

Chapter 4

Coral Reef Biodiversity

Nancy Knowlton^{1,2}, Russell E. Brainard³, Rebecca Fisher⁴, Megan Moews³, Laetitia Plaisance^{1,2}, M. Julian Caley⁵

¹Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA

²Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

³Coral Reef Ecosystem Division, Pacific Islands Fisheries Science Center, National Oceanic and Atmospheric Administration, Honolulu, Hawaii, USA

⁴Australian Institute of Marine Science, The University of Western Australia Oceans Institute, Crawley, Western Australia, Australia

⁵Australian Institute of Marine Science, Townsville, Queensland, Australia

4.1 Introduction

Coral reefs are often called the rainforests of the sea, but not because of their vastness. Being largely limited to warm shallow waters, their extent is surprisingly small – in total only 260,000–600,000 km², or approximately 5% that of rainforests, less than 0.1% of the earth's surface, or 0.2% of the ocean's surface (Reaka-Kudla 1997) – and thus smaller in total land area than France. One might therefore think that assessing the diversity of coral reefs would be far easier than for some of the other realms, geographic regions, and taxonomic groups that make up the 14 field projects of the Census of Marine Life that concern ocean life today. Yet coral reefs are the most diverse marine habitat per unit area, and perhaps the most diverse marine habitat overall – the deep sea being the only other contender, in part because of its huge area. As with rainforests, most of this diversity is not found in the organisms that create the three-dimensional structure of reefs – there are, in fact, fewer than 1,000

species of stony corals (scleractinians) that build reefs (Cairns 1999). Rather, the multitude of small organisms living with corals – the equivalent of the insects in the forest – are responsible for the staggering numbers of species associated with reefs.

Coral reefs share another, more dubious, characteristic with tropical rainforests, namely their vulnerability to human impacts, both global (associated with CO₂ emissions) and local (poor water quality, destructive and overfishing, invasive species). Although today concerns about the future of coral reefs dominate the news and the literature (see, for example, Bellwood *et al.* 2004), alarm about the state of coral reefs was slow in coming (Knowlton 2006). The first modern concerns arose in the late 1960s because of mass mortalities associated with population explosions of the crown-of-thorns sea star, *Acanthaster planci*, in the Pacific (Chesher 1969). The collapse of coral reefs in the Caribbean, caused by diseases (both of branching corals and the keystone urchin herbivore *Diadema antillarum*) coupled with overfishing, further added to the alarm (Hughes 1994), with paleontological analyses indicating that these levels of mortality had no precedents in the past several thousand years (Pandolfi 2002). More recently, surprisingly high losses to Pacific reefs, where

most coral reef diversity is found, have been documented (Bruno & Selig 2007). The dangers associated with rising temperatures have been recognized since the El Niño warming event of 1983 bleached and killed many eastern Pacific corals (Glynn 1993), and more recently the perhaps even graver threat of ocean acidification has come to the fore (Kleypas *et al.* 2006; De'ath *et al.* 2009). In late 2007 and mid-2008, two sobering reports appeared: one declared that one-third of all corals were at risk of extinction (Carpenter *et al.* 2008), making them the most endangered group of animals on the planet, and the other predicted that based on current trends in greenhouse gas emissions, coral reefs would cease to exist meaningfully by 2050 (Hoegh-Guldberg *et al.* 2007). As recently as October 2009, the Center for Biological Diversity petitioned the United States Government to list 83 species of corals under the Endangered Species Act owing to ocean warming and acidification.

Given these dire prognoses, it is surprising that relatively little attention has been paid to threats to coral reef diversity itself. Terrestrial conservation biologists often invoke the specter of a sixth mass extinction, originally based on loss of tropical rain forest to agriculture and livestock, with the disruptive effects of warming gaining more attention of late. However, marine scientists in general, and coral reefs scientists specifically, have been curiously less vocal when it comes to overall biodiversity loss. With a few exceptions (for example, the analysis of reef hot spots by Roberts *et al.* 2002), most scientific studies have focused instead on the loss of fishes, corals, and the ecosystem services that they provide. Indeed, even Roberts *et al.* based their biodiversity analysis on just four groups: fishes, corals, snails, and lobsters. The lack of serious attention to overall biodiversity loss stems not only from the assumption that extinction is less common in the ocean (McKinney 1998), but also from the sheer magnitude of the unknown diversity associated with coral reefs, which makes it difficult to assess its loss. People in general, and conservation groups in particular, tend to focus on what can be more easily measured.

Thus a central purpose of the Census of Coral Reef Ecosystems (CReefs) project has been to make the unmeasured measurable, and thus to make the unknown if not known at least knowable. In this chapter we summarize what we knew about coral reef diversity when we started, what the Census has contributed to our understanding, and what the findings suggest for the future of coral reef diversity, both as a topic of scientific study and as a heritage that may or may not be with us when this century draws to a close. A second and equally important goal is to chart the path that will make assessing coral reef diversity, and marine diversity generally, both locally and globally, a realistic endeavor, one that will contribute both to basic diversity science and coral reef and ocean management.

4.2 Background: The “Known” before the Census

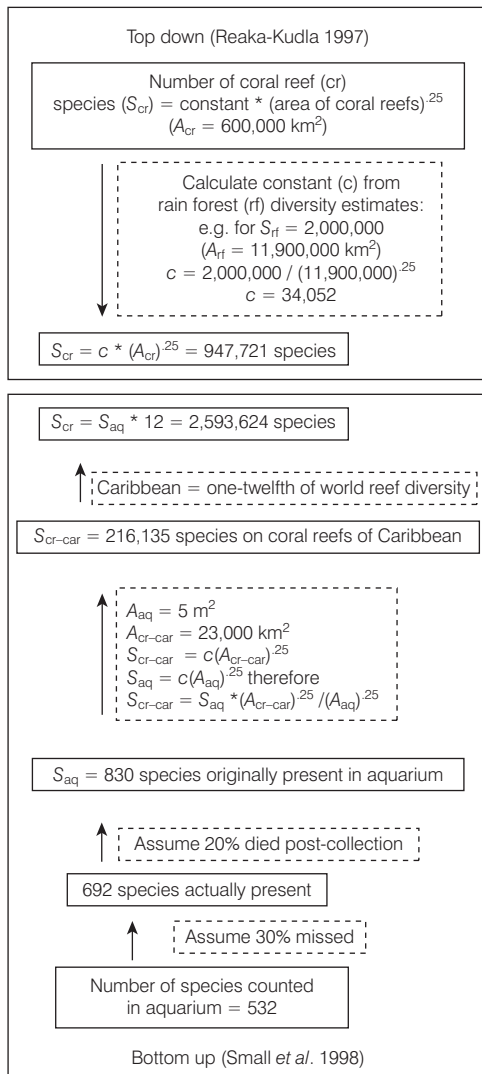
Although the Census began in 2000, CReefs was not launched until 2005. At that time, there were two key studies that attempted to estimate the global diversity of coral reefs. The first (Reaka-Kudla 1997) extrapolated from the diversity of tropical rainforests. The second (Small *et al.* 1998) extrapolated from the diversity of a large tropical aquarium. Extrapolation is of course the only way to make such an estimate: the key issues are the reliability of the assumptions underpinning the extrapolation and the scale of the extrapolation. Thus we explain the logic underlying these two analyses in some detail (Fig. 4.1), because it is important to understand their limits.

4.2.1 Estimates from rainforest diversity

To estimate the likely total number of coral reef species, Reaka-Kudla (1997) started with three estimates of rainforest diversity: 1,305,000, 2,000,000, and 20,000,000 species. Then, assuming that forests and reefs have the same species–area relations ($S = cA^{.25}$), she used the forest estimates to calculate the constant c for each of the three forest diversity estimates, and then calculated a total coral reef diversity assuming reefs occupy 5% of the area of tropical forests. From this she arrived at a reef estimate of approximately 618,000–9,477,000 species. Of course, the estimates of rainforest diversity are just that – estimates, and rough ones – and we know very little about how the diversity of a square kilometer of forest compares with that of a square kilometer of reef, or how heterogeneous that diversity is with distance.

4.2.2 Estimates from a mesocosm

Small *et al.* (1998) took an entirely different approach to estimate the likely number of coral reef organisms. They identified to species or morphospecies the non-bacterial/archaeal occupants of a coral reef mesocosm that was largely created by two collections totaling 5 m² of reef from a single locality in the Bahamas. The analysis occurred seven years after the last addition to the mesocosm, and the number obtained was 532 species. Using the same species–area relation as Reaka-Kudla and an estimate of Caribbean reef area of 23,000 km², they estimated a minimum total Caribbean reef diversity of approximately 138,000 species. Assuming that they missed 30% of the species in the mesocosm tank and that 20% did not survive, the figures increased to approximately 180,000 and 216,000 Caribbean reef species, respectively. Finally,

**Fig. 4.1**

Steps used for estimating coral reef biodiversity based on extrapolation from rainforests (Reaka-Kudla 1997) and a mesocosm (Small *et al.* 1998). S , number of species; A , area; c , constant in species–area equations; cr, coral reefs; rf, rain forests; aq, aquarium; cr-car, Caribbean coral reefs.

assuming that Caribbean reefs have one-twelfth of all reef species, they calculated total reef diversity figures of approximately 1,656,000, 2,163,000, and 2,594,000 species, respectively. If the less conservative estimate of total reef area in the world that Reaka-Kudla (1997) used (600,000 km²) is applied to these calculations, the figure for reefs overall rises to approximately 3.2 million species.

Although it is gratifying, and indeed somewhat surprising, that these two different approaches yield estimates that overlap, the many untested assumptions that went into the calculations make them “guestimates” at best (though they remain extraordinarily valuable efforts). After all, if even

the estimates of total area occupied by reefs vary by a factor of two, the total number of species on reefs is not surprisingly a highly uncertain figure. For this reason, most analyses of reef diversity have focused on patterns (with respect to latitude, longitude, depth, etc.; see, for example, Mora *et al.* 2003) rather than actual numbers.

4.2.3 Estimates of the undescribed

Reaka-Kudla (1997) took a published estimate of the total number of described species of 1,868,000, calculated that approximately 15% of all described species are marine based on taxon-by-taxon reviews, and used another analysis indicating that approximately 80% of all marine species are coastal, to arrive at an estimate of 219,000 described coastal species. She then used the percentage of coasts that are tropical (24%) and a species–area calculation (estimated number of species $S = cA^z$; see Fig. 4.1 legend), with the assumption that tropical coasts are twice as diverse as other coasts) to get an estimate of 195,000 described tropical coastal species. Finally, she used the proportion of tropical coasts that are reefs (6%), and the assumption that reefs are twice as diverse per unit area as other tropical coastal habitats, to arrive at a figure of 93,000 described coral reef species. By these calculations the proportion of coral reef species that remains to be described ranges from 85% to 99%.

Such attempts to estimate reef diversity based on existing data have continued over the lifetime of the Census. In 2005, as part of the Census effort, Reaka-Kudla (2005) updated and refined her analysis using similar methods. She estimated that there were approximately 95,000 described coral reef species, representing 35% of all marine species. Her summary estimate of total coral reef species was 1 million to 3 million (based on global estimated totals of all species of 10 million, 14 million, and 20 million), with approximately 30,000 being found in the Caribbean, and only about 5% described. Combining Reaka-Kudla’s approach with Chapman’s (2009) recent estimate of 11 million species globally would suggest a figure of less than 2 million for all coral reef species. Bouchet (2006) reviewed a variety of methods for estimating global diversity: extrapolation from samples, from known faunas and regions, from ecological criteria (species–area relations), and from taxonomists’ estimates of ratios of known species to unknown species. The estimate that he found most credible was based on brachyuran crabs. With currently 212 species from Europe representing 4% of the world’s total of 5,200 crab species and a total of 29,713 European marine species in all taxa overall, a global estimate of 728,809 marine species results. If one accepts that there are really 250 European brachyuran crabs, 10,000 brachyuran crabs globally, and 35,000–40,000 marine species in all taxa in

Europe, then one arrives at a global estimate of approximately 1.4 million to 1.6 million marine species. If coral reefs contain 35% of the marine total, then the total number of coral reef species is about 490,000–560,000.

We thus have estimates of coral reef diversity that range from approximately 500,000 to nearly 10 million. Yet even the smallest of these numbers greatly exceeds the numbers of species recorded for individual tropical locations. For example, Paulay (2003) reported 5,640 marine species from the Mariana Islands, Wehrtmann *et al.* (2009) reported 6,778 marine species from the Atlantic and Pacific coasts of Costa Rica, and Bouchet *et al.* (2002) reported 2,738 species of marine mollusks from the west coast of New Caledonia. There is essentially no bridge between coarse global analyses and intensive local biodiversity assessments for most coral reef organisms. Moreover, most of these geographically focused studies have used traditional (that is primarily morphological) criteria for recognizing species.

One way to examine the limits of such compilations is to look at a handful of species in detail. Just as CReefs was launched, Meyer *et al.* (2005) published such an analysis, and it clearly indicated that the geographic scale for endemism could be far finer for some marine organisms than traditionally assumed. In a study of the snail *Astraliium* “*rhodostomum*” in the western Pacific and eastern Indian Ocean, they documented two deep clades (estimated age more than 30 million years), which together contained seven subclades (estimated ages 11 million to 20 million years). The subclades themselves comprised evolutionary significant units (with estimated ages of 2 million to 7 million years) that in total represented at least 30 divergent, geographically isolated units, some separated by as little as 180 km. Given that marine organisms separated by the Isthmus of Panama (at least 3 million years ago) are typically reproductively isolated (Knowlton *et al.* 1993; Lessios 2008) and that many of the evolutionary significant units of *Astraliium* also have differences in color pattern, it could be argued (depending on the species concept used (Knowlton & Weigt 1997)) that all the clades/subclades, and perhaps most of the evolutionary significant units merit recognition at the level of species.

The question for global estimates of reef diversity, of course, is how typical is the pattern documented by Meyer *et al.* (2005)? As they note, many previous studies of phylogeography concern groups, like fishes and sea urchins, that have relatively widely dispersing larvae, and in these groups fine-scaled endemism is rarer, although hardly unknown. *Astraliium* has limited dispersal and other species with similar larval dispersal are very likely to have similar patterns. Moreover, limited dispersal is associated with small size (Strathmann 1985; Knowlton & Jackson 1993), and much of the diversity on reefs, as in other biological communities (May 1988), comprises small organisms. For example, Bouchet *et al.* (2002) found that the most diverse size class of mollusks (25% of the species)

was the 1.9–4.1 mm size class, with about one-third of all diversity of that size or smaller. It is not inconceivable that at least 30% of all reef species have patterns of endemism like *Astraliium*.

4.3 The Census of Coral Reef Ecosystems Approach

Because of the limits to previous analyses, a primary goal of the CReefs project has been to develop new methods needed to address the challenge of assessing the enormous diversity of coral reefs and begin to apply these methods. A comprehensive global assessment of the diversity of the world’s coral reefs, both healthy and in distress (that is, at least one-quarter and perhaps over one-third of the diversity of the oceans overall) was clearly beyond the scope of a five-year project, but developing the methods and ground-truthing them was not. The two key methodological components upon which CReefs focused were molecular analyses and standardized sampling.

4.3.1 Molecular analyses

As can be seen from the above summary of previous efforts, the most significant limitation has been the sparseness of available diversity data. It is difficult to extrapolate reliably from a few tiny samples, fundamentally because we do not know the rules for doing so. We cannot develop the rules because it is too expensive and labor intensive to analyze a greater number of samples and we lack adequate scientific expertise for many taxonomic groups.

Molecular methods can reduce these constraints. Instead of depending on taxonomic expertise for species identification, or even sorting into operational taxonomic units of all collected material, one can identify organisms by their genes. This is the concept that underpins genetic barcoding (Hebert *et al.* 2003), and sequencing of the mitochondrial cytochrome oxidase (*COI*) gene works well for many marine organisms, although some technical problems remain. For some groups, such as corals, the *COI* gene is not variable enough to be used at the species level (Shearer & Coffroth 2008), and for others, such as certain crustaceans, there are problems with either amplifying the genes before sequencing (success rates are rarely above 80% overall (Plaisance *et al.* 2009)) or with pseudogenes (extra, non-functional and independently evolving copies in the nucleus; see, for example, Williams & Knowlton (2001); Plaisance *et al.* (2009)). Nevertheless, for taxonomically well-known groups it has great power and potential when applied carefully.

However, even when a gene like *COI* is effective and speeds up the process of sorting organisms to taxonomic groups considerably, it does not by itself provide a name.

For example, in CReefs analyses (described in more detail below) of crustaceans from the Northern Line Islands and Moorea (Plaisance *et al.* 2009), not a single barcode sequence obtained matched any genetic sequence in GenBank at the species level (match of at least 95%), and most matched by only 80–84%; Puillandre *et al.* (2009) similarly found that only one of 24 neogastropod egg cases in the Philippines could be tentatively identified to genus. Given the enormous scope of the unnamed, as reviewed above, this is likely to remain the case for the foreseeable future. Indeed, a mechanism for using standardized barcodes as names as they accumulate, though resisted in many traditional circles and certainly requiring planning for implementation, may well be the only viable solution when so much of biodiversity will remain unstudied by traditional approaches.

Moreover, even though barcoding represents a vast improvement in efficiency compared with visual sorting and traditional identification, it remains an intermediate step towards the goal of obtaining a truly efficient biodiversity assessment methodology. This is because barcoding still involves removing and sampling or sub-sampling individual organisms from collected material, a process that can be time-consuming and costly, especially for small organisms where most of the diversity lies. The next part of the journey towards having a truly efficient method for assessing diversity involves a new and still evolving technology – next generation sequencing – which can be used to obtain large numbers of very short sequences from a sample. This technology has already transformed our ability to study microbial communities (see Chapter 12), and is probably the most significant technological advance for DNA-based studies of diversity since the development of the polymerase chain reaction (PCR).

Although now routinely used in analyzing the genomes of single organisms and environmental samples (“environmental genomics”) of microbes, using this method to study the diversity of multicellular life poses challenges. The essence of the problem is the following: if you throw a sample in a blender at one end and get out a list of DNA sequences at the other, to what extent does the list of sequences faithfully reflect what went into the blender? Although this problem of representativeness affects all environmental genomics, it is particularly severe for multicellular organisms for several reasons. First, many multicellular organisms, including common members of coral reefs such as sponges and tunicates, produce an assortment of substances that interfere with critical reactions needed for amplification of DNA. Second, even ignoring the very large organisms that can better be identified in reef transects, multicellular life varies enormously in size, from clumps of algae or sponge of several cubic centimeters to tiny amphipods and worms, so the amount of DNA from different organisms in a sample will vary correspondingly. Finally, some types of DNA amplify well with standard primers

used to start the amplification reaction, and others amplify poorly or not at all (for example, caridean shrimp in the studies of Plaisance *et al.* (2009)). Thus because of the general problem of inhibition and the fact that some organisms might be over- or under-represented because of differences in DNA amount or amplificability, there is no necessary relation between what goes into the blender and what comes out of the sequencer.

To tackle this problem, CReefs is engaged in experimental analyses to estimate the extent and patterns of bias associated with mass sequencing of coral reef community samples. For example, from a given sample one can remove all mobile organisms (which overall are less likely to produce inhibitory substances), remove one subsample from each for individual amplification (to test for any taxonomically based primer problems), remove another subsample to mix with the collection of other similar-sized subsamples before amplification (yielding a mixture where the amount of tissue is approximately equal for all individuals, to test for the effects of different amounts of DNA), and compare these two methods with results when the rest of the body parts, with their very different sizes, are mixed together before amplification (as would be the case in any large-scale sampling protocol). Results so far suggest that genes that are optimal for species-level diagnosis (for example, *COI*) may not be ideal for getting a representative assessment of the community composition, so some compromise between sensitivity and comprehensiveness may be needed. However, if the extent and pattern of bias are known and relatively constant, it should be possible to compare across space and time, which is what is most needed.

4.3.2 Systematic sampling

The second component of the CReefs strategy is systematic sampling, with samples analyzed using the molecular techniques described above, widely applied. Hand sampling by divers ranging over a reef remains the most efficient way to find species (both already known and new) when they are large enough to be seen (Fig. 4.2). However, the effort required to enumerate diversity properly can be daunting (May 2004), and divers vary enormously in their abilities in this regard, making it very difficult to compare work from different places and times involving different people. Two particular methods have been developed by CReefs: assessments of the organisms (especially crustaceans) living in heads of dead *Pocillopora* coral, and assessments of all marine organisms settling into autonomous reef monitoring structures (ARMS) placed on the reef for one to three years.

Using communities of invertebrates living in dead heads of the coral *Pocillopora* (Fig. 4.3) as proxies for reef diversity had the advantage that it could be implemented immediately, an important consideration given the short time-frame for CReefs from its founding to the close of the

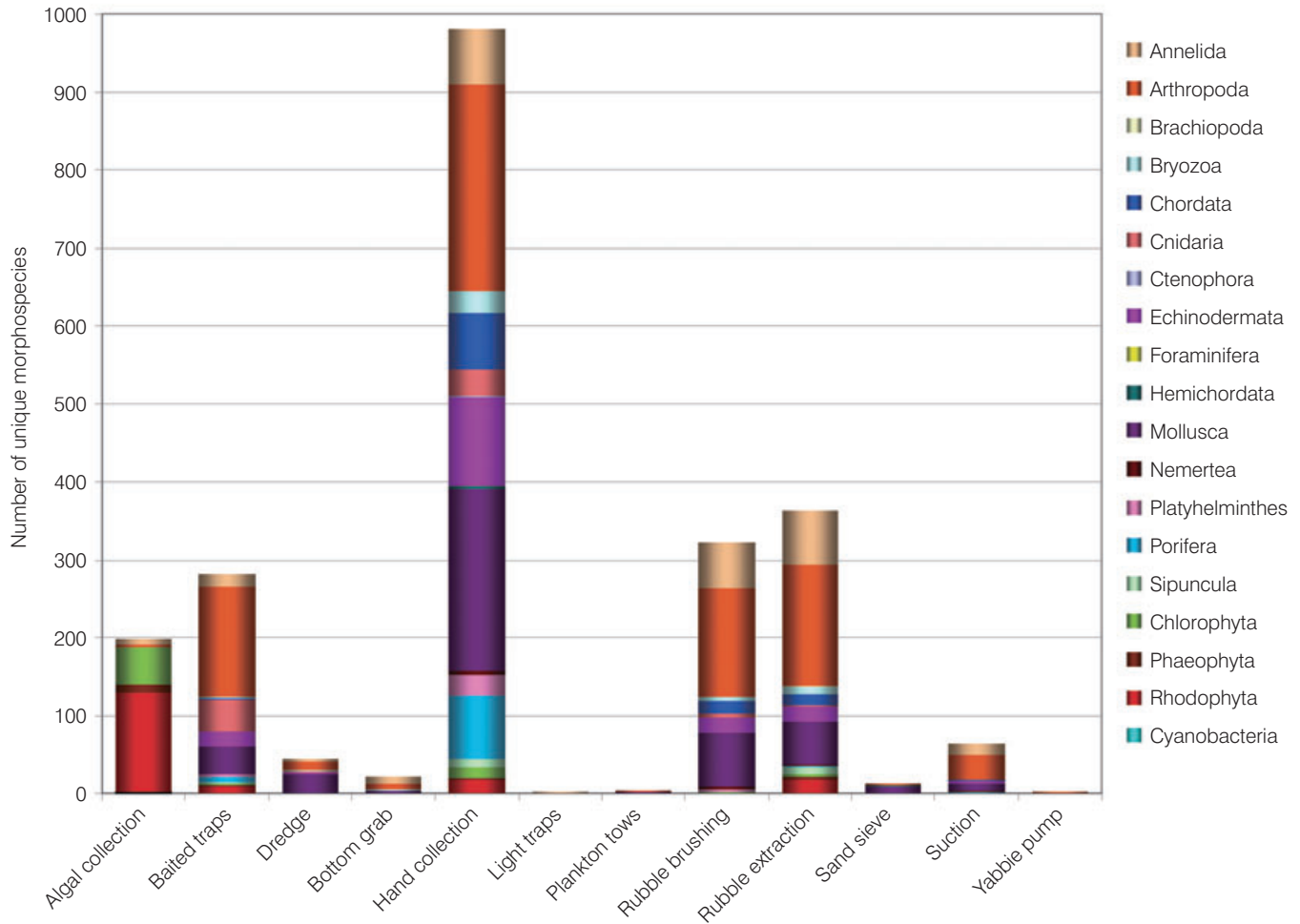


Fig. 4.2

Numbers of species (by higher-level taxon) obtained using different methods on the CReefs cruise to French Frigate Shoals, Northwestern Hawaiian Islands.

Fig. 4.3

Laetitia Plaisance at work on barcoding for CReefs project. **(A)** Preparing to break open dead head of *Pocillopora* coral to extract resident invertebrates. **(B)** Examining extracted DNA before sequencing. A, B, Juergen Freund © FreundFactory.



Census. In addition, because these are natural habitats, interpretation is not confounded by concerns associated with artificial substrates. Assessment of *Pocillopora* heads can be replicated from the Red Sea to the Eastern Pacific (and with some adjustments, reef rubble in the Caribbean can also be compared). On the other hand, the sizes of dead coral heads can be only roughly standardized (for example, fitting snugly into standard buckets), their ages cannot be known with any precision (although one can collect heads that are old enough to be covered with fouling sessile organisms but young enough not to have been substantially bioeroded, to provide some standardization), and all collections involving removal of reef require permits (which, for example, were denied for the initial CReefs cruise to the Northwestern Hawaiian Islands).

The second systematic sampling method, ARMS, has been developed as a standard method to mimic the structural complexity of coral reef habitats and attract colonizing invertebrates and algae. ARMS evolved from “artificial reef matrix structures” (note: this is different from “autonomous reef monitoring structures”), originally designed and tested in the eastern Caribbean to collect as much diversity as possible (Zimmerman & Martin 2004). Their original design involved several layers of concrete with different sized openings and a variety of microhabitats, including a mesh basket containing coral rubble suspended from a PVC frame to allow different occupants to colonize the structure. It was determined that such structures were heavy, over-sampled sites, and that it was difficult, time-consuming, and costly to extract and process specimens.

Rather than attempting to collect and document all of the diversity of coral reefs, CReefs developed the current generation ARMS as a simple, cost-effective, standardized tool to assess spatial patterns and temporal trends of

indices of cryptic diversity systematically on a global scale. After numerous design modifications and test deployments, CReefs settled on an ARMS design consisting of 23 cm × 23 cm gray, type 1 PVC plates stacked in alternating series of open and obstructed formats (created by x-shaped inserts dividing each space into four sectors), topped by plastic pond filter mesh and a final plate, and attached to a base plate of 35 cm × 45 cm, which is then affixed to the reef (Fig. 4.4A). In December 2008, using some experimental ARMS deployed off Oahu, CReefs partners conducted a workshop to develop protocols for retrieval, sampling, and processing, including sample preservation and molecular analyses.

DNA barcode analyses were also conducted to characterize crustacean biodiversity associated with ARMS in comparison to the dead *Pocillopora* heads from other sites in the Pacific. These results suggest that coupling ARMS with taxonomic and molecular analyses can be an effective method to assess and monitor understudied coral reef invertebrate biodiversity. In the long run, ARMS will be a much more powerful tool than assessment of dead *Pocillopora* heads, because they can be deployed nearly anywhere (including non-reef sites), do not involve destructive sampling of natural habitats, are much easier to remove organisms from (especially true for sessile organisms), and can be highly standardized. Permits for deploying and subsequently collecting ARMS are also in general easier to obtain than those for collection of live rubble. ARMS have the disadvantage of not being natural habitats (being made of PVC and lacking many small nooks and crannies), but early assessments suggest that the diversity captured is representative of the communities in which they are placed (Gustav Paulay, personal communication; see also Fig. 4.4B).

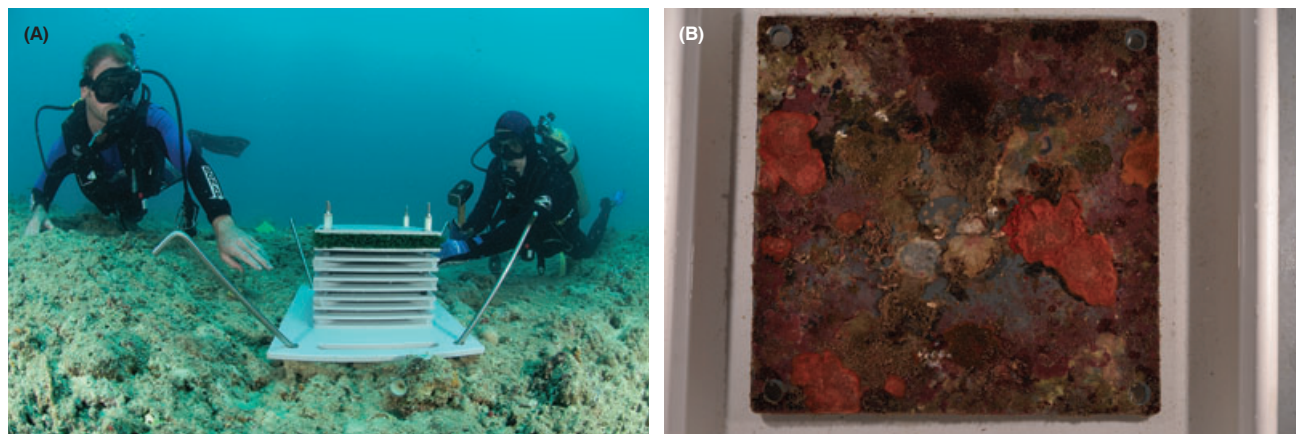
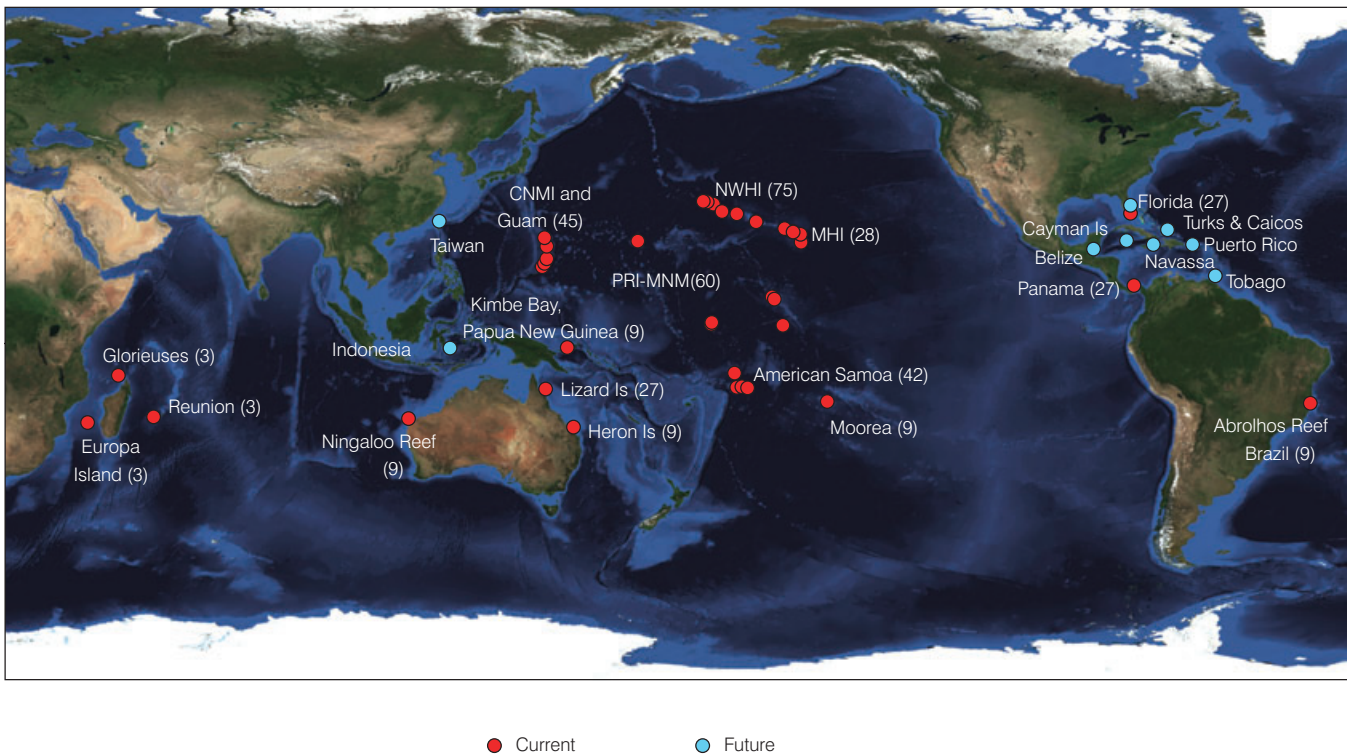


Fig. 4.4

Autonomous reef monitoring structures (ARMS) in Australia. **(A)** ARMS being installed on reef (Juergen Freund © FreundFactory). **(B)** ARMS layer after removal from reef one year after deployment (Gustav Paulay, Florida Museum of Natural History).

**Fig. 4.5**

Map of current and planned deployment sites for ARMS. See text for full listing of abbreviated names.

Over 400 ARMS have been widely deployed throughout the Pacific between 2006 and 2009, with smaller, yet increasing efforts in the Indian Ocean and the Caribbean (Fig. 4.5). They were successfully deployed throughout the Papahānaumokuākea Marine National Monument (MNM) and Main Hawaiian Islands, the recently established Pacific Remote Islands MNM (Line Islands, Phoenix Islands, and Wake Atoll), American Samoa (including Rose Atoll MNM), Australia (the Great Barrier Reef's Lizard and Heron Islands, Ningaloo Reef), Brazil (Abrolhos Reef), Guam, Northern Mariana Islands (including Marianas Trench MNM), French Polynesia (Moorea), western Indian Ocean (Reunion, Europa, and Glorieuses Islands), Panama, Papua New Guinea (Kimbe Bay), and Florida. Additional deployments are planned for 2010 in Puerto Rico, the Cayman Islands, Belize, Taiwan, Indonesia, and other locations within the Coral Triangle. The approach has been adopted as a key biodiversity assessment tool by the National Oceanic and Atmospheric Administration (NOAA)'s Pacific Reef Assessment and Monitoring Program and as a central component of the Smithsonian's Marine Initiative and NOAA's Biodiversity Alternative. To ensure consistency and comparability, and to reduce costs, efforts so far have been led by CReefs' Hawaii Node (NOAA's Pacific Islands

Fisheries Science Center, Coral Reef Ecosystem Division), with ARMS being centrally produced.

Finally, it should be noted that dead *Pocillopora* heads and ARMS are not the only solution to standardized sampling. Analyses of fixed amounts of sediment or of fixed amounts of material vacuumed from a reef (for example, see methods of Bouchet *et al.* 2002) are complementary approaches that target different components of coral reef communities. The key features that all of them share are (1) they can be at least in some sense standardized, so that results from different studies can be compared, and (2) they lend themselves to molecular analyses using either barcoding or environmental genomics.

4.4 CReefs Results

Analyses of dead *Pocillopora* heads from five islands in the Central Pacific (Kirimati, Tabuaeran, Palmyra, Kingman, and Moorea) have now been published (Plaisance *et al.* 2009), and several surprising results from these analyses are already apparent. First, the total number of crustaceans recorded was exceptionally high for such a small sample. A total of 22 small dead coral heads

(combined length + width + height dimensions of each approximately 90 cm, total basal area less than 2 m²) yielded 789 individual crustaceans. Of these, 500 were sequenced (all rare plus representatives of abundant morphospecies were selected), which yielded 403 usable sequences, from which 135 operational taxonomic units were distinguished. Of these, 65 were brachyuran crab species, a number equivalent to approximately 30% of the entire described brachyuran fauna of European seas and approximately 1% of the global total! Second, most species were rare and locally distributed: 44% of all species were sampled just once, and another 33% were only found on one of five islands. Even more surprisingly, 48 of the 70 decapod species found in the Northern Line Islands were not only not found in the Moorea *Pocillopora* head survey, but were not recorded in the extensive cross-habitat collections associated with the Moorea Biocode Project (www.mooreabiocode.org). Third, despite the marked anthropogenic impacts on the abundance of corals and fishes on the two inhabited islands in the Northern Line Islands (Sandin *et al.* 2008), there does not appear to be a comparable negative impact on the diversity of small crustaceans. Therefore, our reliance so far on corals and fishes as surrogates for coral reef biodiversity may need to be re-examined.

Data from other expeditions are still being analyzed, but the patterns remain consistent with these results. For example, the numbers of species/percentage singleton figures from Australian dead coral samples were 58/43% (Ningaloo Reef, 7 heads), 113/47% (Heron Island, 16 coral heads), and 48/60% (Lizard Island, 11 heads). Likewise, at French Frigate Shoals in the Northwestern Hawaiian Islands, one-third of all invertebrate morphospecies collected were singletons or found at only one site, and one-third of the crustaceans from ARMS were singletons.

Finally, from the beginning, one goal of CReefs has been to build taxonomic expertise and information for those groups of coral reef organisms that are poorly known, to complement the molecular approaches. As noted above, most ecological studies focus on corals or fishes; mollusks (and to a lesser extent crustaceans) are better known than most other groups, but even for these groups many gaps remain. Much progress was made possible by several CReefs cruises and expeditions, beginning with the cruise to French Frigate Shoals in the Northwestern Hawaiian Islands. By 2009, scientists from the French Frigate Shoals efforts had already found that of the nearly 400 algal specimens (approximately 160 morphospecies) catalogued, many were not on the list of 179 described taxa previously reported (Vroom *et al.* 2006). Also, at least 50 new invertebrate species and over 100 new records were identified for the region, including probable new species among sponges, corals, anemones, flatworms, segmented worms, crabs, bivalves, gastropods, octopuses, sea cucumbers, sea stars, and sea squirts (six octopuses were collected repre-

senting six different species, three may be new). As a result of the repeated expeditions to Heron Island, Lizard Island, and Ningaloo Reef in Australia, hundreds more new species and records are being identified. The taxonomic papers published under the aegis of CReefs are now appearing, but initial estimates suggest that there are about 100 new species among the 4,150 sample lots and approximately 2,100 morphospecies in Hawaii (PIFSC 2007), and more than 1,000 new species from over 15,000 sample lots from Australia; one new family has already been described.

Initial results from the original French Frigate Shoals ARMS showed that prototype ARMS were most productive in sampling mollusks (28%), ascidians (24%), crustaceans (19%), and bryozoans (11%) in fore reef and lagoonal patch reef habitats. Of the 12 prototype ARMS recovered from French Frigate Shoals, new records for the Northwestern Hawaiian Islands were recorded for two non-native (alien) species of solitary tunicates, *Cnemidocarpa irene* and *Polycarpa aurita* (Godwin *et al.* 2008). The results from the standardized, globally distributed ARMS await 2010–2012 retrieval and analyses. Further analyses will take place beyond 2012 as the ARMS are used for continued monitoring to assess the biodiversity impacts of climate change and ocean acidification.

Identification and analysis of specimens can be a time-consuming process, thus there are likely to be many more discoveries as the specimens from these efforts are further analyzed. Such discoveries will be documented in multiple joint publications and the data placed in the global Ocean Biogeographic Information System (Chapter 17) (more than 400,000 records submitted so far). By providing scientists and managers with a more complete picture of what exists in coral reef ecosystems, they will be better equipped to manage them and in particular watch for and manage changes over time. Furthermore, with the integration of future investigations, there can be a greater understanding of biodiversity over gradients of human disturbance.

4.5 Gaps in Knowledge

There are unlimited questions at various scales that could be asked about coral reef diversity, but CReefs has focused on developing the methods needed to answer these four:

- 1) How many species occur on coral reefs and what are the patterns of species diversity for all reef species across gradients of human disturbance?
- 2) What kinds/percentages of species are obligately associated with healthy coral reefs and how widely are they distributed?
- 3) What are the prospects for maintenance of species diversity on reefs suffering various levels of human impacts?

- 4) How much and what kinds of taxonomic and ecological information are required to manage reef biodiversity effectively, and are cost-effective proxies possible?

Ironically, it is the last of these that we have answered first: we do now have a method that is cost effective for assessing diversity, and it has the potential to work far more effectively than using individual taxa as proxies. Moreover, in a DNA analogue to Moore's Law, sequencing costs have dropped substantially since the start of CReefs, and will continue to drop over the coming decade, further increasing the use of these approaches.

Although we do not have a firm answer to question (3), results so far suggest that moderately impacted reefs continue to support large amounts of diversity but seriously degraded reefs do not. This pattern is easiest to understand as a nonlinear relation between diversity and disturbance such as predicted by the intermediate disturbance hypothesis. For example, human disturbance may initially increase the types of habitat available to small invertebrates living in the reef, by causing corals and algae to coexist more equally (unimpacted reefs have very little macroalgae; see, for example, Sandin *et al.* (2008)). Thus diversity appears to be relatively unaffected by human disturbance in the Northern Line Islands, probably because even the most degraded of the Northern Line Islands are comparatively pristine (Knowlton & Jackson 2008). In contrast, Caribbean reefs show a clear pattern of decreased invertebrate diversity with lower coral cover and three-dimensionality (Idjadi & Edmunds 2006), probably because almost all Caribbean reefs are seriously degraded (Gardner *et al.* 2003; Pandolfi *et al.* 2003).

The first two questions are the hardest to answer – we cannot reliably estimate global reef diversity from 2 m² of dead coral heads from the Central Pacific, any more than Small *et al.* (1998) could from 5 m² of Caribbean reef placed and later sampled in a mesocosm. Just comparing these two analyses points to the many possibilities for error in the assumptions. For example, Small *et al.* (1998) found eight species of decapods and assumed that a comparable sample in the Pacific would be 12 times more diverse, yet in a sample less than half that size from a part of the Pacific not renowned for its diversity, Plaisance *et al.* (2009) found 108 decapod species. The extremely high prevalence of singletons at any site and the related absence of overlap between sites, a pattern characteristic of all the CReefs results, clearly imply that much more geographically dense sampling is needed to determine the level of endemism, which in turn profoundly affects extrapolations from single sites to the world at large. However, despite the challenges now, much better answers to these questions will be possible with the analysis over the next few years of the hundreds of ARMS that are currently deployed (Fig. 4.5).

4.6 Advancing Knowledge

4.6.1 Current limits to our knowledge

The greatest limit to knowledge has been the lack of a biodiversity assessment protocol that can be implemented globally. Both lack of money and lack of agreement on an appropriate method have played a role, but providing the latter would go a long way towards acquiring the necessary financial commitments. This is why CReefs has focused on methodological advancements.

With the establishment of an agreed approach (widely accepted by scientists and managers globally), and an appropriate financial commitment for both the regular analysis of samples and the maintenance of databases, coral reef biodiversity studies could be standardized at a global scale, with adequately dense sampling. Given estimates that coral reefs represent perhaps over one-third of all the diversity of marine life, it is ludicrous to assume that this diversity can be understood by tiny and unsystematic assessments – 22 heads of dead corals from the central Pacific, 5 m² of reef from the Bahamas, species lists or numbers from a handful of well-studied locations, or even a many-month expedition in the heart of reef diversity, the Coral Triangle. Alone, these do not begin to provide enough information, even to know what the appropriate geographic scale of sampling is.

4.6.2 Why the knowledge is needed

The scientific justifications for estimating coral reef diversity go well beyond simple curiosity about the total number of organisms living on reefs – given the threats that reefs face, better knowledge of how reef diversity is likely to be impacted by loss of living coral is clearly essential for conservation and management. Although diversity may remain poorly understood, the nature of the threats is far clearer. Three are globally pervasive – overfishing and destructive fishing, poor water quality, and the effects of carbon dioxide in the atmosphere. A fourth threat – invasive species – also represents a serious problem in an increasing number of locations (for example, seaweeds in the Pacific and lionfish in the Caribbean). This combination of human-induced impacts has resulted in a situation where globally about 60% of coral reefs have been degraded or lost (Jackson 2008).

Coral reefs have sometimes been referred to as the equivalent of a canary in a coal mine, an unmistakable warning that humankind is in the process of doing irreparable damage to the planet. Although it is certainly true that coral reefs are among the first victims of the combined onslaughts of local impacts and global change, it is worth

remembering that coral reefs represent far more than a “canary”. Whether the number is 0.5 million or 2 million or 10 million species, accepting the potential demise of the ecosystems that support perhaps 35% of the global marine diversity of the planet seems ill-advised at best. Even in strictly economic terms, the value of coral reefs has been assessed at approximately 30 billion US dollars annually (Conservation International 2008). This does not include the potential medical and industrial benefits associated with compounds yet to be discovered from reef organisms.

The loss of corals and the potential threat to biodiversity in general terms is well established, but we still remain largely ignorant of the details. The efforts of CReefs have provided a means to begin rapidly narrowing some of the key gaps in our knowledge. As noted above, conservation priorities are often based on what can be measured. Providing a reliable method that estimates biodiversity across space and through time is essential for motivating the public to protect biodiversity, for designing the specifics of marine protected areas, and for monitoring their effectiveness. In addition to assessing changes in diversity generally, these approaches can also be used to detect invasive species (see, for example, Godwin *et al.* 2008).

4.6.3 Ideas to move the goal posts

On land in general, and in particular for rainforests, two approaches have transformed our understanding of their diversity, both originating in the tropical forests of Panama. On the one hand, Erwin (1982) catapulted studies of rain-forest diversity forward by counting the total diversity of insects associated with 19 trees, and then extrapolating from these results to the world at large. Although Erwin’s estimates remain highly debated and the inspiration for continuing research, the basic principle is sound – an intensive survey of a limited area. The closest analogue to the Erwin approach, amplified by the use of molecular methods and a broader taxonomic and spatial base, is the Moorea Biocode Project. The second approach is the establishment of the Center for Tropical Forest Science, which involves the repeated census of trees greater than 5 cm diameter at breast height in 50-hectare forest plots now scattered around the world. This provides a different kind of data, more superficial in terms of diversity estimates in any one location, but with the potential to monitor differences and similarities across space and through time. In the sea, no comparable program exists; the establishment of such a program would be transformative.

4.6.4 Blueprint for the future

We will never know every life form that lives in the oceans, or on coral reefs. However, with standardized

sampling at many locations and intensive sampling at a few, both made newly practical by molecular techniques, we can extrapolate from what we know to reefs at large. This approach involves far more than a static inventory of DNA sequences. By selecting sites strategically, we can address specific questions about factors responsible for biodiversity patterns that have challenged scientists for decades. Repeated samples will detect biodiversity loss and change and the arrival and impact of invasive species. Conservation biologists will know where biodiversity is concentrated, where it is disappearing, and where it is most resilient.

A blueprint for the future would thus consist of the following elements.

- 1) A global array of ARMS sites. At any site, these are currently deployed in three groups of three: three closely spaced ARMS constitute a replicate set, and three sets of three in one general location can be used to assess very fine-scale replicability of results. If comparisons of ARMS deployed for one versus two versus three years are desired, then three sets of the array of nine are needed. At broader scales, the results of Meyer *et al.* (2005) clearly indicate that every archipelago should have at least one set of nine (or 27 for multi-year analyses). As part of the international OceanObs ’09 conference, systematic assessments and monitoring of biodiversity using standardized ARMS were included in Community White Papers on global ocean observing systems for coral reef ecosystems (Brainard *et al.* 2010) and ocean acidification (Feely *et al.* 2010).
- 2) Two or three all-taxa inventories, on the model of the Moorea Biocode Project but with the addition of bringing a sequencer on site to analyze everything as it comes ashore. These are essential to ground-truth the ARMS, that is, to determine the relation of diversity measured by ARMS versus diversity measured by much more intensive efforts (which cannot be practically implemented at many sites or many times).
- 3) An ARMS biodiversity database. Sequence data, including environmental genomics data, can be stored in GenBank, but many additional data are associated with ARMS. Moreover, if ARMS are placed in permanently established quadrats, essentially small versions of 50-hectare forest plots, the sequence data become connected with other ecological data, greatly enhancing the value of the diversity measures. Fifty-hectare plots have stimulated many other ecological observations and experiments, and ARMS themselves could be elaborated upon, and even be subjected to manipulations designed to test hypotheses.

4.7 Conclusions

Coral reefs are enormously diverse marine ecosystems, perhaps harboring one-quarter to one-third of all marine species. Most coral reef species are undescribed, and will remain so for the foreseeable future. Because coral reef diversity is so hard to measure in any comprehensive fashion, almost all previous attempts have focused on a very limited number of taxonomic groups, or have been very limited in geographic scope. This has resulted in our knowledge of coral reef diversity being based on groups that do not represent most of the diversity on reefs (for example, especially fishes and corals) or on a patchwork and idiosyncratic set of broader analyses that are almost impossible to compare with one another.

Molecular approaches, applied systematically on a global scale, offer reef scientists the ability for the first time to assess coral reef diversity. The key to success will be to use techniques that can quickly survey a representative fraction of biodiversity, and to use these techniques across a dense global sampling grid. CReefs has developed autonomous reef monitoring structures (ARMS) that can be deployed, recovered, and analyzed by next generation sequencing methods in a cost-effective way. When combined with more intensive ground-truthing surveys at a limited number of sites, these methods will allow us to better understand diversity patterns generally, and to evaluate, monitor, and enhance the effectiveness of management strategies for coral reef ecosystems.

Acknowledgments

We thank the scientists from around the world who took part in the numerous workshops and meetings held to determine priorities and implementation for the CReefs Project. We acknowledge those who volunteered their time and resources for the coral reef censuses in the Northwestern Hawaiian Islands Marine National Monument and Australia, joined forces to create globally standardized protocols for ARMS and DNA sequencing, and assisted with the processing and identification of specimens. Special thanks go to Gustav Paulay, Scott Godwin, Joel Martin, Philippe Bouchet, Christopher Meyer, Amy Driskell, Andrea Ormos, Tito Lotufo, and in particular, Ian Poiner and Jesse Ausubel, for their guidance and support throughout the CReefs mission. Our appreciation further goes out to Vaiarii Terorotua, Florent Angly, Mary Wakeford, Shawn Smith, Penny Dockry, the NOAA Pacific Islands Fisheries Science Center, University of Hawaii Joint Institute for Marine and Atmospheric Research, and the University of Hawaii Institute of Marine Biology for their extensive field, outreach, and administrative support. We gratefully acknowledge the Alfred P. Sloan Foundation, the Gordon and Betty Moore

Foundation, the Moore Family Foundation, the National Geographic Society, the Australian Institute of Marine Science, the Great Barrier Reef Foundation, NOAA's Coral Reef Conservation Program and Pacific Region Integrated Data Enterprise, the Scripps Institution of Oceanography, and the National Museum of Natural History for their resource and monetary support of the CReefs efforts.

References

- Bellwood, D.R., Hughes, T.P., Folke, C., *et al.* (2004) Confronting the coral reef crisis. *Nature* **429**, 827–833.
- Bouchet, P. (2006) The magnitude of marine biodiversity. In: *The Exploration of Marine Biodiversity: Scientific and Technological Challenges* (ed. C.M. Duarte), pp. 32–64. Madrid, Spain: Fundación BBVA.
- Bouchet, P., Lozouet, P., Maestrati, P., *et al.* (2002) Assessing the magnitude of species richness in tropical marine environments: exceptionally high numbers of molluscs at a New Caledonia site. *Biological Journal of the Linnean Society* **75**, 421–436.
- Brainard, R., Bainbridge, S., Brinkman, R., *et al.* (2010) An international network of coral reef ecosystem observing systems (I-CREOS). In: *Proceedings of OceanObs'09: Sustained Ocean Observations and Information for Society (Vol. 2), Venice, Italy, 21–25 September 2009* (eds. J. Hall, D.E. Harrison, D. Stammer). ESA Publication WPP-306.
- Bruno, J.F. & Selig, E.R. (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS ONE*, **8**, e711.
- Cairns, S.D. (1999) Species richness of recent Scleractinia. *Atoll Research Bulletin* **459**, 1–46.
- Carpenter, K.E., Abrar, M., Aeby, G., *et al.* (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**, 560–563.
- Chapman, A.D. (2009) *Numbers of Living Species in Australia and the World*, 2nd edn. Australian Biological Resources Study, Department of the Environment, Water, Heritage and the Arts.
- Chesher, R.H. (1969) Destruction of Pacific corals by the sea star *Acanthaster planci*. *Science* **165**, 280–283.
- Conservation International (2008) *Economic Values of Coral Reefs, Mangroves, and Seagrasses: A Global Compilation*. Arlington, Virginia: Center for Applied Biodiversity Science, Conservation International.
- De'ath G., Lough J.M. & Fabricius K.E. (2009) Declining coral calcification on the Great Barrier Reef. *Science* **323**, 116–119.
- Erwin, T.L. (1982) Tropical forests: their richness in Coleoptera and other arthropod species. *The Coleopterists Bulletin* **36**, 74–75.
- Feely, R.A., Fabry, V.J., Dickson, A., *et al.* (2010) An international observational network for ocean acidification. In: *Proceedings of OceanObs'09: Sustained Ocean Observations and Information for Society (Vol. 2), Venice, Italy, 21–25 September 2009* (eds. J. Hall, D.E. Harrison, D. Stammer). ESA Publication WPP-306.
- Gardner, T.A., Côté, I.M., Gill, J.A., *et al.* (2003) Long-term region-wide declines in Caribbean corals. *Science* **301**, 958–960.
- Glynn, P.W. (1993) Coral reef bleaching: ecological perspectives. *Coral Reefs* **12**, 1–17.
- Godwin, S., Harris, L., Charette, A., *et al.* (2008) The marine invertebrate species associated with the biofouling of derelict fishing gear in the Pāpāhānaumokuākea–Marine National Monument: A focus on marine non-native species transport. Hawaii Institute for Marine Biology, Kaneohe, HI, 26 pp.

- Hebert, P.D.N., Cywinska, A., Ball, S.L., *et al.* (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270, 313–321.
- Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., *et al.* (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318, 1737–1742.
- Hughes, T.P. (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science* 265, 1547–1551.
- Idjadi, J.A. & Edmunds, P.J. (2006) Scleractinian corals as facilitators for other invertebrates on a Caribbean reef. *Marine Ecology Progress Series* 319, 117–127.
- Jackson, J.B.C. (2008) Evolution and extinction in the brave new ocean. *Proceedings of the National Academy of Sciences of the USA* 105 (Suppl. 1), 11458–11465.
- Kleypas, J.A., Feely, R.A., Fabry, V.J., *et al.* (2006) *Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research*. Washington, DC: NSF, NOAA, and the US Geological Survey.
- Knowlton, N. (2006) Coral reef coda: what can we hope for? In: *Coral Reef Conservation* (eds. I. Côté & J. Reynolds), pp. 538–549. Cambridge, UK: Cambridge University Press.
- Knowlton, N. & Jackson, J.B.C. (1993) Inbreeding and outbreeding in marine invertebrates. In: *The Natural History of Inbreeding and Outbreeding* (ed. N.W. Thornhill), pp. 200–249. Chicago, Illinois: University of Chicago Press.
- Knowlton, N. & Jackson, J.B.C. (2008) Shifting baselines, local impacts, and global change on coral reefs. *PLoS Biology*, 6, e54.
- Knowlton, N. & Weigt, L.A. (1997) Species of marine invertebrates: a comparison of the biological and phylogenetic species concepts. In: *Species: the Units of Biodiversity* (eds. M.F. Claridge, H.A. Dawah, M.R. Wilson). *Systematics Association (UK) Special Volume Series* 54, pp. 199–219. London: Chapman and Hall.
- Knowlton, N., Weigt, L.A., Solorzano, L.A., *et al.* (1993) Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260, 1629–1632.
- Lessios, H.A. (2008) The Great American Schism: Divergence of marine organisms after the rise of the Central American Isthmus. *Annual Review of Ecology, Evolution and Systematics* 36, 63–91.
- May, R.M. (1988) How many species are there on Earth? *Science* 241, 1441–1449.
- May, R.M. (2004) Tomorrow's taxonomy: collecting new species in the field will remain the rate limiting step. *Philosophical Transactions of the Royal Society of London B* 359, 733–734.
- McKinney, M.L. (1998) Is marine biodiversity at less risk? Evidence and implications. *Diversity and Distributions* 4, 3–8.
- Meyer, C.P., Geller, J.B. & Paulay, G. (2005) Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. *Evolution* 59, 113–125.
- Mora, C., Chittaro, P.M., Sale, P.F., *et al.* (2003) Patterns and processes in reef fish diversity. *Nature* 421, 933–936.
- Pandolfi, J.M. (2002) Coral community dynamics at multiple scales. *Coral Reefs* 21, 13–23.
- Pandolfi, J.M., Bradbury, R.H., Sala, E., *et al.* (2003) Global trajectories of the long-term decline of coral reef ecosystems. *Science* 301, 955–958.
- Paulay, G. (2003) Marine biodiversity of Guam and the Marianas: overview. *Micronesica* 35–36, 3–25.
- Pacific Island Fisheries Science Center (PIFSC) (2007) Oscar Elton Sette/Cruise OES-06-11 (OES-47). U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS), PIFSC Cruise Report CR-07-007, pp. 11–36. Honolulu: NOAA NMFS PIFSC.
- Plaisance, L., Knowlton, N., Pauley, G., *et al.* (2009) Reef-associated crustacean fauna: biodiversity estimates using semi-quantitative sampling and DNA barcoding. *Coral Reefs* 28, 977–986.
- Puillandre, N., Strong, E.E., Bouchet, P., *et al.* (2009) Identifying gastropod spawn from DNA barcodes: possible but not yet practicable. *Molecular Ecology Resources* 9, 1311–1321.
- Reaka-Kudla, M.L. (1997) The global biodiversity of coral reefs: a comparison with rain forests. In: *Biodiversity II: Understanding and Protecting Our Biological Resources* (eds. M.L. Reaka-Kudla, D.E. Wilson & E.O. Wilson), pp. 83–108. Washington, DC: Joseph Henry Press.
- Reaka-Kudla, M.L. (2005) Biodiversity of Caribbean coral reefs. In: *Caribbean Marine Biodiversity: The Known and the Unknown* (eds. P. Miloslavich & E. Klein), pp. 259–276. Lancaster, Pennsylvania: DEStech Publications.
- Roberts, C.M., McClean, C.J., Veron, J.E.N., *et al.* (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* 295, 1280–1284.
- Sandin, S.A., Smith, J.E., DeMartini, E.E., *et al.* (2008) Baselines and degradation of coral reefs in the northern Line Islands. *PLoS ONE* 3, e1548.
- Shearer, T.L. & Coffroth, M.A. (2008) Barcoding corals: limited by interspecific divergence, not intraspecific variation. *Molecular Ecology Resources* 8, 247–255.
- Small, A.M., Adey, W.H. & Spoon, D. (1998) Are current estimates of coral reef biodiversity too low? The view through the window of a microcosm. *Atoll Research Bulletin* 458, 1–20.
- Strathmann, R.R. (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics* 16, 339–361.
- Vroom, P.S., Page, K.N., Peyton, K.A., *et al.* (2006) Marine algae of French Frigate Shoals, northwestern Hawaiian islands: species list and biogeographic comparisons. *Pacific Science* 60, 81–95.
- Wehrtmann, I.S., Cortés, J. & Echeverría-Sáenz, S. (2009) Marine biodiversity of Costa Rica: perspectives and conclusions. In: *Marine Biodiversity of Costa Rica, Central America* (eds. I.S. Wehrtmann & J. Cortés), pp. 521–533. Springer.
- Williams, S.T. & Knowlton, N. (2001) Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution* 18, 1484–1493.
- Zimmerman, T.L. & Martin, J.W. (2004) Artificial Reef Matrix Structures (ARMS): an inexpensive and effective method for collecting coral reef-associated invertebrates. *Gulf and Caribbean Research* 16, 59–64.