Chapter 11

Sampling the Marine Realm

by

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Abstract

The marine environment is the largest ecosystem in the world and includes a vast array of habitats. Except for the Micrognathozoa and Onychophora, all animal phyla are represented in the marine realm. We comment briefly on the most commonly used sampling methods for the study of pelagic and deep-sea benthic biodiversity, but focus on sampling methods of marine benthic biodiversity in coastal areas, because >75% of known marine species are from these waters. To gain an accurate idea of the magnitude of species richness, massive collecting efforts are necessary. It is more effective to concentrate on relative small areas (100-300 km²) where diverse habitats are present, than to spread studies across extensive zones. Discrete, representative stations based on macrohabitats should be selected within the sampling area, and each station sampled by intertidal collecting, scuba diving and/or dredging. At each station complementary techniques should be deployed, including hand picking (to collect sessile and large motile species and pieces of substratum), suction sampling, brushing rocks or rubble for epibenthos, breaking hard substrates for endolithic organisms, hand-towed nets for motile species, sieving, and dredging. Rubble brushing and suction sampling have been the most effective methods for collecting small species (the major component of the marine benthic biodiversity) on hard substrates. Special techniques are required to study certain taxa, especially fragile, rare, symbiotic, or minute interstitial organisms.

Key words: sampling methods, biodiversity, coastal area

1.   Introduction

The marine environment is the largest ecosystem in the world and includes a vast array of habitats. Most of the planet (71% of the world's surface) is covered by ocean waters, with an average depth of ~3,800 m. Oceans thus hold an overall volume of some 1.370×10^6 km³ (97% of the water on the planet) capable of supporting life. However, surprisingly, species diversity appears to be far lower in the sea (around 250,000 known species) than on land (between 1.4 and 1.7 million known species), probably because dispersal is more wide-ranging in water than on land and genetic connectivity is maintained over vast expanses (but see Paulay & Meyer, 2006). This may be partly the result of broader geographic ranges and consequently lower rates of speciation for marine versus terrestrial species. Furthermore marine environments are physically much less variable in space and time than terrestrial ones. Finally, the most diverse group of macroorganisms, the insects (within the animal kingdom) and the angiosperms (within the plant kingdom) are largely restricted to terrestrial and freshwater environments. Nevertheless, the diversity of major lineages (phyla and classes) is much greater in the sea than on land or in freshwater, reflecting the ocean as the cradle of life.

Of the 34 currently recognized animal phyla (Table 1), all except two occur in oceanic waters: 16 are exclusively marine; 16 occur in both marine and freshwater, while only one phylum is exclusive to freshwater (Micrognathozoa), and one restricted more or less to land (but with marine fossil record: Onychophora). Many exclusively marine animal phyla are relatively obscure and have few species. The major exceptions is the Echinodermata, with 7,000 described species. A number of other major animal phyla including the cnidarians, sponges, as well as the non-metazoan brown and red algae (Phaeophyta and Rhodophyta, respectively) are largely marine, each with only a small number of non-marine (usually freshwater) representatives. A summary for the world view of species has been published by Chapman (2009), while a complete review of marine species was given by Bouchet *et al.* (2006).

Although knowledge of marine biodiversity has increased enormously in the past few decades, marine life remains far less well documented than terrestrial biodiversity. The main reason is that most of the marine biosphere is difficult to access. The oceans are tantalizing from the shorelines, but their great depths and remote reaches make them challenging to study. Study of any part below the top few meters requires specialized equipment and is expensive and time consuming. Knowledge of most of the sea is thus based on remote-sensing and sampling techniques, and remains limited and less precise. As these techniques become more sophisticated, so does our understanding of marine ecosystems, especially for areas away from the coastal zone.

Although research on biodiversity has greatly increased in recent decades, these efforts are dominated by studies on terrestrial environments. Between 1987 and 2004, only 9.8% of published research dealt with marine biodiversity (Hendriks *et al.*, 2006). This severe imbalance is also evident in international programs.

Table 1. Extant Animal Phyla.

Phylum	Notes	Marine	Holoplanktonic members
Porifera	sponges	yes	no
Placozoa	Tricoplax	only	no
Cnidaria	hydroids, jellyfish, anemones, corals	yes	yes
Myxozoa	aff. Cnidaria?	yes	as parasites
Ctenophora	comb jellies	only	yes
Orthonectida	"Mesozoa"	only	no
Dicyemida	"Mesozoa"	only	no
Chaetognatha	arrow worms	only	only
Platyhelminthes	flatworms, polyphyletic?	yes	yes
Gastrotricha	minute worms	yes	yes – semi-pelagic
Entoprocta	= Kamptozoa	yes	no
Gnathostomulida	minute "jaw" worms of hypoxic habitats	only	no
Rotifera	=Syndermata, incl. Acanthocephala	yes	yes
Micrognathozoa	Microscopic worms, Limnognathia	no	no
Cycliophora	lobster lip worms, Symbion	only	no
Nemertea	ribbon worms	yes	yes
Sipuncula	peanut worms	only	no
Annelida	segmented worms, incl. Pognophora & Echiura	yes	yes
Mollusca	Snails, clams, chitons, squid	yes	Yes
Phoronida	horseshoe worms	only	no
Bryozoa	= Ectoprocta, moss animals	yes	no
Brachiopoda	lamp shells	only	no
Nematoda	round worms	yes	as parasites
Nematomorpha	horse hair worms	yes	no
Kinorhyncha	minute "mud dragons"	only	no
Priapula	carnivorous worms	only	no
Loricifera	"girdle-wearers", minute	only	no
Tardigrada	water bears	yes	no
Onychophora	velvet worms	no	no
Arthropoda	Insects, myriapods, crustaceans, spiders, incl. Pentastomida	yes	yes
Xenoturbellida	Xenoturbella	only	no
Echinodermata	stars, urchins, sea cucumbers	only	yes
Hemichordata	acorn worms	only	no (presumably)
Chordata	tunciates, vertebrates	yes	yes

For instance, only about 10% of the First Open Science Conference of the Diversitas Programme (November 2005 in Mexico) that dealt with biodiversity science, addressed marine biodiversity (Hendriks *et al*., 2006). This disproportionally small research effort on marine biodiversity is in sharp contrast

to the large phyletic diversity in the oceans compared to land. The phyletic and genomic richness of the ocean also remains an underutilized resource for biotechnology, pharmacology, and other resources.

The global inventory of the marine realm is far from complete, especially for minute and rare species, and commensals and parasites, which together represent the largest number of species in complex ecosystems (Bouchet *et al.,* 2009). Besides, a rich fauna of some neglected habitats still remains overlooked (Denis & Alfhous, 2004; Mendoza *et al.*, in press). Despite this deficit, most integrated studies on marine biodiversity focus on a few well-known indicator taxa (fishes, corals), neglecting most other groups, often because of a reputation of being too diverse or difficult for non-specialists. Nevertheless, close to 1,800 new marine species are described each year (Bouchet *et al*., 2002).

The aims of this chapter

A complete review of methods for the study of all marine biodiversity is outside the scope of this chapter. The most commonly used sampling methods for the study of the pelagic and deep-sea benthic biodiversity are commented upon briefly, and we focus on sampling methods for marine benthic biodiversity in coastal areas, where >75% of recorded marine biodiversity is concentrated. Microscopic organisms are also outside the scope of this chapter. We principally focus on the study of marine metazoans and macroscopic seaweeds.

2. Pelagic Biodiversity

The oceanic pelagic zone is dominated numerically by plankton in euphotic surface waters. Plankton are by definition drifting or weakly swimming organisms, and include a wide range of small to microscopic animals, protists and bacteria. Free-swimming pelagic organisms are collectively termed nekton. Both tend to concentrate along major circulation currents (gyres), contact zones and upwelling regions, and this causes significant local variations in abundance and diversity.

The marked vertical gradients of light, temperature, pressure, nutrient availability and salinity within the pelagic realm create vertical structuring of pelagic species assemblages into several depth zones that tend to fluctuate in time and space. Some components of the epipelagic and mesopelagic nekton and even plankton perform remarkable diel migrations: ascending to surface waters at night to feed and descending, sometimes over 1 km, during the day (Groombridge & Jenkins, 2002). With few exceptions, the only food source for organisms in the aphotic zone is the 'rain' of organic matter (faeces, moulted crustacean exoskeletons, corpses) from the euphotic zone.

2.1. Plankton

Plankton refers to the assemblage of passively floating, drifting, or somewhat motile organisms occurring in the water column, primarily comprising bacteria, protists, tiny algae, small animals, and developmental stages (eggs, larvae, etc) of larger organisms. Planktonic organisms range in size from microbes (under 0.001 mm) to jellyfish with gelatinous bells >1 m in diameter and tentacles up to

10 m long. Plankton can be loosely grouped as producers (phytoplankton, including prokaryotic and eukaryotic algae) and consumers (zooplankton as well as heterotrophic bacteria and protists). Many protists are both producers and consumers, and may account for a large proportion of primary production.

Planktonic assemblages are strongly affected by physical and chemical characteristics of water masses on scales ranging up to entire ocean circulations. The vertical structure of the water column is also important, especially the depth of the mixed layers, as this influences nutrient and light levels that control phytoplankton growth and assemblage composition. Although plankton is most abundant in the photic zone, it is found at all depths. At least 40% of the world's primary production occurs in the open ocean, and much of this production is initially consumed by planktonic crustaceans (mainly copepods). These organisms are relatively well studied, and many have been assumed to be cosmopolitan.

In surprising contrast to their globally high biomass and productivity, the diversity of planktonic organisms is low, with only ~3,700 described species of holoplanktonic zooplankton (Groombridge & Jenkins, 2002). This has been attributed to the dynamic mixing of oceans limiting geographic differentiation. Nevertheless most animal phyla are represented in the plankton as many benthic species have a planktonic larval phase. Zooplankton is dominated numerically and in total mass by animals that spend their entire lives as plankton. Such animals are termed holoplankton, while temporary residents of the plankton (such as eggs and larval forms) are called meroplankton. Of the 34 marine animal phyla only 13 have representatives in the holoplankton (Table 1).

Sampling methods

There are many comprehensive books on sampling methods for plankton (*e.g.* UNESCO, 1968; Harris *et al*., 2000; Goswami, 2004; Suthers & Rissik, 2009, among many others). Towed nets are still the primary means of collecting many plankters. Plankton nets vary in size, shape and mesh size but all are designed to capture drifting or relatively slow-moving organisms retained by the mesh. The simplest nets are conical in shape, with a wide mouth opening attached to a metal ring and a narrow tapered end fastened to a collecting jar known as the "cod end". This kind of net can be towed vertically, horizontally, or obliquely through the desired sampling depths. Such nets will filter water and collect organisms during the entire towing period. More sophisticated nets can be opened and closed at selected depths, and a series of such nets may be attached to a single frame to allow sampling of different discrete depths during a single towed operation. Analyses of the collected samples permit a more detailed picture of the vertical distribution of plankton.

Zooplankton pumps can also be used; these pull water from a selected depth and pass it through a mesh. The Moored Automated, Serial, Zooplanktic Pump (MASZP) is designed to make moored, time-series collections of small planktonic species.

Each discrete plankton sample is usually filtered over a portion of mesh, which is covered by another piece of mesh, and the two strips are wound together on a

spool residing in a preservative bath for *in situ* storage. The material collected is later washed from the mesh, and the organisms sorted by hand for microscopic identification. To expedite sample processing new technologies have been developed for recognizing species in mixed populations through species-specific immunofluorescent markers (Garland & Butman, 1996).

In recent decades attempts have been made to observe zooplankton directly in the field, by scuba diving or from submersibles. Such direct sampling has enabled the collection of delicate species, especially large-bodied jelly-plankton (colonial radiolarians, medusae, ctenophores, salps, etc.) that were undersampled or destroyed using traditional methods, but are important components in pelagic environments. Recent development and refinement of acoustic and optical technology has also enabled better quantitative estimates of biomass and the distribution of the more mobile members of the plankton. Many of the holoplanktonic species can be identified by acoustic or optical images. Autonomous sampling buoys, autonomous underwater vehicles (AUV, essentially oceanographic robotic systems), autonomous surface vehicles, gliders, drifters, among other, are also being used in the study of the plankton.

New instruments, such as the Video Plankton Recorder (an underwater video microscope attached to a Remotely Operated Vehicle) are bringing new insights to the study of these small animals. Today global-scale analytic methods for all marine zooplankton groups are being developed using new technologies, including molecular, optical and acoustical imaging, and remote detection. By 2010 the coordinated multinational effort Census of Marine Zooplankton (http://www.cmarz.org; within the Census of Marine Life) seeks to complete both the morphological and DNA barcode analyses of at least the ~6,800 described species of marine metazoan and protozoan plankton. DNA barcoding is underway in laboratories in Japan and the USA (O'Dor & Gallardo, 2005), including DNA barcoding of existing specimens in collections as well as identified cryptic species among cosmopolitan groups. The Census of Marine Zooplankton will provide the first global synthesis of the biodiversity and biogeography of the species that make up the greatest animal biomass on the planet. It is likely to double the number of known zooplankton species and will provide DNA barcodes for their reliable and rapid identification. On the other hand, Venter *et.al.* (2004) identified at least 1,800 new species of microbes using "whole-genome shotgun sequencing" to microbial populations of the Sargasso Sea.

2.2. Nekton

The nekton comprises the large, pelagic, marine animals able to move independently of water currents. Fish make up the largest fraction of the nekton, but some crustacean (some euphausiids, shrimps, and swimming crabs), many cephalopods (such as squids), marine turtles, and marine mammals are also important nektonic components. There are ~1,200 nektonic fish species compared with ~13,000 coastal ones, >300 species of nektonic cephalopods, and five species of marine turtles (Angel, 1993). Wholly aquatic mammals are confined to two orders, the Cetacea and the Sirenia. The cetaceans comprise some 78 species, all except five marine, distributed throughout the world's seas.

It has generally been assumed that pelagic biomass below the euphotic zone is low. Recent studies based on a variety of surveys have indicated that the global biomass of tropical mesopelagic animals may be surprisingly high (Groombridge & Jenkins, 2002). Around 160 fish genera in 30 families are recognized as important components of the mesopelagic fauna (usually small species less than 10 cm in length).

Study of the pelagic fauna requires the use of expensive high-seas research vessels (Figure 1A). The sampling methods are mainly those employed in fisheries and oceanography. In fact, nektonic species are usually studied within the branch of marine science that is called "fisheries oceanography". There is an extensive bibliography and entire journals (*e.g. Fish Biology and Fisheries*) devoted to this discipline. Tagging and real-time tracking of many large pelagic animals using new technologies are making it possible to provide unprecedented estimates of the global distribution and abundance of the largest animals in this realm.

A review of the techniques to study this pelagic fauna is outside the scope of this chapter. We refer to comprehensive publications on the subject such as those of Roper & Rathjen (1991), Sibert & Nielsen (2001), Gabriel *et al.* (2007), among many others.

3.   Deep-sea biodiversity

Around 50% of the Earth's surface is covered by ocean >3,000 m deep. Despite their enormous volume, the deep oceans were initially thought to be relatively simple ecosystems that made little contribution to global species diversity. However thorough quantitative samples of infauna have shown that deep sea is surprisingly species rich, even rivalling the diversity of coral reefs (Grassle & Maciolek, 1992). As more of the deep-sea is surveyed with increasingly sophisticated gear, it is apparent that the environment itself, in terms of substrate features and/or current regime, is more variable than was once thought. Environmental diversity in the form of microhabitats (small areas having slightly different characteristics) can in itself lead to higher diversity in animals. Indeed, the deep-sea benthos has a patchy distribution, with significant aggregations of animals that have been detected in different taxonomic groups on scales ranging from centimeters and meters to kilometers. This patchy distribution makes representative samples difficult to obtain for assessing biomass and species diversity of deep-sea animals. In addition, discoveries during the past decades have shown that there are some deep habitats with unusual benthic diversity, such as seamounts and rock outcrops, submarine canyons, beds of manganese nodules, deep-water reefs of ahermatypic corals, hydrothermal vents, cold seeps, and other chemosynthetic ecosystems such whale skeletons or sunken wood.

Sampling methods

Open-sea and deep-water work imposes procedures substantially different from those required for near-shore surveys. Deep-sea sampling is costly and time-consuming. Collecting a sample from 8,000 m depth with towed gear, for

example, requires a very large winch with at least 11 km of cable in order to allow for the towing angle. It takes up to 24 hours to let out that much wire, obtain a sample, and retrieve it. Cost of shiptime can easily exceed 20,000 € per day.

Fig. 1. Surface based sampling. A. French research vessel Alis of the IRD center; B. Dredge haul off the coast of Cortes; C. Trawl haul from the deep; D. Off shore plankton tow. (Photo A - C by Panglao Marine Biodiversity Project 2004; D by Chris Meyer).

Holmes & McIntire (1984), also provide a guide to relevant publications on the study of marine benthos until 1984, while Gage & Tyler (1991) review methods to study organisms of the deep-sea floor, giving detailed description of traditional gears and sampling techniques. Wenneck *et al.* (2008) review recent technological advances. An overview of organization and procedures of a survey of the deep-water fauna is given by Richter de Forges *et al.* (2009).

Comprehensive surveys have utilized trawls, bottom sledges, dredges, grabs, box samplers and corers, as well as a variety of acoustic and optical approaches. Large trawls and nets give snapshots of life sampled across a mile or longer stretch of bottom. In contrast box cores deployed from surface vessels provide samples that are precisely spaced and come from a single spot. A specialized deep-sea fauna living in the lowest strata of the water column are bottom-dependent, swimming animals that may perform daily or seasonal vertical migrations above the bottom, the supra- or hyper-benthos. Suprabenthic fauna essentially consists of crustaceans from the superorder Peracarida (amphipods, cumaceans, isopods and mysids). The suprabenthic sled was designed for such

near-bottom sampling operations with a number of nets that fish at different heights above the substrate.

Sampling the deep-sea benthos from surface ships does not provide a close-up view of the system. To understand relationships of organisms with the environment *in situ* studies are useful, as well as the ability to return to the same spot. This can be achieved with manned or unmanned vehicles equipped with precise navigational capabilities and visually operated sample manipulators and/or video recorders. Submersibles or remotely operated vehicles (ROVs) are the only way to: 1) precisely sample small-scale features such as sediment forms, rocks, or individual organisms; 2) sample repeatedly with respect to specific experiments or features of the bottom over time spans up to several years; 3) push sampling devices and other instruments into the bottom without disturbance of the sediment-water interface; 4) locate objects and sample in complex rocky topography where tethered devices could not move over the bottom without encountering obstacles; 5) sample specific layers in the water column; and 6) sample delicate organisms that are destroyed by traditional sampling gear.

Submersible-based sampling was accelerated with the use of the *Alvin* by the United States and *Archimède* and *Cyana* by France during the French-American Mid-Ocean Underwater Study (FAMOUS) project in the 1970's (Heirtzer & Grassle, 1976). The discovery in 1978 of new and abundant sea life around deep-sea hydrothermal vents near the Galapagos Islands greatly increased research in this special environment as well as the use of manned submersibles. Most submersibles require a mother ship to assist in moving it to the dive location and for recharging energy sources, checking equipment, and housing diving personnel. In a normal operating dive a deep-sea submersible will stay submerged for 6 to 10 hours, in waters up to 3 km deep (the rate of ascent and descent is about 2 km/hour). It can move over the bottom at a speed of 1 to 2 knots and can cover a path of several kilometres.

Several types of unmanned, remotely operated vehicles (ROVs) or autonomous underwater vehicles (AUVs) can carry a variety of recording equipment to document deep sea organisms. These can be remotely operated from surface vessels, or pre-programmed to do their jobs independently of direct human control. Some have manipulators that are able to take samples. Clarke (2003), Chave (2004), or Divas (2004), among many others provide glimpses of deep sea exploration by submersibles.

4. Benthic biodiversity in coastal areas

Marine biodiversity is much higher in benthic than pelagic systems, and is also thought to be higher in coastal waters rather than in the open/deep sea, since there is greater range of habitats near the coast (but see Grassle & Maciolek, 1992). Continental shelves cover <10% of the ocean's area, but contain most of the documented marine biodiversity. In fact, more than 75% of known marine species are concentrated in coastal areas, especially in the tropical regions (Bouchet, 2006). For this reason and because key coastal habitats are lost globally at rates 2 to 10 times faster than those in tropical forests (Reaka-Kudla,

1997), special attention and effort must be paid to their study and conservation. The highest coastal marine species diversity is in the Indo-Malayan archipelago and decreases both longitudinally and latitudinally from there (Hoeksema, 2007).

Representative coastal benthic habitats include:

- **Mangroves -** Mangroves are a "hybrid" terrestrial/marine ecosystem, unique in that terrestrial organisms occur in the canopy and marine species at the base (Figure 2). Mangroves, or mangals, are a diverse collection of shrubs and trees that live rooted in soft, intertidal marine sediments. Mangroves dominate deltaic and low coastal areas, and are restricted to the tropics and subtropics. Global area occupied by mangroves slightly exceeds 180,000 km^2, covering 60-70% of the tropical and subtropical coastline (Groombridge & Jenkins, 2002).

- **Coral reefs -** Coral reefs are accumulations of solid calcium carbonate matrix developed by stony corals and co-occurring organisms. Coral reefs are tropical shallow water ecosystems, typically with very high biodiversity, although they are also known (but are more limited and less diverse) in some deep and high latitude environments. They dominate shallow, clear, warm, nutrient-poor waters with limited terrestrial sediment runoff in the tropics. The global extent of coral reefs has been estimated at around 285,000 km² (Groombridge & Jenkins, 2002).

- **Seagrass meadows -** Seagrasses are flowering plants adapted to shallow marine and estuarine environments across a wide range of latitudes. About 58 living species are recognized. They occur from the littoral region to depths of 50 or 60 m and cover extensive areas on shallow soft substrates. Globally seagrass beds cover between 200,000 and 500,000 km² of the continental shelves (Spalding *et al.*, 2003).

- **Rocky bottoms -** Rocky substrates can be of biological or geological origin. The former are referred to as reefs and include coral reefs, the latter are characteristic of tectonically active areas such as convergent margins and volcanic islands. Rocky bottoms provide considerably physical complexity and tend to harbour diverse biota. Different habitats and complex communities are usually identifiable and can be characterized according to a combination of physical and biological attributes.

- **Kelp forests -** Kelp forests are subtidal macro-algal communities dominated by kelps (large brown algae of several genera, including *Laminaria*, *Saccorhiza*, *Ecklonia* and *Macrocystis*) in cold temperate to subtropical regions. They form distinctive lower intertidal to shallow subtidal communities, especially in areas with currents or surf. Kelps usually require hard bottom for attachment, and grow off rocky shores to depths of 20-40 m. The net primary production of kelp forests is comparable to tropical rainforests.

- **Soft sediments -** Soft sediments are the most widespread coastal marine ecosystem type. Virtually the entire seabed away from the coastline is covered by marine sediments.

- ***Anchialine caves -*** Anchialine caves are defined as bodies of hyaline water with more or less extensive subterranean connections to the sea. They show noticeable marine as well as terrestrial influences. Such habitats include land-locked open pools, pools in caves, and entirely submerged cave passages, which are known to harbor a number of fascinating organisms, such as the primitive crustacean class Remipedia.

4.1. Planning

Most of the general methods and procedures described here for fieldwork in coastal marine areas are being deployed in the Moorea Biocode Project, an effort to build the first comprehensive, voucher-based, genetic inventory of all non-microbial life in a tropical ecosystem (http://www.mooreabiocode.org). These general methods and procedures are also those basically employed in a long term project conducted by a group led by Philippe Bouchet (National Museum of Natural History of Paris), the purpose of which is to address the magnitude of species richness in coral reefs and associated environments by selecting sites through the Indo-Pacific biodiversity gradient (see Bouchet *et al*., 2002), including surveys at Lifou in Loyality Islands, 2000, Rapa in southernmost French Polynesia, 2002, Koumac and Touho in New Caledonia, 1992, Panglao in the Philippines, 2004, and SE corner of Santo in Vanuatu Islands, 2006. While these large-scale biodiversity survey expedition(s) usually are carried out over a relatively short time interval, planning for them can take years of preparation, including obtaining permits, coordinating participant travel, etc. (for more information see the first chapter on the concept, challenges and solutions of planning an ATBI+M).

4.1.1. Choosing the area and stations

If the objective of an All Taxa Biodiversity Inventory is to maximize the potential biodiversity encountered, then the selected site should have high habitat heterogeneity. It is most effective to choose a relatively small coastal area (no more than 100-300 km^2) so that all of it is accessible within one hour from the field lab by boat or vehicle, at a location that includes the greatest diversity of habitats characteristic of the region. Covering more extensive areas from a shore-based field lab becomes logistically difficult and inefficient. The depth range surveyed should range from the intertidal fringe to about 50 m when limited to SCUBA, or to greater depths (*e.g.* to 100 m) when boat-based sampling via dredges, trawls, and grabs is available. A number of discrete sampling stations should be selected, spanning the range of habitats, at each of which a broad range of sampling techniques are utilized. Background information, including a planning visit to the area and preliminary sampling, are very useful in scoping out a region, selecting the survey site, as well as choosing some of the stations to be sampled.

Importantly, the selected site will need to have sufficient facilities and infrastructure: boats, support staff, diving support, meals for the participants, etc., in place by the time the project starts. It is necessary to establish a field lab in a

place from where teams can go sample (mainly in small boats) and return for the sorting process.

4.1.2. Defining the task

An ATBI is a tall order in the marine realm because of the diversity of organisms present. Thus it is important to define the taxonomic scope of the project and plan accordingly. It is not feasible to study all groups of organisms in an area. Most early integrated studies on tropical marine biodiversity have focused on a few indicator taxa (especially fishes and corals – both groups that live largely exposed and are thus visually immediately apparent) and neglected others, because of logistic constraints and sampling and taxonomic challenges. The greatest challenge for marine ATBIs is that most taxa live concealed, and this "cryptofauna" harbors most of the species richness. Useful additional taxa to include in a limited ATBI include seaweeds, sponges, octocorals, mollusks, decapods, polychaetes, bryozoans, echinoderms and tunicates.

These include most of the other macrobiota that lives exposed, as well as the large-bodied, conspicuous, or taxonomically relatively well-known groups. The Moorea Biocode project includes most taxa with a goal to cover most macrofauna (>10 mm), a good portion of mesofauna (1-10 mm), and explore microfauna (<1 mm).

In an ATBI, sampling at any station is normally qualitative or semi-quantitative, with collecting effort usually proportional to species richness and habitat heterogeneity as perceived empirically in the field. Quantitative sampling is not nearly as effective as qualitative sampling carried out by a specialist at capturing maximum biodiversity. For instance, in parallel studies of fore reef decapod diversity in Moorea, a semi-quantitative approach sampling replicate dead coral heads yielded 50 species, whereas specialized collecting in the same habitat over the same amount of time recovered 210 species, with 23 in common between the methods (Plaisance *et al.,* in press).

4.1.3. Building a team

A massive collecting effort requires a team composed of biodiversity specialists and support personnel. Participants need to include taxonomic experts, who contribute both by planning and participating in collecting thus contributing their expertise in finding species, as well as in sorting catches to morphospecies (see below). Support personnel help with field work (boats, diving, collecting), processing samples, photography, and the general operation of the expedition. Volunteers and students can help and gain as well as provide expertise, while local fisherman can provide field knowledge about habitats, organisms, and effective sampling methods. A major effort can easily include more than 50 participants.

4.1.4. Time required

About 500 person-days of field work can provide reasonable coverage for 2-4 phyla in a high diversity area. Typical efforts in such medium scale expeditions take 4-6 weeks of field work with 10-20 field workers. It is also useful to repeat such an effort in a different part of the year, because many organisms are annual or have seasonal cycles.

4.2. Sampling methods

Below we give an overview on general procedures for fieldwork in marine coastal areas. A detailed description of all gear and techniques is beyond the scope of this chapter and we recommend more specialized literature or a handbook on methods for the study of marine benthos, such as Holme & McIntire (1984). A useful picture on sampling design in a coastal area is provided by Bouchet *et al.* (2009)

4.2.1. Intertidal sampling

The coastal intertidal is a rich and easily accessible habitat, as no snorkelling or diving skills are required; thus most taxonomists can pursue field collecting there. Intertidal habitats include rocky shores, reef flats, sand and mud flats, beaches, seagrasses, and mangroves. Effective methods include visual searches for larger organisms that live exposed or under rocks, yabbie-pumping for burrowing species and their associates, digging and sieving for soft bottom infauna, sifting through algae, using baited traps, hand dredges, and/or examination of residues of rock/algal wash.
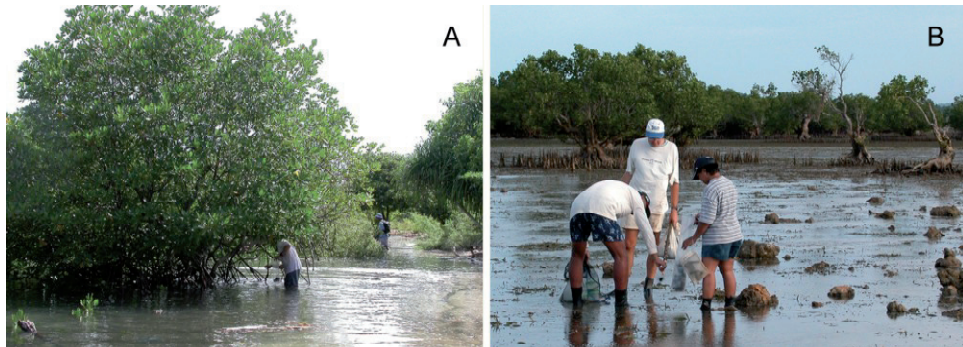

Fig. 2. Shore based sampling. A. Collecting in mangrove; B. intertidal sampling on mudflat and seagrass bed. (Photos by Panglao Marine Biodiversity Project 2004).

Most intertidal sampling happens at low tide. It is useful to also collect during night low tides, as many animals are nocturnally active, are buried in the sediment during the day, and much more easily found at night when they emerge. Tides during the new and full moon periods are the largest, but some habitats (high intertidal, estuarine, river/mangrove transition) do not require extreme tides to be properly sampled.

4.2.2. Underwater collecting

Scuba diving and/or snorkelling allow very selective sampling and choice of specific places and microhabitats. Underwater sampling is also very useful for observing species in their natural state and for obtaining more detailed information about the structure of benthic communities and other valuable ecologic data.

Fig. 3. Scuba based sampling. A. Underwater brushing for micromolluscs; B. Brushing rubble for cryptic species; C. Hand collecting among rubble and coral on forereef; D. Investigating gorgonians for associated mollusks; E. ARMS pre-deployment; F. Vacuum set-up; G. Vacuum suction in operation. (Photos A., D. & G. by Panglao Marine Biodiversity Project 2004; B. by Jenna Moore; C. by Sea McKeon; E. by Rusty Brainard; F. by Chris Meyer).

The most common methods used in diving are:

- **Hand collecting of motile species**. Larger motile organisms (>1 cm) are often best collected by hand, or by hand-held devices like nets or slurp guns. Although some motile species live exposed on the bottom and are readily encountered, others live concealed in the substratum. On hard bottoms turning loose rocks reveals a broad array of cryptofauna, as does searching in soft sediments by fanning or just by feeling with hands. Crevices and small caverns are also good places to search for cryptofauna. Night diving is very useful, because numerous cryptic, motile species emerge at night, making them easier to find and collect. Scuba hand sampling is usually also an effective way to look for symbiotic associates on larger sessile and mobile organisms like sponges, cnidarians, and echinoderms (Fig. 3D).

- **Hand-towed nets**. Using hand-towed nets in seagrass meadows is done to collect the motile fauna that live on the leaves. Nocturnal sampling is recommended, as the number of specimens collected may be up to five fold higher in nocturnal samples than those obtained during the day (pers. obs., JT).

- **Hand collecting sessile biota**. Hard bottoms have a rich sessile flora and fauna. While some sessile species are large, exposed, conspicuous and thus readily noticed and collected, many more are small, cryptic, encrusting, living in crevices, in the reef matrix and under rocks. Thus, as for mobile fauna, sampling under rocks and in crevices is important to get a representative coverage of the sessile fauna. A hammer and chisel, small drywall saw, scrapers, and clippers are useful tools for removing sessile organisms.

- Many sessile species have very useful field morphological characters, such as their growth form, shape, color, etc., that can be rapidly changed or lost upon collection or fixation. Some are so fragile that their form and even color can alter rapidly with collection (*e.g.* sponges, ascidians), while encrusting forms (like many bryozoans, worms, etc.) can be difficult to collect intact because of their broad attachment to solid substrata. Thus it is especially important to photodocument sessile species *in situ*. It is best to take both whole colony and close-up (such as 1:1 magnification) photographs before disturbing them, and to keep good records of form, color, and pattern in the field. Many sessile species are associated with mobile micropredators or symbionts, like nudibranchs and crustaceans, and it is important to search for these before disturbing the host.

- **Suction sampling**. An over-sized mechanical aspirator is an efficient tool for sampling small organisms on hard and complex substrates, and can be equally rewarding on soft bottoms (Fig. 3F & G). Aspirators can be powered by compressed air from one or more SCUBA tanks, or by motorized pumps. Depending on the size of the unit, one or two divers are needed to operate it. Brushing the vacuumed area can facilitate dislodgement of tenacious motile fauna. An area of about 5 m^2 can be sampled in one effort, depending on depth and the rate at which filters become clogged.

- **Brushing**. Brushing fine debris and associated motile biota from rubble into large nets or mesh-lined brushing baskets is an effective way to collect micromolluscs, crustaceans, and other invertebrates (Fig. 3A). The brush bristles should be soft enough not to damage the specimens, but hard enough to dislodge them. The opening of the collecting device should be closed after each brushing if possible to prevent more motile specimens (*e.g.* shrimp) from escaping.

- **Extractive sampling**. Most motile species are small and cryptic, difficult to notice, and live concealed within complex benthic communities. This cryptofauna represent the bulk of the reef biodiversity (Dennis & Aldhous, 2004). An effective way to sample these is to take samples of their habitats (rubble, soft sediment, algae, debris, sessile organisms, etc) to the lab and extract the organisms from these bulk samples. Pieces of rubble can be collected into buckets or bins underwater, transported back to the lab, and broken apart and picked over (Fig 5B). Soft sediment can be sieved or picked over for microfauna (Fig 5A). Weak (~10%) solution of ethanol in seawater, isotonic $MgCl_2$ and other narcotizing agents can be used to extract animals from a variety of substrata by letting the sample soak for a few minutes, then shaking a decanting over a mesh. Letting disassembled substrata sit in a bucket for a day or more provides an alternate extracting method. As the oxygen is used up, many organisms crawl out and up to the air-water interface, where they can be readily picked. This is an especially useful way to collect long worms that are otherwise difficult to extract whole. Alternatively, the broken rubble can be placed in a tray with a thin film of water. As the rubble drains and dries out, some animals retreat to the shallow layer of water accumulating in the tray.

- **Deployed Collecting Devices**. A useful method for inventorying especially small sessile organisms in an area is to deploy settlement plates and to periodically check these for species. In temperate areas, it is important to check plates in each season. If they cannot be deployed for a whole year, then deployment in late spring to early summer is ideal in the temperate zone. Most species become recognizable on the plates as soon as 2 weeks after deployment, but become more easily identified after one to three months. Settlement plates are extensively used in marine ecology, with considerable standardization. Thus it is useful to check the literature for settlement plate designs proven to be useful for scoring the flora and fauna in the region, and for which comparative data may be available. For example grey, 14x14 cm PVC plates, deployed horizontally at 1 m depth, are used for biodiversity monitoring by a large variety of organizations along Western Europe, NW and NE America, Hawaii, and New Zealand. Because settlement plates are usually hanging on lines that are attached above water, they can be easily retrieved. A small sized plate is also easy to photograph in the lab under controlled conditions, and can be preserved whole in ethanol if desired. If ethanol is not easily available, one can also use "sun-dry" plates, which still enables the identification of many Bryozoa, sponges, bivalves, barnacles, and some algae, ascidians, tube-worms and corals. When deploying several plates per locality, and scoring the species compositions per plate, one can

use a species accumulation plot to check whether many more species are still to be expected if one would deploy more plates, or whether most potentially-associated species have been sampled.

- Automated Reef Monitoring Structures (ARMS) that have been developed by the CReefs consortium (http://www.creefs.org) as part of the Census of Marine Life are a novel deployable method for quantitatively sampling marine diversity of not only sessile, but also mobile fauna. ARMS are a standardized stack of large settling plates (9 x 9 inches) separated by alternating fully open or compartmentalized layers (Fig. 3E). The ARMS are attached to a basal plate and anchored to the bottom. On CReefs efforts they are deployed at a standard 15 m depth on forereef habitats, currently for one year intervals, although tests are ongoing to determine the effects on community structure with longer intervals. Upon retrieval the ARMS are disassembled and each plate (top and bottom) are photo-documented, mobile fauna separated, and sessile and clinging fauna scraped clean. At this time there are over 200 ARMS deployed worldwide. Current efforts are aimed at developing technologies to enable efficient molecular sampling of this diverse community in parallel with traditional techniques.

In a dive intensive ATBI it is useful to have two groups sampling each station, thus allowing the use of all major methods per station. One group (ideally in a separate boat) pursues bulk sampling (brushing baskets, suction sampler), with experienced divers, but who do not need to have detailed knowledge of the organisms. A second group comprised of taxonomic specialist collectors focuses on hand collecting to take advantage of their experience and better search images for target species groups.

Having marked jars, bags, and coolers on the dive or in the boat allows separation of collections from distinct habitats and microhabitats, and tracking samples. It is useful to keep animals that interfere with each other in separate containers: some molluscs slime (a problem in closed containers), crabs rip, and many nudibranchs and flatworms poison.

4.2.3. Trawling, towing and dredging

Smaller dredges, grabs, traps, plankton nets, and other sampling equipment can be deployed from small boats (Fig. 1D). These equipment can be sufficiently small so that expensive research vessels are not necessary for their deployment in smaller efforts (although the addition of a major research vessel greatly enhances the potential of ship-based sampling). Small boats can be rigged with a modified arm and pulley system, and gear retrieved with a motorized line hauler. Local knowledge can be quite helpful in determining trap design (Fig. 4A) and locations (see below), and can be hired to assist in such sampling or to set and retrieve baited traps.

Local fishermen can be useful sources of uncommon or larger species, especially those of commercial importance (*e.g.*, mollusks, crustaceans, fishes). They may also use specialized techniques that would not otherwise be utilized by the survey, and can be a useful source of interesting bycatch. For instance, both

tangle nets and lumun lumun (Fig. 4B) were adopted from traditional Filipino fishermen and used effectively in Panglao (Ng *et al.,* 2009) and subsequently adopted during the later Santo expedition. Fishermen also have a wealth of local knowledge about habitats, natural history, tides and currents that can greatly facilitate planning the site, station choice, and method selections.

Fig. 4. Artisanal sampling. A. Deployment of locally made traps; B. Retrieval of lumun lumun off Balicasag Island. (Photos by Panglao Marine Biodiversity Project 2004).

4.2.4. Meiofaunal sampling

Marine sediments hold an abundance of microscopic life, the smallest of which attach to individual sand grains or live in the interstices between grains. A variety of bacteria, archaea, and protists share this habitat with minute metazoans, the meiofauna. Meiofauna ranges from <0.1 to a few mm in size, and is a major component of seabed ecosystems, particularly in the deep sea. About half of the animal phyla are represented in the meiofauna, and some (*e.g.*, Loricifera, Kinorhyncha) are confined to it. Nematodes are typically the most numerous component, with harpacticoid copepods, foraminiferans, and various worm groups also abundant.

Because the density of the interstitial organisms can be high, smaller samples are usually adequate and can be examined in their entirety. Simple corers, small diameter (5-10 cm) metal or plastic tubes driven into the sediment by hand or, if necessary, with the aid of a hammer, are the simplest and most effective sampling tools. If the vertical distribution of the fauna is to be studied it is essential that the sample should be divided into appropriate sections immediately on collection, since change within the sample can produce rapid alterations in the vertical distribution of the fauna.

In order to examine and count meiofauna, the samples are usually brought back to the laboratory for extraction from the sediment. Preservation and extraction techniques depend on the type of taxa studied and level of identification desired. "Hard" meiofauna, such as nematodes, copepods, ostracods, and kinorhynchs remain identifiable after rough preservation within the sediment using 4% formaldehyde, but are of little value for genetic studies if formaldehyde is used. "Soft" meiofauna such as turbellarians and gastrotrichs require live extraction. Extraction methods also vary according to the type of sediment and depend on whether extraction is to be qualitative (to obtain representative specimens) or

quantitative (to extract every organism possible for detailed count). Techniques of extraction fall into two categories: 1) those like decantation, elutriation and flotation, which rely on density and the differential rates of settlement between organisms and sediment particles and are suitable for both living and preserved material, and 2) techniques which employ an environmental gradient (*e.g.* temperature or salinity) to drive the living animals out of the sediment. A useful overview of meiofaunal sampling is provided by Higgins & Thiel (1988).

4.2.5. Rapid assessment survey approach

Emphasis on macrofauna is a useful approach when only limited collecting and sorting resources are available in the field. In addition, the taxonomy of macrofauna is relatively better known. In some groups most species can be readily identified in the field by an experienced collector, and this is regarded as a more environmentally friendly approach in conservation studies. Rapid visual survey techniques are useful as preliminary background information, and can provide fairly accurate species lists in taxa whose species are exposed and thus visible to divers (*e.g.* Roberts *et al.,* 2002).

4.2.6. Sediments sampling for quantitative assessment of diversity of skeletonized biota

Bioclastic sediment is composed of fragments of organic skeletal material. All marine sediments have a bioclastic component, and some, especially on oceanic reefs, are composed largely of this. In their study on the species richness of molluscs at a New Caledonian site, Bouchet *et al.* (2002) pointed out that among species encountered at only one station, 52% are represented only by empty shells. These species either live in a habitat that is difficult to sample, are exceedingly rare, or are seasonal/episodic. Rather than background noise to be discarded, skeletal remains of molluscs, brachiopods, forams, ostracods, and other skeletonized taxa can be used as an indicator of how much diversity is missed by the survey in taxa that lack *post mortem* remains, such as flatworms, polychaetes, meiofauna, peracarid crustaceans, etc. (Bouchet *et al*., 2002). Sediment samples of 1-2 liters can provide a good estimate of the diversity of micromolluscs and other small skeletonized taxa of the area. A single such sample often contains well over 100 species of molluscs.

The following standardized sampling design can be used in reef systems. Select a site on the fore reef at 15-20 m depth in a sand patch at least a meter in diameter and within one meter of hard bottom. Secondary sites can be added to cover other habitat types as widely as feasible. Useful secondary sites could include samples from ca. 100 m on the reef talus (if dredges or grabs are available or from deepest scuba depth if not), protected or lagoonal sites at 10 m and 20 m, and shallow (<3 m) sites from moats, sand /mud flats, or reef flats, and samples from well-developed caverns/reef crevices or caves. For statistical comparisons of quantitative samples, 3-5 replicate sites of the same habitat type (*e.g.* 15-20 m fore reef) are useful, with sites within the same physiographic area, 10's of meters apart. Three 1+ liter samples for replicates are also useful per site. Exact volume is not important, and lesser volumes are better than none. In caverns often only limited sediment may be available, but these can be quite diverse. Record approximate distance among samples and the nature of the

bottom (size of sand patch, obvious macrophytes, etc). Each sediment sample should be gently washed in freshwater, so that the fine size fraction is not lost, then dried. In the case of excessively muddy sediments, it is useful to wash out the <0.5 mm size fraction to reduce sample bulk, but to preserve a 50-100 ml subsample for granulometric documentation. Label each collection on heavy, ideally waterproof stock (waterproof paper is ideal) with non-water soluble ink. Most sediment retains sufficient moisture after field drying to rapidly rot poor label material.

4.3.   Sorting process

At the field lab, bulk samples and residues can be sieved in fresh seawater, and fractioned through a set of sieves from 10 to 0.5 mm, so that the coarse and fine fractions are separated (Fig. 5A). Obvious, and especially fragile, macrobiota (nudibranchs, polyclad flatworms, etc.) should be separated prior to sieving to minimize damage. The coarse fractions are sorted by eye, while fractions below 3 mm are sorted with the aid of dissecting microscopes (Fig. 5C-D). Picking of smaller fractions can be very time consuming, and if field time is more limiting than post-field lab time, then they can be preserved unsorted for later picking and study. A washing/sieving area should be located close to the field lab and a source of seawater assured.

Fig. 5. Field lab sample sorting. A. Fine sieving of bottom samples, Panglao 2004; B. Breaking rubble, Moorea Biocode 2008; C. Specimen sorting, Panglao 2008; D. Field lab for Panglao Biodiversity Project 2004. (Photos A, C & D by Panglao Marine Biodiversity Project 2004; B by Chris Meyer).

Samples collected in the field can be processed along two different routes. An important consideration for deciding which route to emphasize is the cost and availability of field vs. home lab time and desired data. Any specimen that has specific associated data (a photo, tissue sample, field observation) needs to be separated and tracked as a single specimen object. If photo documentation or genetic subsampling is a high priority in the survey, then as many specimens as possible should be so tracked.

Whenever possible it is useful to sort samples to morphospecies, because color and other useful field characters which allow for rapid species level sorting can fade or be lost upon preservation, making field sorting much more efficient for many taxa. The general workflow for processing a single fish collecting station is shown in Figure 6. After morphosorting, representative samples from the species were tissue subsampled (not shown), prepped, photographed and then tagged with unique identifiers. Efficient collecting yields many more specimens than can be processed at this level of detail while in the field, and remaining material can be bulk fixed, tagged only with a station number, and sorted back in the home lab.

Fig. 6. Fish sorting and workflow. A. Morphosorting a fish station sample; B. Preparation for photography; C. Photography; D. Tagging vouchers. (Photos by Chris Meyer).

Photography and Illustration

In situ or lab photographs of living or fresh animals capture distinct features and color patterns that are lost upon preservation. As such, live photos are important

for most marine taxa. Even for taxonomic groups where the appearance of live animals has not been used much for taxonomy (*e.g.* shelled molluscs — most books deal only with dead shells), living characteristics may reveal cryptic diversity or provide distinguishing features that differentiate closely related species whose dead remains are less descript. All digital photographs should have an unique identifier that connects them to the specimen photographed. A scientific illustrator may be a luxury, but is helpful for prized specimens, and camera-lucida drawings of selected live/fresh small individuals can be of great value for taxonomic work.

Genetic sampling

Molecular sequence data are becoming an increasingly important character set for delineating biological diversity. In 2003, researchers proposed that species could be identified by the sequence of just a single gene (Cytochrome Oxydase 1 or COI for animals), and that identification of animals and plants could be accelerated by these molecular characters (Hebert *et al.*, 2003). The capacity to identify all living organisms from a specific sequence of their genome is known as "DNA Barcoding" and is currently organized through CBOL (Consortium for the Barcode of Life: www.barcoding.si.edu) with membership from across the globe.

While controversy still exists as to the precision of this method (Meyer & Paulay, 2005), sampling biodiversity in the field would be remiss not to accommodate preserving at least a portion of the specimen for future genetic work.

The extensive fieldwork described above provides unique opportunities to create DNA collections from well vouchered collections for a vast array of tropical marine organisms (Fig. 7). Vouchers identified by taxonomic experts are essential for an effective DNA barcoding campaign. A major marine barcoding campaign (MARbol) is run through the University of Guelph (www.marinebarcoding.org), and readers are directed there for more information. Certain marine groups pose unique challenges for preservation. A special difficulty for snails is that for proper fixation, the animal must not be retracted deep inside the shell, especially if it closes with an operculum; yet, species-level taxonomy often requires examination of the intact shell. A combination of approaches should be used to ensure proper fixation and preservation of shell characters. This may be done by either breaking the shell of one specimen and conserving it side by side with an intact specimen of the same species from the same sample; or by relaxing an extended animal in extension with magnesium chloride; or by carefully extracting the snail out of its shell with a bent needle, or through niku-nuki (Fukuda *et al.*, 2008), a method that uses flash boiling to remove the animal. For crustaceans, the problems are less but still require that interesting species be specially preserved in alcohol. Freezing or relaxation prior to preservation is advised to prevent autotomization of appendages.

Fig. 7. Tissue subsampling for molecular work. A. Sorted micromollusks relaxing prior to tissue sampling; B. Tissue subsampling straight into digestion buffer for DNA extraction onsite, Morrea Biocode 2008; C. Barcoding Alley, tissue subsampling, Santo 2006; D. Echinoderm subsamples in 2D barcoded tubes. (Photo A by Chris Meyer; B by John Deck; C by Yuri Kantor and D by John Starmer).

Fixations for anatomical work

In parallel, further specimens should be relaxed and fixed for anatomical or microscopical work in appropriate fixatives (glutaraldehyde for electron microscopy; Bouin's solution, formalin or alcohol for dissecting) (see Appendix I for description of preservation methods and Appendix II for procedures by taxon). For macrobiota the same specimen should be prepared for both genetic and morphological analysis, with a tissue subsample for DNA taken from the organism prior to anatomical fixation.

Sorting after fieldwork

Samples from individual stations can be sorted to morphospecies and identified generally to the family level in the field (Fig. 6C). Bulk samples are sorted back in the home lab/museum to morphospecies or to the finest level possible. The lowest sortable level should be at least to a taxonomic rank that corresponds to the typical expertise of taxonomists. Specimens are then identified to the lowest taxonomic level readily doable (usually family to species), depending on available expertise. The taxonomic limits of field-based morphospecies designations should be verified by a network of taxonomists and/or by DNA analyses. After segregation to morphospecies/taxa gross measures of abundance (number of

specimens per taxon per station) can be captured if quantitative estimates are desired and the sample appropriate. The resulting information is stored in a relational database. Because of the combined qualitative and semi-quantitative methods employed in marine ATBIs, statistical analyses based on relative abundance data can only be constructed from bulk samples processed to the specimen level. Thus, projections of total species richness at the study site are accordingly difficult.

4.4. Data Management

The utility of specimens generated from a biotic survey is only as good as the associated data included with the collection. As such, information concerning the collecting event and specimen(s) should be managed with utmost care. The volume of material generated during a large-scale inventory can quickly become overwhelming if a consistent data management scheme is not in place. Field data includes the two major categories: event data (where and when) and specimen data (what). Each of these data types should be associated with a table of particular, standardized fields to make data portability and accessibility as easy as possible. In general, these data conform to standards adopted by GBIF and OBIS. In general there is usually a one-to-many relationship between events and specimens as many individuals are collected during a single event (dredge, dive, etc.). Event data should include primary location (*e.g.* island), secondary location (specific site on island), coordinates, habitat type, sampling method, depth, date, and collector. On reef systems, major habitat types to be tracked include: outer reef slope/fore reef, reef crest, outer reef flat, inner reef flat, inner sand flat, mangrove, moat (<3 m deep), lagoon (>3 m deep), lagoon slope, lagoonal patch reef, etc. Specimen data should include identity of taxon to lowest level known, microhabitat, any associated specimens and association type (symbiotic, etc.), fixative and preservative used, whether photographed and subsampled for genetics, and specific notes about sample and specimens. Microhabitats include whether animal lived in sand, attached under rock, loose under rock, in reef framework, boring in rock, commensal associations, etc. Specific notes should include particular aspects about individual specimens where this is important (color in life, texture, smell, etc. — these are often taxon specific, see Appendix II). Other notes about taxa may include abundance or specific behaviors. Large-scale ATBIs should enter this information in spreadsheet form and a database manager should compile each day's activities into a single database. An example template for such field expeditions can be found at http://biocode.berkeley.edu/batch_upload.html.

In addition to station/sample data, one should consider keeping more general field notes. There is a compromise between sampling and taking field notes in terms of effort in any field situation. As time allows, notes about the habitat are useful, both for recording the environment in association with the samples collected and for describing for future workers the environment so that they are able to recognize changes over time. Field notes can cover gross site description (exact location, site map, nature of site in terms of bottom type, topography, benthic cover, dominant species in community, etc.), notes about what you focused on (so future workers can judge what you would have likely recorded if it

was there), and notes about the taxa/communities studied. For the latter a list of species with relative abundances can be useful – but only as useful as identification skills and photo/specimen vouchering allow. Haphazard notes about taxa not focused on are less useful, unless the particular species noted is unusual in its occurrence. If time is limiting, field notes can be captured on voice recorder, and saved as a voice file with the station data. Much of this information can be captured in the database and associated with the collecting event.

Labels and notes

An average day in the field with a large group of participants yields numerous collecting events and hundreds to thousands of specimens. With such a large number of samples and specimens, together with associated photographs and documentation, accurate labelling is critical. It is recommended that a principal field coordinator keeps a master list of all collecting stations/events. Unique event identifiers should be posted centrally at the field lab so that all participants can see and use them. Multiple labels with the collecting station number can be pre-written and then placed within each of the samples and subsamples as bulk samples get split and sorted to various taxonomic levels. A specimen number series can be pre-designated for taxonomic teams or working groups so that labels can also be pre-written and assigned as the material is fractionated along the processing pipeline down to morphospecies lots or individual specimens, when tissue samples or photographs are taken. These labels should be made with sufficient room on the card stock to allow addition of information about the sample (*e.g.* taxon name, color, sex, notes); such extra labelling also provides excellent error checking in cases of confusion. Data can be entered directly into spreadsheets or onto pre-printed sheets, with just the basic minimum fields (EventID, specimenID, lowest taxon, PhotoID(s), tissueID, notes). Care should be taken to be sure to log these data every day into the database, so as not to get too far ahead and lose track of pertinent information and details. Backups, preferably offsite, of the central database should be made each day to insure against disk failure. All paper records and notebooks should be archived (digital images of these are useful) as well as any maps with marked stations. All field labels should be on appropriate sturdy, archival paper stock with pencil or permanent ink that can withstand various media. Make sure field labels are sufficiently large relative to the sample so that that they do not get easily lost or overlooked.

5. Acknowledgements

6.   Recommended references

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7. Appendix I. Preservational aspects

7.1. Relaxation

Purpose is to anaesthetize a specimen so it is unable to respond or contract when placed in fixative. Important for humanitarian reasons, as well as because in many groups identification is hampered or made impossible if fixed in a contracted form; in other groups (*e.g.* crustaceans, some echinoderms) autotomy may occur if dropped straight into fixative. When relaxing make sure the animal: (1) expands if it started out contracted (ascidians, anthozoans) (this may not always happen) and (2) is fully relaxed and does not respond to even strong poking. Do not poke strongly until you are sure it is fairly unresponsive, as otherwise an initial poke will send specimen into real contraction from which it may not come back out. Relaxants are often group specific; some useful chemicals are listed below (there are many others). You may need to experiment with various methods before you find one that works for a specific taxon. Even closely related groups may relax better with different relaxants.

MgCl$_2$: prepared in freshwater at 7.5% weight – which is isoosmotic with seawater. Note that MgCl$_2$ crystals are highly hydrophilic, so if they have been stored poorly and absorbed water, you will need to mix a generously greater amount. Exact percentage is not critical. MgCl$_2$ works by competing with Ca in muscles and nerves, making animals unable to contract. MgCl$_2$ works well with most marine organisms, but can take an hour or more for larger animals. A 50:50 mixture of isotonic MgCl$_2$ solution: sea water is a good general mix to use; MgCl$_2$ solution should be gradually added to seawater for especially sensitive animals.

Menthol: add to dish with animals by either sprinkling crushed crystals on top or adding drops of concentrated menthol solution prepared in ethanol. Menthol works especially well for cnidarians and ascidians.

Chloretone = **chlorobutanol:** Chloretone is not readily miscible in water, so it is prepared in a saturated ethanol solution (a large amount of the chloretone can be dissolved in a volume of alcohol). A couple of drops in a bowl or a pipette full to a bucket works well on echinoderms, including large holothurians.

Clove oil = eugenol: knocks out most crustaceans rapidly. Prepare a saturated solution in sea water, and add to bowl containing animals. Can glom up finely setose appendages of small crustaceans if used straight. A 25% solution of clove oil in ethanol is a useful field anaesthetic and will flush and stun cryptic crustaceans such as stomatopods from crevices.

Cooling/freezing: cooling can facilitate (and enhance chemical-based) relaxation in warm water organisms. Freezing is an effective and humane way of killing animals that can be photographed or subsampled in a freshly thawed state, especially useful for strongly skeletonised crustaceans, like crabs, and shelled molluscs. Slow freezing (but not cooling) can be bad for anatomy and histology so DO NOT use it for soft bodied groups where anatomical information is desired.

Propylene phenoxitol: A nowadays difficult to get but excellent relaxant for bivalves and some other invertebrates. A couple of drops added to a bowl go slowly into solution and rapidly knock out animals.

7.2. Fixation

The purpose of fixation is to fix tissues for long term storage and study. Formalin or similar strong fixatives (Bouin's fixative, glutaraldehyde, etc.) are necessary for histological quality fixation and for most groups where detailed anatomy or histology is needed for identification (*e.g.,* ascidians, most worms, cephalopods, opisthobranchs, etc.). Formalin makes tissue difficult to use or unsuitable for DNA sequencing however. Ethanol is fine as a fixative for groups where only external characters or gross anatomical features are used in taxonomy (*e.g.,* most crustaceans and sponges), and is more pleasant and less hazardous to work with. It is also preferred for groups (like holothurians) where the greater potential acidity introduced by formalin may etch or destroy tiny calcareous sclerites used in taxonomy. An ethanol solution of 70-80% is ideal for fixation, because the alcohol penetrates more readily and does not cause too much tissue shrinking. Lower concentrations may not prevent all microbial activities, while higher concentrations can greatly shrink specimen and make specimens (especially crustacean legs) brittle. It is very useful to take a small (1-3 mm) tissue sample from larger animals and fix it in ample (>10x tissue volume) as a genetic subsample, as it will yield better quality DNA than bulk-fixed samples. Preferably change the alcohol in the field a day or two after initial fixation. Taking a tissue subsample is essential for formalin-fixed specimens if future genetic study is considered.

When fixing large animal (such as large sponges or sea cucumbers) in ethanol, it can be useful to initially fix in 95% ethanol to balance the water content of the animal — you can eyeball this volumetrically. You should always use plenty of fixative fluid — at least 3x volume of specimen, to make sure that the final concentration of fixative is adequately high to do the job (70% for ethanol, 5-10% for formalin). If you are fixing larger animals (> 2-4 cm in all dimensions) it is important to make sure that the fixative penetrates. This is best achieved by injection with a hypodermic needle into the body cavity or body, or by cutting the animal open. Be careful not to blow up the animal or unduly destroy anatomy when doing this. Some fixatives, like Bouin's, have chemical agents to facilitate tissue penetration. Changing the alcohol after a couple of days also improves preservation.

Formalin is used generally at 5-10% strength of the industrial "formalin" mixture — which itself is ca. 38% formaldehyde gas dissolved in water. Thus 10% "formalin" is 3.8% formaldehyde. For marine animals, formalin should be mixed with sea water to make it isoosmotic; for freshwater animals it is mixed with freshwater. At least for taxa with calcareous parts (but is good practice for any taxa) formalin needs to be buffered, as it turns acidic (forming formic acid) with age. Buffering can be achieved with laundry borax (sodium borate), or in a pinch by adding calcium carbonate powder/sand. For good histological/anatomical fixation you may want to use buffer recipes, or use special fixatives like Bouin's.

Bulk alcohol usually comes at 95% concentration; absolute (100%) ethanol is considerably more expensive. 70-80% ethanol is used for routine fixation. 95-100% ethanol is often used for genetic fixation of small subsamples, but whether subsamples are better fixed at this high or at 70-80% ethanol concentrations has come into question. Mix ethanol with distilled (deionized, or otherwise fairly soft) water, as precipitates can form with hard water. In field situations pure ethyl alcohol can be difficult to obtain, but denatured spirits (~95% ethanol + methanol + odor and sometimes color) are available in most places and provide a good alternative. For genetic fixation remove excess water from the specimen as much as possible, add ethanol equal to 5-10x the tissue (not counting shell) volume of the sample, and change at least once in the field, and again back in the lab

7.3. Preservation

Once a specimen is fixed (takes a day or so for small (<1 cm) animals to a week for big ones, then the animal can be transferred into a different, and more benign medium for long term preservation. Thus formalin fixed samples are often transferred to alcohol. To do this, the formalin needs to be soaked out by letting the specimen sit in water (or seawater) for couple of hours to days, then transferred to alcohol. Initial alcohol fix should also be replaced with fresh alcohol after a couple of days, to bring ethanol concentration closer to target and remove debris and solutes from the jar (although retaining solutes in original alcohol may be desired if chemical study of secondary metabolites is desired). For final voucher storage, specimens should be in the smallest jar/vial they fit comfortably into without, and filled to the top with the preservative. Filling the container is important because: 1) more preservative takes longer to evaporate, thus giving more time to discover a faulty seal, and 2) this sets a standard, so that evaporation can be immediately noticed in a collection and addressed by replacing lid or jar. The amount of alcohol relative to specimen volume is no longer important at this stage.

8. Appendix II. Procedures by taxon

Procedures are given here for some of the macrofauna most commonly targeted in surveys. This overview is not meant to be exhaustive for either taxa or methods.

Porifera: No relaxation needed, fix in ample volume of 70-95% alcohol depending on size, transfer to clean alcohol in a days-week. In addition to basic field data, record color (external and internal, if possible), texture, surface feel, odor, mucus production, and any other obvious live character of the sponge. *In situ* photos are very useful for sponges and should be linked to the voucher specimen.

Hard corals: as most characters are based on the skeleton, corals are usually bleached with a solution of sodium hypochlorite to remove all tissues, then washed and dried. However a scraping or small piece of the colony should be preserved in ethanol or one of the specialized coral DNA fixative cocktails to provide a genetic subsample. *In situ* photos are very useful and should cover colony shape as well as a close up of undisturbed polyps.

Soft corals: are not usually relaxed, and can be fixed in alcohol or buffered formalin (former often preferred for taxonomy to minimize etching of ossicles, latter preferred if histological fixation is desired) and stored in alcohol. *In situ* photos are very useful.

Gorgonians (sea fans): are fixed in alcohol or fixed in buffered formalin then quickly dried. If colony is dried, it is useful to have a small portion fixed and stored in alcohol. *In situ* photos are useful.

Black corals: should be fixed like gorgonians, with a good portion pickled. Ethanol is an adequate fixative, formalin is required for histology only. Color notes and *in situ* photos, especially of the expanded polyps are very useful for taxonomy.

Anemones: should be relaxed well with menthol, fixed in formalin ideally when expanded, and stored in alcohol. Photo of live animal is very useful.

Flatworms: Large turbellarians can be challenging to fix because they are fragile, readily contract and can disintegrate. Good fixation can be achieved by allowing animal to crawl and expand on a piece of moistened paper, then placing the paper and animal gently onto frozen formalin to fix; preserve in ethanol. Taking a snippet of tissue with a razor from the end of the crawling worm provides a subsample for DNA and can be fixed in ethanol. Photos of living animal are essential for colourful species.

Other worms: Relax with $MgCl_2$ generally, fix in formalin, preserve in EtOH. Taking tissue subsamples or fixing duplicate animals in ethanol is needed for DNA work. Fixing long nemertean worms straight is important to facilitate sectioning. Photos are useful for colourful species.

Crustaceans: Larger, tough specimens are easiest to kill by freezing, smaller ones relax well with clove oil. Fixation and preservation in alcohol is ideal. Photos of colourful species are very useful. Crabs are best photographed freshly killed with legs spread; for shrimp and other translucent species living photos are much better.

Mollusks: While shells from dead (or live) species can be sufficient for identification, live specimens should be fixed in fluid when possible. $MgCl_2$ and propylene phenoxitol are good relaxants for many molluscs. Fixation can be in alcohol or formalin, with the latter much preferred/essential for opisthobranchs and cephalopods. Photos are most useful for opisthobranchs and cephalopods, but also for soft body of any species.

Bryozoans: Like hard corals, bryozoan taxonomy relies on the skeleton, so dried specimens are fine for taxonomic study, but alcohol-fixed or subsampled animals are best for genetics. Photos are the best way to record colony shape, especially in fragile species.

Ophiuroids, asteroids, echinoids: Best relaxed with $MgCl_2$ in a flat pan for stars so they spread out, then fixed in ethanol or formalin. Ethanol fixation is preferred if specimens will be kept wet, while formalin provides better fix for specimens destined to be dried out. As most/all taxonomic characters are skeletal, dried specimens are a good way to keep especially large specimens. Associated field photos and genetic ethanol biopsies important. Tube feet make easily accessible subsamples in echinoderms.

Crinoids: Best fixed by pushing animal oral surface down into a pan of ethanol, allowing the arms to spread while pushing the animal into the alcohol. This way they die in seconds and fix in a spread-out position. Preserve in alcohol. Field photos are useful.

Holothuroids: Relax with $MgCl_2$ or chloretone, inject with and fix in 70-95% EtOH depending on size (to dilute down to 70-80% with body fluids); preserve in EtOH. Field/live photos extremely useful.

Urochordates: Relax with menthol, fix in formalin, store in formalin. Ideally *in situ* photo is extremely useful. Tissue subsamples for genetic studies should be preserved in alcohol.