apparently resilient genotypes into our nursery. These are about 1.5h far away by boat. If I understood you right, you wouldn't recommend that?

This is probably fine. A single large isolated colony may or may not be a very old genotype. It makes sense to include it if it is doing well. Would want to be cautious to not *only* source from very large colonies (that might be very old). Again, the goal should be *mixed* provenance.

13. Do you recommend working with fragments that are broken off and which cannot be traced back to a parent colony?

Yes, but carefully. It is important to keep track of the genet once it is in the nursery, but it does not matter too much where it originally came from per se. One should refrain from collecting many unattributable fragments from a single location as this would increase likelihood that newly collected fragment turns out to be from the same genet. Collecting single fragments of opportunity from each site (or fragments separated by tens of meters) should be fine.

14. Are there any other methods for genotyping corals in the field? At least to be able to differentiate one to the other.

Sadly we cannot yet genotype corals "in the field" (meaning, on the boat or in the water) - that would be so awesome! [We have recently proposed a potential solution to this challenge and hope to get the funding we need to develop such a method.]

Of the feasible lab-based methods alternative to SNP-chip that Iliana's group developed (Kitchen et al. 2018 doi:10.1101/2020.01.21.914424), Misha would say that RAD (Restriction site Associated DNA) approach has the most promise (and of course his favorite implementation of it, 2b-RAD, https://github.com/z0on/2bRAD_denovo). One nice thing about RAD is that it will work on any coral species right away (there is no need for development of species-specific genotyping tools), and it can be really inexpensive. The problem, however, is how to make this method accessible to reef restoration practitioners. We are looking into options right now.

15. A tool was mentioned that could be used onboard a boat to test genetics...this would be amazing! Does this exist yet or is it the pipeline?

Not at the present time, unfortunately. This is at the proposal stage and we really hope we get funded :)

16. There is research suggesting that newly settled corals acquire multiple symbionts, and that over time they select one to be more dominant. This selection seems to occur in less than a year, even less than 4-6 months. Also, symbiont acquisition happens within 2-3 weeks. Isn't the mortality too high if you place <2 weeks old corals in nature? Should we expose them to local symbionts is the lab (through symbiont cultures or adults from the locations they will be moved to)?

Those are very important questions, really key to restoration based on sexually produced recruits (hence, AGF). We don't currently know how big of a problem (survival-wise) it is to outplant a recruit with "wrong" symbionts, and at what age the recruit can no longer easily adjust away from such 'wrong' sets of symbionts. If it is a problem, how can we "feed" the coral the "right" symbionts (sample them somehow from the intended outplant location perhaps?). Studies have been undertaken testing laboratory and field approaches to seeding recruits with different symbionts and monitoring the longevity of these symbionts in the field, and their physiological tradeoffs, e.g. thermal tolerance and growth (Quigley et al. papers on GBR, Williamson et al. manuscript), but the particular symbionts that are the most appropriate likely vary by site and may also be contingent on unpredictable future events, e.g. thermal stress.

17, 31 - 32. You recommend that sexual recruits only be cultured ex situ for a minimal amount of time, but if survival is very high after return to the reef, does the benefit of the increase in new recombined juveniles added to the population outweigh the risk of adaptation to tank conditions?

If you are suggesting outplanting at earlier stages, so that frags can recruit Symbiodinium spp. from the actual reef and to avoid acclimation to nursery/lab conditions, will this require any secondary management tactics in order to keep more vulnerable corals alive? i.e. turf removal, specialized plot design to protect from algal overgrowth?

At what point after successful spawning is it suggested to outplant these baby corals to the reef, rather than grow them all in the lab/nursery?

Certainly, survivorship of early stage outplants is compromised by many different factors and a major challenge for making restoration more efficient is developing means to improve this.

Post-settlement survivorship is a factor that is highly variable according to species and the quality of the receiving habitat/location. Improved quality of this habitat by conventional management, along with the sorts of secondary management suggested by the questioner may be helpful. Additional strategies such as microhabitat enhancements (e.g. substrates for larval

'delivery devices' may help protect very young settlers from predation and competition) could also be beneficial. Indeed, developing methods to improve early post-outplant survivorship is a focal priority of the CRC Larval Propagation Working Group. In some cases, greater investment in post-settlement husbandry may be necessary for meaningful numbers of larval recruits to survive. In the interest of avoiding selection for aquaculture environments, such husbandry should be undertaken in the most 'natural' condition possible, including exposure to 'natural' pools of Symbiodineacae during infection periods.

Overall, we recommend outplanting settled recruits as early as is feasible and to a variety of different sites, not just the site of original collection.

There is ample evidence that incidental and/or targeted grazing by parrotfishes can be a big source of mortality for recruits (Nozawa 2008; Baria et al. 2010; Edmunds et al. 2014, Linden and Rinkevich 2017; Shantz et al. 2020). In places like the Keys with lots of big parrotfish munching on the reef it may be worth deliberately placing recruits (or the tetrapods they're on) in areas that provide refuge from big parrotfishes or even experimenting with protecting recruits for a short period of time after outplanting. It's likely to be more labor intensive but there is evidence that, at least over the first few months, survival can be dramatically higher in some areas (see the Baria paper cited above).

18-19. In regard to the source locations for collection, I understand the concept of sourcing environmentally diverse patches but is there a minimum distance that is recommended? 100 meters, more? How far is 'local'?

What is the practical distance delineation among local, near, distant for broodstock sourcing? Is there a definitive measure or is it variable depending on area/island, reef distribution etc.?

The simple rule of thumb is, any reef patch that looks visually different (i.e. contains a different community of organisms or different environmental conditions) is probably special in its own way and necessitates at least some local adaptation. We feel that much of the environmental variation to which corals would be adapted is available on a local scale - back vs fore reef, turbid vs clear, deep vs shallow, high vs low wave energy. It is more a question of habitat heterogeneity than absolute distance.

There is more discussion in our paper https://doi.org/10.1002/eap.1978

On our long list of topics to tackle as a working group is the need for better quantification of abiotic factors on scales that matter for local adaptation of corals. We will need the help of engineers and oceanographers so if you know of any who would like to get involved, let us know.

20. Do I understand correctly that you recommend out-planting genets from different gradients areas? If so, what are the main 3 gradients you would recommend using? We are in Barbados - would you recommend coral sharing with another island?

Please see the answer above: we feel like the meaningful adaptive variation can be sampled well by collecting corals from visually different reef habitats within local reach. It would be prudent (and logistically easier) to incorporate the variability present within local reach first, before importing from nearby islands (but that would be a reasonable next step where and when authorized).

22-23, 25. You talk about 3-6 genets per species, but in non-Caribbean regions there is usually much higher species diversity so how many _species_ do we need for a successful restoration project?

If you work with fragmenting of corals of opportunities only and frag only a small number of each different genotype you collected, is it better to focus on many species to enhance high diversity or to focus only on few species. Christian, marinecultures.org

To be sure, the fact that selecting only 3 colonies capture 50% of the diversity is for one species. So if we want to do AGF for "entire reefs" we have to do all these steps for each target species right?

The question of which species to address is indeed a dilemma in diverse systems. In our paper, we suggest that species should be selected that are functionally most important (e.g., important reef-builders or endangered species) and that are experiencing the most severe declines. These would still be important considerations, but there are no specific rules of thumb regarding the number of species necessary for 'successful' restoration. These questions delve into the next level of restoration, that of ecological restoration. While our paper does not really address this, these are important questions that need to be answered during the planning of your short- and long-term goals for your restoration program. Are you worried about shoreline protection? Then focus on reef-crest building species. Fish habitat? Then include branching species that are important to all life phases of fishes. Etc.

The bulk of our developed guidelines applies at the species level and would need to be replicated for each species addressed. In other words, having multiple species in the restoration project would not compensate for lack of genetic diversity within each. We feel that restoration of one or few species (chosen for fulfilling key ecosystem functions) with good population genetic planning and management would be more valuable and successful in the long-term than addressing multiple species opportunistically.

24. Many of the strategies presented are specific to Acropora in the Caribbean, the reproductive biology of many Pacific corals is more tricky… additional challenges include identifying rare, endemic, or endangered corals as well as invasive species. Are there plans to expand the working group to encompass these issues?

The very real challenges of identifying corals to species in the diverse Indo-Pacific fauna is not particular to the realm of coral restoration, and hence at this time we have not identified it as a priority for our working group.

26. If 3-4 colonies can retain ca. 50% of the allelic diversity, how much do bleaching events actual curb the potential diversity in a natural population?

Excellent question. We documented the loss of alleles in a natural population of *Acropora palmata* in the Florida Keys (Williams et al (2014) doi:10.1007/s00338-014-1157-y.) that ceased to recruit sexually a while ago. However, as a result of a die-off event you only completely lose variants that are rare enough not to be present within the surviving individuals. This means, unless your surviving population is less than 50 genets, you basically lose only rare and very rare variants (less than 1% frequency). Is this a problem? Probably not in the short term, since all the *currently* adaptive variants are likely to be not that rare (which is our main assumption for the "3 is enough" calculation). This might become a problem in the longer term, however, when *entirely new* adaptations would be necessary - these would be based on currently rare variants or even new mutations, both of which are in short supply in small populations that are no longer sexually producing at high rates. We address this problem by incorporating AGF - bringing new adaptive variants from other populations. In other words, sampling 3 genets per patch from locally diverse environments takes care of the short-term (i.e the next couple of decades) adaptive potential, whereas AGF promotes longer-term adaptation.

An interesting spin on this question is, can we actually measure the decline in genetic diversity due to bleaching events in natural coral populations that are still producing and recruiting sexual offspring? This would be hard. Think of it this way: right after the die-off, the surviving corals still represent a sample from a perfectly healthy large population, before the die-off. It would take many consecutive generations of small-population breeding for the genetic diversity to decline measurably (well, unless we sequence all or nearly all members of the surviving population). And if the population rebounds back to the original numbers via sexual reproduction soon enough (after a few coral generations, i.e. a couple of decades), the die-off might not leave any detectable trace at all in the genetic diversity of the species.

27. Are there specific traits that are likely to be inherited from mom/dad donors? If yes, do you consider this factor in designing recombination strategies?

Aha! Glad you asked! We cut it out of the presentation in the interest of time, but maternal effects are definitely a thing. Mothers likely influence the offspring's survival much more than dads because they supply yolk, mitochondria and other good stuff in those large eggs (dads just supply DNA), so it is expected, but not yet directly demonstrated, that 'local moms' would be best. So, if you are doing AGF, you may consider using a local mother and imported dad (cryopreserved sperm) - this will likely improve the chance of the recruit surviving in the mother's habitat (and is also quite convenient logistically, since it is much more feasible to freeze and move sperm than eggs).The new CRC cryopreservation working group is tackling these questions.

28. What are the major risks to biodiversity of carrying out nurseries in the open sea without the possibility of genotyping (with emphasis on developing countries without the option of genotification)?

The main risk is ending up with only a few genets or even just a single genet in the whole nursery, the fragments of which happen to grow best in the nursery, instead of a good representation of genetic diversity of a species being restored. That said, this risk can be largely averted by ensuring that the initial stock is unlikely to come from clonal individuals (i.e. collected from widely dispersed reef patches) and tracking the origin of every fragment.

29. Assuming legal frameworks are put in place, and playing devil's advocate, what is the worst-case scenario of such "foreign" transplants?

Worst-case scenario would be that a hitch-hiker metazoan or microbial/viral pathogen gets transported and established at the destination. In regards to this, though, there have already been detrimental introductions (including from the Pacific, e.g, the lionfish), so it would be naive to think that 'foreign' microbes have not already been transported. Ballast water from the international shipping industry is a large culprit in biological introductions. Nonetheless, we want to minimize this risk, preferably by importing just the genes (e.g. frozen sperm), not whole organisms that are more likely to carry hitch-hikers. Moving coral larvae among locations would also represent less risk than moving adult corals.

Next worse is that all the transplants die and it is a big waste of money and energy. Our paper discusses outbreeding depression. Range edge populations often carry increased mutational loads (deleterious recessive alleles) and may be more inbred than populations in the center of the species range. Recent studies on terrestrial organisms (very different life histories from corals) suggest that introducing individuals from large, outbred populations into small, inbred populations can hasten the extinction of the small population due to the introduction of recessive deleterious alleles. In other words, the risk of a reduction in fitness in >F2 generations increases. So far for corals, we have no evidence that the range edge populations are more inbred than other populations. While probably low, a formal assessment (via modeling) of this risk for corals is as yet outstanding but is certainly feasible (PhD thesis project, anyone?).

30. Can you identify the parents of a sexual recruit if you have SNP analysis of all the potential parents?

Oh yes, absolutely. This is easiest if the recruits are the result of a two-parent cross. However, even when four parents were used, we were able to assign larvae to parents (Baums et al. 2013 doi:10.1007/s00338-013-1012-6.). Beyond four potential parents, it does get more difficult.

33. During fertilization, are you suggesting making specific crosses or larger batch-style fertilization within the combinations of local-local, local-outside, etc.?

The goals of the project need to be clearly defined. If the goal is to assess the heritability of certain traits or creating a coral stud book, you would want to do two-parent crosses. But, but for the purposes of restoring local coral populations with maximal adaptation potential, you want to mix gametes from as many genets as possible together (multi-parent crosses).

34. Does Florida have the legal framework to allow coral to be shared with countries in the Caribbean and has this occurred?

Sadly, not yet. There are ongoing discussions toward evolving management paradigms about this, but it will not be quick. Experimental applications of AGF are an important first step - to verify expectations regarding feasibility and risks. For an example of an experimental application, please see Hagedorn et al. (2019; https://www.biorxiv.org/content/10.1101/492447v1).

35. Some coral species have life history strategies that do not require successful recruitment annually and persist even with fairly rare recruitment. How does one infer reproductive viability for these species?

Such low recruitment may not happen often naturally, at least it is not supposed to. In Indo-Pacific, everything recruits like crazy (100s of recruits per settlement tile). Even in the Caribbean some long-lived corals (*Montastrea cavernosa*, *Siderastrea siderea*, to lesser extent *Pseudodiploria strigosa*) still recruit regularly. Also, recruitment gaps would not be too surprising on a local scale (any given location might not get recruits for few years in a row, as long as at least some other locations do), but we are looking at *multi-decadal, region-wide* recruitment failure of the major reef builders (Acropora and *Orbicella*) in the whole Caribbean. I do believe that it is profoundly not natural and is the major reason for Caribbean-wide reef decline.

Assessing reproductive viability is perhaps most difficult and important for species that are locally naturally rare such as *Dendrogyra cylindrus*. It looks like this species has never been particularly common (with a few exceptions such as the US Virgin Islands) throughout the fossil record. Here, experimental crosses using field collected gametes can at least help to determine whether the existing genets produce viable gametes and larvae (which appears to be the case). This is a long way away from showing that the remaining genets can produce new natural recruits though.

Overall, the reproductive viability of a population can be judged according to whether it is maintaining itself. A decades-long, ratcheting pattern of decline, as observed for Caribbean Acroporids and Orbicellids, seems to fail this criterion.

36. Given that coral metabolism is the consequence of a multi-gene product system/network, how do you reconcile the strategy which focuses on singe genes and their representation in any population? For example, a gene that seems to encode a new substrate transporter would normally evolve in tandem with intracellular enzymes in the same metabolic pathway.

We actually don't think that variation in ecologically relevant traits in corals is due to single or a few genes; as you say, it is much more likely that the whole genomic makeup plays a role. Our upcoming paper (Fuller et al, Science some time later this year) strongly supports this "polygenic" scenario for bleaching tolerance. In such a situation, thinking in terms of additive genome-wide genetic variation (as implied in our advice) is justified. Single-gene studies are

very valuable to figure out how coral genes actually work, but in terms of practical advice we have to think genome-wide.

36. Hello! Thanks for the presentation! I would like to now how do you know which populations need more genetic diversity? And if we assume that every population, how do we prioritize which population needs more genetic diversity?

Thank you! I assume you are asking about AGF - which populations likely need *imported* genetic diversity? (because, of course, every population needs genetic diversity to efficiently adapt, see Misha's "evolution is not slow" rant). Populations most in need of AGF would be those that are changing most rapidly away from their historical conditions, have low population size, and low connectivity.

37. I largely work in terrestrial plant restoration (though I have a marine bio educational background). There is a convention in native plant restoration to use site specific propagules, which I have thought for a while is short sighted in light of climate change. The strategies for mixing local and non-local genetics and the evolutionary arguments presented here are compelling. Is anyone aware of papers presenting similar concepts for terrestrial vegetation?

Prober et al. 2015. Front Ecol Evol. 3:65 (and references herein)

38. Given what is happening with COVID-19 in terms of closed borders etc., what resources should a practitioner in the Caribbean have to get genetic work done to contribute to global database?

It is really hard to say about the virus situation right now, hopefully it will be resolved in a few months. But then the question still stands - our main challenge right now is to make genotyping and data handling accessible to actual reef restoration practitioners, not just academic scientists. We are working on this, it is a really high priority for us. Iliana's group SNP-chip is the best example so far.

Meanwhile - please do track your genets (fragments coming from the same original colony), and do archive a genetic sample of all source fragments by preserving a small piece (0.25 g) of each genet in 100% ethanol (or Bacardi 151 if you don't have it)! Please keep it in the freezer or as cool as logistically possible until the genotyping capacity is fully established. We feel like these samples will become super important in just a year or two.

39. Are there any reefs you know of that showing more resilience to coral bleaching naturally?

Places with a mechanism that ameliorates the temperature stress: Reefs with good water flow, periodic upwelling, high diel thermal variability, and moderate levels of dissolved organic carbon (that absorbs solar irradiance) have all been shown to experience less severe bleaching during high-temperature anomalies.

Yes, corals living in hot, variable places do not bleach at the same temperatures than their neighbors, and throughout the range of any coral species they are adapted to their *local* temperature, which might differ quite dramatically among locations. This argument in favor of coral capacity to adapt was nicely presented in Hughes et al 2003 Science paper, https://science.sciencemag.org/content/301/5635/929.

40. Instead of moving corals around, or trying to propagate resilient corals, why not just try correcting the Iron and Manganese deficiency of the symbionts that seems to be occurring at high temperatures, thus causing coral bleaching?

Beyond the scope of our working group.

41. Thermal stress seems to be the dominant physical parameter driving the extirpation of corals and associated communities. Our group at the Rowland Institute is working on floating mirror arrays with the potential to stabilize local ocean water temperatures to preindustrial levels. One side effect is a 10% reduction in solar radiation. What are some ecosystem-level concerns that you can see for such a reduction solar irradiance beside an associated reduction in primary production?

Beyond the scope of our working group.

Current status of these sorts of environmental interventions are reviewed by the National Academies Panel on coral interventions; https://www.nap.edu/catalog/25424/a-decision-framework-for-interventions-to-increase-the-persistence-and-resilience-of-coral-reefs

For additional questions, please email Coral.Restoration@noaa.gov.