The quest for an animal model of coral health and disease

Could a 'lab rat' for the coral field help researchers conserve these threatened animals in the wild?

Ellen P. Neff

How can we understand a disease such as cancer without first understanding, for example, the basic mechanisms of cell division, asks Annika Guse, a molecular cell biologist at the Centre for Organismal Studies in Heidelberg. That work doesn't usually start with a human—it starts with cell lines and animal models. "At the end of the day, you need something that is simpler and faster," she says.

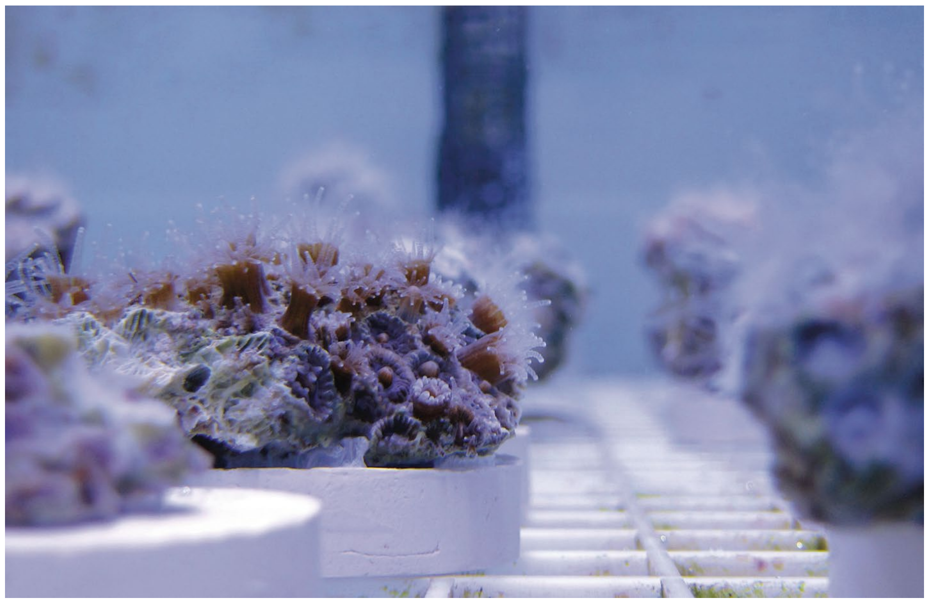
That holds true not just for cancer and other human conditions; it can also apply to other animals, such as corals.

Corals are in trouble. "On a global scale, especially in the past few years, coral reefs have been devastated by disease and bleaching," says Koty Sharp, a marine microbiologist at Roger Williams University in Rhode Island. According to a 2019 report from the National Academies of Science, Engineering, and Medicine[1], tropical coral reef coverage has declined between 30 and 50% globally over the past 30 years, the result of a combination of the building pressures of climate change, which is bringing both rising water temperatures and increasing ocean acidity, along with the anthropogenic stresses of pollution, overfishing, and habitat degradation. Corals—and the millions of people that depend on reefs for food, tourism dollars, and shoreline protection against storms—face an increasingly uncertain future.

"We're in this time of urgency," says Randi Rotjan, a marine ecologist at Boston University. "Everything's changing really, really fast…We need a lab rat."

Though some can grow to the size of a house and the larger reefs they form can be monitored from space, corals are animals—tiny, colonial cnidarians. A wild reef, however, is a challenging and uncontrollable environment in which to attempt to study the cellular and molecular mechanisms that contribute to coral health and disease.

The search has been on for an animal model of these animals, one that's not threatened nor endangered, and that can be disentangled from the larger, complex pressures facing tropical reefs and studied under more controlled laboratory conditions. "I don't want to harvest the reef for every itsy bitsy question," says Rotjan.



Live from the lab | The temperate coral, *Astrangia poculata*, growing in culture. Credit: Sharp Lab, Roger Williams University

Just as when researchers rely on animal models in biomedicine, the goal is ask questions in the model first and see what translates to a larger organism: in this case, the coral reef.

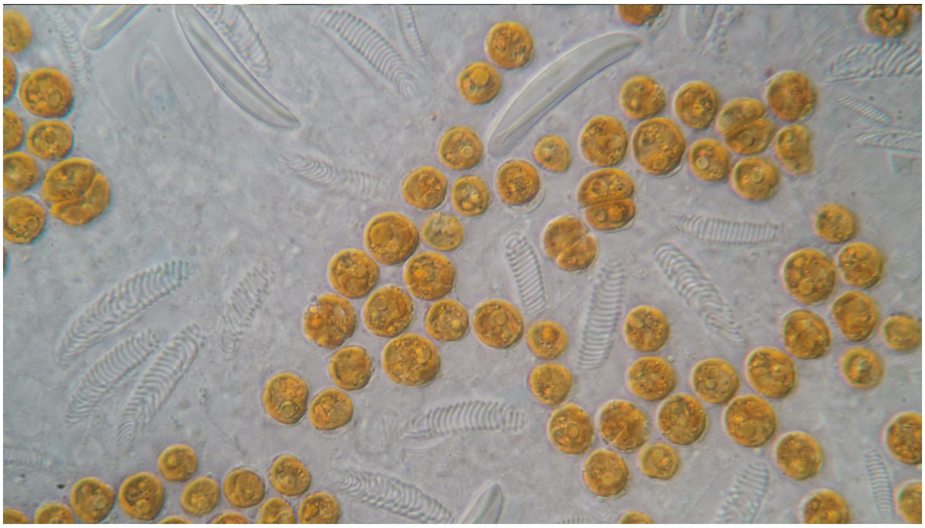
The trouble with the tropics

"The essential being of a coral is symbiosis," says Virginia Weis, a biologist at Oregon State University. Sessile, shallow-water corals evolved to thrive in the nutrient-deplete waters of the tropics thanks in large part to the close relationship they have formed with photosynthetic microalgae. These algae are unicellular dinoflagellates of the family *Symbiodiniace*[3] that take up residence within the corals' tissues and provide the animals with an added source of energy, as well their vibrant colors.

The individual polyps of a coral grow as colonies together, forming large calcareous skeletons and transporting and sharing their energy and other resources across the colony. In a large coral, the microalgae symbionts can even vary across the organism, says Weis, depending on environmental variables such as how much shading there is over one area versus another.

Should a coral become stressed or sick however, it tends to expel its symbionts, 'bleaching' to a ghostly white. Bleached corals are not yet dead corals, but their prognosis is poor. Should a second stressor hit a bleached coral before it can re-establish its energy-providing symbiosis, it might not make it through. Bleaching events are becoming more frequent—leaving inadequate time for meaningful recovery[2].

Why corals bleach—and how they establish that relationship with their symbionts in the first place—is still being determined. "There are a lot of open questions that essentially need a more standardized framework," says Christian Voolstra of the University of Konstanz.



In symbiosis | The *Symbiodium* found in Aiptasia. Credit: J.C. Koch & S. Maruyama

Coral symbiosis was long thought of as a very mutual relationship, with everyone getting their fair share; that might not however always be the case. "Our understanding of symbiosis needs to be broader," he says. "Depending on the environment, your best friend can become your worst enemy."

Understanding corals is the aim, but there is a bigger, fundamental principle to be studied: the interaction of two distinct organisms. Guse likens it to a cell biology problem: there are symbiont cells, which must find and gain entry into the cells of a larger host; both must then communicate and coordinate their functions.

But why is the symbiont not cleared by the host's immune system? How does the host know it's getting something good from its intracellular 'invader'? What does the symbiont get out of the relationship? "Uncovering the underlying fundamental mechanisms of the interaction between corals and their symbionts will allow us to better understand what goes wrong under environmental stress," she says.

And in addition to their photosynthetic symbionts, corals also harbor a microbiome, with bacteria, fungi, and archaea in residence too. These multi-partner interactions all likely contribute to the health and resilience of reefs as well, says Sharp.

Tropical corals, however, just don't take all that well to the lab. Corals get big and they grow slowly, and the animals require pristine water quality and water chemistry, a narrow temperature range (for many species, between 23°–29°Celsius), and a specific lighting regimen. "They're very, very fussy," says Weis.

Permits are often required to collect them from the field in the first place, and their reproductive cycles and growth and development are long and complex too. As sessile organisms they rely on broadcast spawning, coordinated once annually via environmental cues such as the lunar cycle. Larvae in turn need their own mix of internal and external signals to dictate when and where to settle down and start the metamorphosis process, says Cawa Tran at California State University Chico.

A more tractable option was needed, one that could be kept in aquaria and more easily manipulated as needed. Over the past decade, a couple candidates have emerged.

Friends and anemones

Corals can be sampled and brought into the lab for a time, but much work has been done in the field. "Coral reef biologists tend to be interested in ecologically contextualized questions on very big scales," says Weis.

With & without | Aiptasia, in aposymbiotic (left) and symbiotic (right) states. Credit: C. Renicke

Comparative studies on reefs have long been a focus, she says, but there had been less interest in the community for developing a true model system. She was met with not a few eye rolls when she first brought up Aiptasia at a 2004 coral reef symposium.

Aiptasia, *Exaiptasia pallida*, is an anemone—a cnidarian relative, but not a tropical reef building coral. It can however happily exist in both symbiosis with its photosynthesizing algae and in a symbiont-free aposymbiotic state—turn out the lights or turn up the temperature and the anemone will 'bleach,' leaving an animal than can then be recolonized with specific symbionts. "Not just the homologous native type, but you can challenge the animals with types they would never see in nature," says Weis, "What does it look like when they are challenged with the *wrong* type of symbiont?"

Despite initial reticence in the field, some—mostly symbiologists and cell biologists at first—heard the call, says Weis. She, with Simon Davy from Victoria University of Wellington, John Pringle from Stanford, and others convened a workshop that brought together coral biologists with cell biologists and parasitologists used to working on model systems. This culminated in a 2008 opinion in *Trends in Ecology and Evolution* detailing the argument for developing the Aiptasia model to study the cellular and molecular mechanisms of symbiosis[4].

The field has been growing since, says Davy, encompassing cell and molecular biologists as well as coral biologists and educators too. "It's been quite valuable," says Tran, "Not only as a research model, but one that we're really using to train the next generation of biologists to have conversations about coral reef conservation, about symbiotic interactions." In her work, Tran transitioned from tropical corals to Aiptasia in the lab as post-doc with Pringle and has now set up her own lab around anemones. "It's quite beautiful in that sense," she says.

The anemone is a cosmopolitan species, found globally in tropical waters throughout the Caribbean and Indopacific. It's a hardy one too, such that it's even made a name for itself as a pest in the aquarium trade. "In a way, the more you neglect them, the better they do," says Weis. "We can grow them with very little care—way easier than a coral."

They're small animals—about an inch long—that can be maintained in small dishes with artificial sea water. They can produce genetic replicates via prolific asexual budding or ample larvae in response to blue light that mimics the moon. The budded adults can be variously 'bleached' and recolonized, and the aposymbiotic larvae

Looking at larva | An aposympiotic Aiptasia larvae, stained to show various body structures. Credit: C. Tran & D.K. Simmons

can be mixed with different combinations of partners as well. As most corals don't pass their symbionts on to their progeny each generation, this is a way to explore how relationships with symbionts are first established in an animal host, says Guse.

Unfortunately, those larvae just won't settle down—yet. "There's one gigantic hurdle," says Weis, "We haven't closed the life cycle."

"We can get them to spawn and generate larvae, but the larvae are missing something, something critical to cue them to settle and go through metamorphosis," she says. "They basically swim forever." The Aiptasia genome has been sequenced[5], various microinjection and electroporation protocols are in the works, but the life cycle needs to be closed for true genetic manipulations, such as via CRISPR gene editing, to make it into the adults.

Don't be *Astrangia*

Aiptasia is a solitary polyp that lacks a calcareous skeleton—beneficial for microscopy purposes relative to their stony cousins and for considering symbiosis without the energy-demanding influence of calcification—but it is not a coral. Taking cues from the success of Aiptasia community, there is another growing group of researchers converging around a closer cousin to tropical species: the temperate coral *Astrangia poculata*.

From a small roundtable in 2012 held in an attic at New England Biolabs led by Sharp, and Rotjan, the *Astrangia* community's workshops—annual events at Roger Williams since 2016—now count over 120 participants, including graduate students and postdocs, eager to adopt the coral and expand the toolbox for working with it in the lab.

It was hard to believe *Astrangia* was not already a model, says Colleen Cavanaugh, a microbiologist at Harvard who is working on a proposal with Sharp to further push the coral's potential as a model species. The corals can be found on rocky surfaces all along the western Atlantic coast from Connecticut down through the Gulf of Mexico. Cavanaugh's recent subject of interest, chemosynthetic symbiosis in giant tube worms found at deep sea hydrothermal vents, require a research vessel and submarine to acquire—collecting the corals just takes getting wet.

In addition to their wide geographic range, *Astrangia* can be found from the intertidal down to mesophotic depths, naturally existing with and without photosynthetic symbionts. "It makes it just a really interesting creature in and of itself to try to understand how it works and where the limits of plasticity versus adaptation lie," says Rotjan.

Relative to the narrow temperature band inhabited by their tropical relatives, *Astrangia* at their northern range can experience considerable temperature swings. "They can go from the cold New England winters to the hot New England summers, and everywhere in between," says Sharp. "They're extremely resilient to thermal stress." Researchers can manipulate the temperate coral and observe how the *Astrangia* holobiont responds in a way they can't currently do with tropical corals, she says.

As *Astrangia* has northern and southern populations with distinct symbionts, there's natural plasticity to compare in the field too, and eventually back in the lab with colonies sampled from those different areas. "I think it lends itself well to a lot of hypothesis testing," says Sean Grace, a marine ecologist at Southern Connecticut University who co-organizes the community's workshops with Sharp and Rotjan.

The animals naturally enter a state of quiescence during the winter, during which time their microbiomes shift notably, says Sharp. As they start to feed again, the microbiome becomes more active and organized—independent of *Symbiodinium* fluctuations[6]. "This gives us this outstanding opportunity to really narrow in on that specific timeframe and say, 'what is happening in the coral?' Both from the host side and from the microbiome side to reestablish that community," she says. "It gives us the opportunity to identify organizing principles that govern microbiome assembly in the animals and symbiosis in general."

They're relatively easy going in the lab, says Rotjan. They need running sea water and a specific water balance for their calcareous skeletons, plus some supplemental feeding, but they can handle a range of temperate and light regimes. They're small too, about the size of a fist. "It's not going to answer questions about large, reef building corals," she says. "But a mouse is never going to be as tall as a human either, so I can live with that."

Tentacles out | Up close with *Astrangia*. Credit: A. Schickle, Sharp Lab, Roger Williams University

Recently, they've closed the life cycle as well, says Sharp, an important step on the way to CRISPR-ing the animals. Like tropical corals, *Astrangia* only reproduce once per year and then the larvae window closes. After years of efforts and the borrowing of techniques from shellfish culture, they have been able to scale up the number of larvae produced per spawning event into the 10s of thousands—there's much to improve says Sharp, but they have been able to get a small number of those settle and metamorphose into juvenile corals.

Coral tool building

Both the Aiptasia and *Astrangia* fields have spent much initial energy on community building and tool development, in order to make it possible for newcomers to bring the animals into their facilities and have the means to manipulate them.

With over a decade now under their belts, the Aiptasia community has many resources at their disposal. From the start, Weis has emphasized and encouraged cooperation among the labs adopting Aiptasia; in addition to discussions at their periodic workshops, much tool building and lab exchange happens on the community's Protocols.io site. "Instead of waiting for the paper to be published, people can quickly get that information and move forward," she says.

There are genomes, transcriptomes, and metabolomes either available or in progress for both species. That the animals are 'metaorganisms' with multiple symbiotic partners means that researchers have to take care with attributions. "You always have to make sure that you keep your player straight," says Rotjan. "This gene belongs to bacteria, this gene belongs to the photosynthetic symbiont, and this gene belongs to the host."

Until recently, transcriptomic analyses were largely done at the whole organism level, but not all cells are symbiotic, says Guse. Working with Aiptasia larvae, her lab has been working on a protocol to dissociate the small, transparent organisms into individual endoderm cells; from there, they can hand pick groups of cells with and without symbionts for transcriptomic analysis to see what pathways and mechanisms drive the difference during symbiosis establishment. This has yielded a large, hypothesis-generating dataset, she says.

Tran's postdoctoral work led to a microfluidic device to isolate larvae for live imaging[7]. They even caught a bleaching event in action, following addition of a common herbicide, she says. Paired with fluorescently labeled bacteria, a similar device designed around adult anemones will let her lab now explore how the microbiome changes in the living animals, without the need to preserve and fix them for imaging.

Reach for it | Aiptasia anemones. Credit: J.C. Koch

Voolstra has been using Aiptasia to ground truth observations from mobile 'stress boxes' that are used in the field to test the fitness of particular corals and evaluate the differences between more and less fit specimens. "Does this coral have some specific genes, do their specific symbionts? Do they have specific bacteria?" he says. "It goes a little bit back to the roots—that nature does it best."

The challenge now is to start merging the observations in the lab with those from the field, says Voolstra—to combine the model systems framework, with all its controllable advantages, but to make it flexible enough to put the ecosystem variation back into the equation.

Translating to the field

Aiptasia and *Astrangia* might be the furthest along, but other model candidates—the upside down jellyfish, *Casseiopeia*, another temperature coral called *Oculina*, or even a few tropical varieties—float around the coral field regularly. The Betty and Gordon Moore Foundation has launched a program to support tool development in the marine symbiosis field, and this summer, the 14th International Coral Reef Symposium will feature a session dedicated to the topic, to continue discussions of the need for model species and how the different emerging options can each contribute to the bigger picture of coral reef conservation. "We're down at the discovery level to try to boost these potentially translational tools that might be use as we as a field are trying to grapple with a very urgent concern," says Weis. "It's not one solution—it's many."

Ultimately—not unlike moving from mouse (or other model organisms) to man—observations from the lab need to be extended out to the field. That includes both to the models' wild counterparts and, eventually, to their tropical brethren. "Not everything we can do in the lab we can do in the field because it's much harder to work with coral, but certain experiments can be done in a comparative way," says Guse, who travels annually to work with collaborators in Japan. For example, samples can be collected from wild animals for metabolomics or transcriptional analysis, or researchers can perform simple perturbations with coral larvae to see if what's been observed in the models can be found in corals as well.

Some patterns are emerging. For example, elements involved in the innate immune system with roles in symbiosis have been identified and studied in Aiptasia, says Weis; transcriptional work from field samples of tropical species had indicated the same pathways at play in stressed animals.

Davy's lab is using proteomics and metabolomics to study the symbiosis and its physiological response to environmental stress; they have been able to extend these techniques for use in the field. "It's very good for tool development, which can then be used with corals," he says. If pathways and mechanisms are conserved, that could yield, for example, targets for genetic engineering. In the field however, researchers should be wary of drawing conclusions from samples of just single species, says Davy—there are just so many corals with so many different symbionts.

Rotjan's lab is adding a third element: computer modeling. "We try to manipulate and understand the drivers of those patterns in the lab, and if we're right, then we try to contextualize those patterns via mechanisms that we postulate in a mathematical model," If they converge, it suggests a solid understanding and could be a means to generate predictions for field testing. "When they disagree, then either the model's wrong—our experiment isn't testing the thing that we think we're testing for—or there's something really strange about the pattern in the field."

"The field is always right—that's nature showing us the way it is," says Rotjan. "We have to try to understand why and how."

Catching glimmers…

These efforts continue against a darkening backdrop. "No matter what we do with a model, and no matter what we do with our science as coral reef biologists, we are 100 hundred percent dependent on the rest of the world doing their part," says Rotjan.

Model organisms may get us to answers about the fundamentals faster, such as identifying pathways that could be tapped via probiotics or 'superfoods' to encourage symbiont resettlement after bleaching. They may provide a quicker route to tool development that couldn't be worked out in the field, be it for genetic engineering or assisted evolution to save even small patches of targeted reefs. Findings from models might just buy some time, says Davy, but the larger threats to coral reefs remain. "You can catch glimmers of hope, but the pace of tool development within the field is vastly outstripped by the pace of decline of the reefs," he says.

"I oscillate, like every coral biologist. There are moments that take your breath away," says Rotjan. "You can come across a reef that is so stunningly beautiful, and it just makes you feel like 'Okay, this still exists, we can do this.'… And there are moments that just take your breath away from their absolute devastation."

"I'm hopeful," says Rotjan, "but that doesn't mean that I don't have moments of tragic sadness." No one, however, plans to stop any time soon. ❐

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