

FIELD METHODS

Preamble

The world is changing. Much of what you see today will be gone or very different tomorrow. The window for recording what the biosphere was like before excessive human impact, although closing for millennia, is now rapidly slamming shut. One of the greatest priorities for field research is to record (in specimen, audiovisual, field note, and other archival formats) as much of the remaining biocomplexity of the planet as possible for future generations. Many species and ecosystems will be documented for the last and often only time by a single willing naturalist of our generation who was there and recorded it before it disappeared. As you see a reef bleach severely or a forest gets logged, most of what was not recorded from that area will remain unknowable forever. Our understanding of Earth, and our descendants ability to reassemble quasi-natural ecosystems, will hinge on the archival record we leave. Having the taxonomic and field skills for making the most meaningful records, and capturing information in the most complete, efficient, and useful manner possible is thus paramount. The guidelines below are meant as an introduction to some basic field methods, especially as they pertain to the marine fauna of coral reefs, to help you do this. Recording as much as you can, as well as you can, while you can, is a good principal to go by.

Collecting for diversity

Balancing habitat coverage and accurate field data can be a challenge. On the one hand you want to sample as many habitats as possible to increase diversity of species encountered. On the other hand you want to keep the number of habitats / microhabitats sampled per dive or field excursion to a manageable number, to make keeping track of the provenience of each specimen easier during subsequent processing. Creating a field plan by considering the number of days available for the number of habitats to be covered is useful. If you have sufficient time and are not doing a 1-2 dive blitzkrieg of an island, focus on a fairly narrow depth range and 2-3 microhabitats at a time. You can always keep your eye open for other unusual critters and grab them beyond this focus. If time is limited though, then it is worth loosening up on data precision in exchange for better overall coverage – so go over as many habitats as possible. If you are after diversity then visit a variety of sites and also consider how others have sampled before you. Reef flats and moats are among the best sampled reef habitats overall, while silty, murky areas in lagoons, subtidal parts of mangroves, deep fore reefs are much less sampled and thus more likely to yield undocumented diversity. Specialized habitats and methods practiced by taxonomists can uncover a diversity of taxa missed by others

Having numbered jars / Ziploc bags (or labels that you can put inside them) to separate collections from different stations / habitats / microhabitats facilitates keeping track of samples. You can also separate collections from different stations/dives into different coolers as a quick way of tracking. Keep animals that mess each other up in separate containers. Some mollusks slime (that's no big deal to other mollusks, but can really foul and kill a crab – a pain for photography, etc), crabs rip, many nudibranchs and flatworms emit toxins that kill other organisms confined in the same container, etc. Do not put too many/large organisms in a sealed container as they can suffocate. If collecting above water (intertidal, ship-based), leave ample air in each container. Most animals do better moist with minimal water than in a jar full of water, as they have access to ample oxygen. Some algae or other material in a jar can keep animals like crabs from fighting. Tiny organisms get lost in large containers with other large animals; have vials available to separate these. Putting important and fragile animals in their own container is also very useful.

Transparent plastic containers are great, but most float, which can be a pain during dives. You can string plastic jars together and attach them to your belt. Glass containers (with metal lids) sink nicely but break (I prefer these). PET (Polyethylene Terephthalate) vials are great, they do not break and are denser than seawater; use with lids with polypropylene inserts (Wheaton 986756).

Once out of the water quickly stabilize and organize the collection. Make sure all containers have ample oxygen (exchange water if necessary), are kept at appropriate temperature, and stations/habitats are tracked. You may wish to divide the contents of crowded containers, add station labels, and keep jars and bags in a cooler. A portable airpump is useful for oxygenating larger open containers, especially if you have a long trip to the lab. Special plastic bags that let oxygen through can also be useful – these have to have their surface exposed to air (not stacked or submerged).

Collecting methods

Two rather different approaches for field sampling are to go after individual specimens in the field (specimen/hand collecting) or to collect samples of substrata or communities and then extract specimens from these samples in the lab (mass sampling). Hand collecting is particularly useful for large organisms, uncommon organisms, or those that live in specialized microhabitats or habitats that cannot or will not be sampled otherwise. Knowledge of the biology, habitats, habits, and signs of organisms is useful/essential for finding these.

Hand collecting

- sessile biota
- symbionts
- tools: forceps, knives, brushes, hammer/chisel, scraper, nets, yabby pumps,

Mass sampling

- brushing
- vacuuming
- sieving – dry and wet
- coring
- substratum removal: rock, reef, gravel, finer sediments, algae, brush/vacuum samples

Shipboard sampling

- dredges; anchor dredge, beam trawl
- trawls
- epibenthic sled
- grabs
- plankton net
- traps: baited, light, emergence

Extraction methods

- disassociate - sieve - pick
- sweating
- sediment swirling (gentle and coarse); decant on 200 um (or finer)
- bubbling (and blotting) for hydrophobics – many Ecdysozoa
- chemical extraction
 - irritants, e.g. 10 ml cc formalin to bucket of rubble sample for 3 minutes (White 2013)
 - relaxants, e.g. MgCl₂, 20-30 minute, decant, back in SW
 - freshwater shock
 - seawater ice
 - light attraction
- fix and pick, Rose Bengal

Recording data – field notes, station & specimen labeling

Stations

Most collecting efforts are tied to a small area, one or a few habitats, and one or a few methods. Assigning a Station Number (Event Number) to each collecting event is an efficient way to track associated data. Make sure Station Numbers are uniquely project specific (e.g. FHLIZ16_###), as Station 12 sitting on a shelf a year later could be confused among projects. In group projects it is best to have station numbers assigned on a board – so new numbers are created as different people need them and thus not duplicated. The station board should be captured into a FIMS (Field Information Management System; see below) regularly (e.g. end of each day) by someone assigned to the task. It is also useful to periodically photograph the board so it could be examined in case inconsistencies are discovered later. Each station should minimally have a location (text, lat/long (range/precision), depth (range), agent list (collectors), date (range), collecting method, habitat(s), microhabitat(s)). Additional data fields are useful, but many of these are easier to add in the station table in the FIMS. Getting folks to regularly check and add to data in the FIMS during the field work is the best way to capture this information. Have the FIMS available in a public computer and get people to use the working copy. Back this up regularly. Ideally get location from a GPS; you can also use Google Earth/Maps if collecting near recognizable landmarks (e.g. complex shores). Marking stations physically on a map, then depositing a copy of the map along with the specimens in a natural history collection is

also an excellent idea. It is useful to have assign station data oversight to a single person, who makes sure data are captured daily. Poor station data recording is the single worst problem in large surveys and is a major pain to deal with later.

Field notes

It is useful to keep field notes for each station, as well as general description of the area at the end of the trip. As time allows, notes about habitat are useful, both for recording the environment you worked, and for describing for future workers the nature of the environment you saw, so that potential temporal changes can be recognized. 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 – this helps interpret absence data), and notes about the taxa / communities you studied. For the latter a list of species with relative abundances can be useful – but only as useful as your identification skills and photo / specimen vouchering. Haphazard notes about taxa that you did not focus on are less useful, unless the particular species noted is unusual in its identity or occurrence. Making written field notes is time consuming and can take precious time from other tasks in intense field work. In contrast voice recording field notes is field-expedient, and can be done immediately after sampling, allowing you to record details you are likely to forget. Make sure you start out with date, location, station number, etc so the recording is easily associated with the relevant station. Photographs of habitats and communities at the station are also useful. Field notebooks are also useful for making diagrams, maps, etc. A combination of notebook, voice, and photos provides excellent coverage. These can all be archived in a collection database, attached to the relevant station/specimen(s).

Specimen stabilization & tracking

Once back in the lab specimens need to be stabilized: (1) removed from confining field containers to open containers (trays, Tupperware, 6-well trays) that allow exchange of oxygen, (2) further sorted if necessary to prevent potential negative interactions (e.g. isolate particularly delicate animals), and (3) labeled with Station Numbers. Note that in the field you can track stations as 1, 2, 3, but proper Station Numbers are assigned once you are back in the lab (see *Stations* above). It is useful to have the person who collected a sample do the stabilization and also partake in sorting, because someone else may miss a hard-to-see/small/symbiotic specimen when emptying/sorting a container that the collector will be aware of and can look for.

Sorting

Specimens can be fixed unsorted (bulk station sample), sorted to morphospecies (specimen lots), or separated as individuals (to track with subsamples or photos). Separating lots or specimens is useful for tracking additional associated data, but at the cost of increasing field burden. Which method you will want to follow depends on your objectives and field setting – are you trying to record the most diversity in a limited amount time, or a smaller number of specimens with detailed information? Mixing these three approaches can be useful, as you can triage specimens into those you sort and deal with as morphospecies/individuals (essential for tracking photos and tissues), those you bulk fix from one microhabitat (and/or station) unsorted, and those you do not end up processing and release.

When bulk fixing from a station, sort animals into groups that preserve well together, i.e. need the same fixative and will not entangle or damage each other in bulk samples. Some groups do not do well fixed in bulk, e.g. shrimp tent to loose many legs, so if species are mixed up you may not be able to associate important legs for identification with the specimens they came off of. Bulk samples are best labeled with just the Station Number and fixative used (e.g. FHLIZ16-13; 80% ethanol). Unsorted samples are usually only entered into the FIMS after they are later sorted back at the museum.

Sorting live/fresh specimens to morphospecies is very useful, as species can often be separated or identified quickly from live appearance/color, but take detailed examination in the lab to sort and identify. Also, your sorting impression can give additional information to the person later studying the collection. Thus if what looks like one species in a faded pickle was separated into two species in the field, then the taxonomist studying the collection is alerted to the possibility that cryptic species are involved. Sorting to morphospecies/specimens allows easy tracking of associated data such as (1) subsamples (tissue for DNA, histology, larval stages, etc), (2) photographs, (3) specific information about (exact microhabitat or depth, verbal description of color, smell, texture, associated symbionts, etc.).

Labeling

Once sorted add a pre-printed Specimen Number (= Field Numbers) to each lot, and record data on field sheet or FIMS. You can keep all specimens of a species from a station together under one lot, or split out some specimens that are further documented (photod, subsampled) into their own lots with separate Specimen Numbers. It is efficient to print field numbers ahead of time for a field project in sets of two, e.g. BRNG-0001 / BRNG-0001_DNA; BRNG-0002 / BRNG-0002_DNA. Double labels can be separated to go with each of voucher and tissue sample, or both included with the voucher, which also indicates that no tissue was yet taken from that specimen. The labels are co-photographed with the specimen, facilitating photo tracking. Specimen labels should be printed on sturdy, archival stock, with lettering that does not come off. Note that most printers do not produce archival quality labels; e.g. laser printed letters have a tendency to rub off unless specially treated. We print labels thermally on plastic stock and can supply these for projects. You can also create hand-written field labels to accompany each sample on appropriately sturdy, archival paper stock, with pencil or alcohol-fast ink (India ink, Micron pens, etc). As inks sink into rag paper (and pencil marks adhere well), these are of archival quality. If you use pencil press strong enough so that your writing is clearly visible. However hand labeling is inefficient for large projects. It is also useful to have preprinted labels about fixative to add to specimens when multiple fixatives are used in a project. For example we use “fixed in formalin” labels (or a red plastic strip) in our projects, but do not add a label to specimens placed in alcohol, as that is the go-to preservative for most.

Make sure labels are sufficiently large relative to the sample so that they are not easily lost / overlooked. Our preprinted labels are small because they need to fit in DNA vials and because most specimens are small; however this makes them prone to loss with large specimens. It is useful to add a second, hand-written, larger label (with same Specimen Number) to accompany large specimens. It can also be useful to physically attach labels to large specimens with monofilament line of bell wire, as this can facilitate processing. Thus corals with attached labels can be bleached in the same container, large echinoderms with attached labels can be fixed and stored together in the same large container.

Specimen data is captured in field sheets and FIMS. It is inefficient, inaccurate, and costly to transcribe detailed hand-written labels into the collection database, so avoid these (or use them only in conjunction with Specimen Numbers to duplicate data otherwise already captured in FIMS). Pre-printed field sheets used in conjunction with spreadsheets are a field expedient method for data capture. Field Sheets should capture at a minimum the Specimen Number and source Station Number; it is also useful to capture some level of identification, whether photos, tissues, and vouchers were taken from that lot, whether the morphospecies matches that in another lot (when not identified to species), and specific notes such as, exact microhabitat or depth, smell, texture, associated symbionts, unusual fixation, number of specimens taken, etc, as desired. See the Specimen Table entry below for guidance on this. A typical field sheet is shown below. Field sheets are printed on thick, archival paper, held on a clip board, and filled out by pencil or water & alcohol proof pen (Micron pens, etc).

Date: 12.VII.2016		Project: FHL dock survey		species	Entered by: GP		
Spec #	Station #	Morphospecies	match	Photo	Tissue	Voucher	Notes: depth, microhab
BFHL-	FHL16-	(lowest taxon level)	BFHL-	Y / N	Y / N	Y / N	associations, fix...
5820	12	Eunicidae red antenna		Y	Y	Y	1X, HCHO fix
5821	12	Eunicidae red antenna	5820	N	N	Y	21X, EtOH
5822	13	Pachycheles		Y	Y	Y	rare, only 1 seen

In this example Station Numbers are FHL16_###, Specimen Numbers are BFHL_###, Morphospecies are combination of lowest taxonomic identification plus field morphospecies name, Species Match identifies a sample that is the same species as another sample in the project (here BFHL_5821 is considered to match BFHL-5820). Photo, Tissue, Voucher are Yes/No fields, Notes: include shorthand of information on fixatives (HCHO, EtOH), number of specimens (1X, 21X), abundance, symbionts, microhabitat, etc.

FIMS

Station and Specimen information should be entered into a Field Information Management System (database) as soon as convenient. Agents making the collection should either enter or proof their own data. In large operations it can be useful to send images of station boards and field sheets to a data entry person to speed up data entry and

access without adding additional people to the field party. In this way the FIMS can be further edited and added to by expedition participants quickly while in the field.

Several collection databases and projects have developed FIMS designed to match desired data structure. For example the Specify project that runs many museum collection databases has a Workbench function that generates a FIMS. These database-generated FIMS can be pretty clunky because they have to match the data model of their source database. For example Specify Workbench requires separate field for each name of each collector, so 15 fields if up to 5 people are listed as collectors. A more field-expedient FIMS can be used for first data entry, as long as data fields can easily be matched (or parsed) from that. Make sure however that fields used in FIMS match fields used in final database or can be readily parsed or converted into these fields. Station and Specimen information should be kept in separate data tables; see Field_template.xlsx for a model. Below is a brief overview of some of the fields in these tables.

Station Table

Location is typically parsed into in 3-4 hierarchical fields: primary location (e.g., country / island), secondary location (state / specific site on island), tertiary location (county/town), etc

Geospatial data: lat/long coordinates (ideally in decimal format; 5 decimal digits gives sufficient precision (meters), more is false precision); source of coordinates (GPS, Google Earth, etc), datum, precision (in meters – radius of error around specific location).

Habitat: useful to parse this into macrohabitat and microhabitat. Latter or both are ideally duplicated in the Station and Specimen Tables, so that information is captured at the appropriate level. This way you can capture broader, more general habitat info at the station level, but add specific information about particular specimens in specimen table when appropriate.

Macrohabitat is gross habitat type, e.g.: mangroves, intertidal rocky shore; on island reef systems include: outer reef slope / fore reef, reef crest, outer reef flat, inner reef flat, inner sand flat, mangrove, moat (<3m) deep), lagoon (>3m deep), lagoon slope, lagoonal patch reef, etc.

Microhabitat includes specific information about where the organism was found: in sand, on sand, attached under rock, loose under rock, in reef framework, boring in rock, commensal associations with species X, etc. When collecting much material from a single microhabitat, it can be useful to assign different stations (or substations) to each microhabitat, so that microhabitat information is captured at the station table level.

Depth: min, max, units. Capture at Station level, but include in Specimen Table to allow more precision: e.g. 1-30 m (whole dive), specimen X taken at 10-15 m.

Collector(s)

Date (range)

Specimen Table / Field Sheet

Field Sheets are paper forms filled out while processing; Specimen Table is the spreadsheet table in the FIMS where this information gets transcribed.

Taxon: field identification to lowest practicable level. A “morphospecies designation” for unidentified species can also be very useful, especially when applied consistently (e.g., red, branching axinellid sponge; *Pagurixus* w/ blue chela). This way a species you recognize but cannot yet identify can be consistently labeled.

Morphospecies match: Useful field for tracking unidentified but recognizable morphospecies. These can be associated by reference to a field number voucher – e.g. same taxon as BRNG-1234, or by consistent use of morphospecies name – e.g. *Pagurixus* w/ blue chela.

Voucher, tissue, image: three yes/no fields about whether voucher, tissue, and image were taken from sample. Can also make more complex and parse type of preservative (for voucher), whether DNA or RNA fix (for tissue).

Several other important fields are captured in a FIMS, but are usually kept in a single cell on field sheets because of lack of space. In field sheets we keep a “Note” field for such observations, so the pertinent info can be captured there, and parsed out later when they are copied into the FIMS. Ideally the collector will do the parsing and can also add additional information into FIMS at the time. For example if all specimens were fixed in 10% formalin, then it is most efficient to enter this into the FIMS, but not onto field sheets, but you will want to note fixative specifically for a specimen that was fixed in alcohol. Of course capturing all this information directly into the FIMS is great, but is usually not field expedient. Notes that are especially useful to capture immediately onto field sheets include:

- fixative (if the fixative used is not the standard one for taxon)
- habitat, microhabitat, depth (if more precise than for Station as whole)

- association (with other specimens)
- type of association (parasite, epibiont, etc)
- notes proper: e.g., color in life, texture, smell, etc.; behavior; abundance, etc.

Photo tracking

For lab photography include the Specimen Label in some of the shots with the specimen, so they can be easily matched. If your labels are standard sized, this also provides a scale. Adding a scale and color sheet is also useful, but not as field expedient. For *in situ* field photography make sure you note which photos are of which specimens, so they can be associated later; discussing this in voice recorded field notes at the end of a day is an effective way to capture this quickly. Photos should then be databased with unique identifiers that associates each photo with a field number, or with ancillary information if the specimen photographed was not collected. As always it is best to give photos unique identifiers for tracking, and track the PhotoID with the Specimen Number in the FIMS, to be then imported into a photo database. A photo page for this purposes is included in the Field_template.xlsx. See separate notes on photography for details.