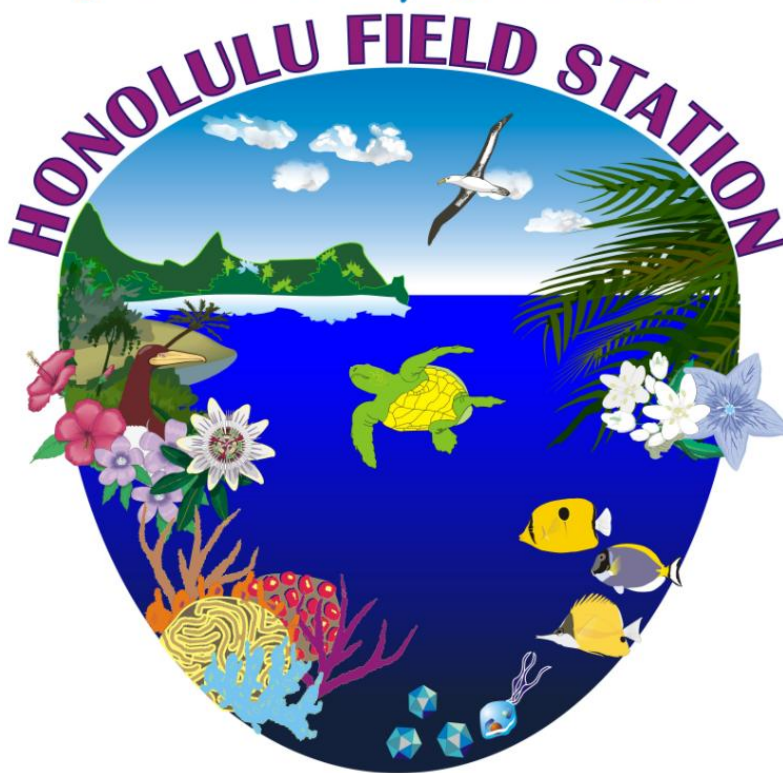


Collecting corals for histopathology. A practical guide

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Why do histopathology on corals?

Coral reefs are declining globally at an unprecedented rate, and disease is a major contributor to these declines in many regions. Unfortunately, we know very little about causes of coral diseases because too few people are focusing on what is killing corals at the tissue level. Histopathology can be very helpful in this regard in that it allows you to visualize, at the cellular level, potential agents associated with disease and the host response to those agents. Histopathology is used routinely to diagnose potential causes of disease in a variety of wildlife. However, few animal disease diagnosticians are looking at coral lesions. A significant barrier to getting veterinarians and other animal disease diagnosticians involved in coral disease has been the lack of user-friendly guides on how to collect and process corals for histopathology.

This manual intends to rectify this by focusing, in an approachable way, on what is needed to properly collect corals for histopathology. This guide provides NO information on ways to census corals for disease or to look at the broader picture of coral health. Coral disease investigation is a broader topic best done as a partnership between animal disease diagnosticians and coral ecologists.



IMPORTANT: Please note that corals are protected in many areas and cannot be collected without proper permits. Users of this manual are **STRONGLY URGED** to familiarize themselves with the laws and regulations of the region or country they are working in to ensure that they have all proper local, national, and international permits **PRIOR** to carrying out the activities described herein. It is also assumed here that users are familiar with SCUBA diving and specifically, the demands required of scientific diving and associated hazards. The procedures herein require use of tools and photographic gear underwater, so the user must be very comfortable with diving procedures.

Materials needed to collect and process corals for histopathology

Item	Purpose
Goodie bag	Holds your sample bags underwater
Rongeurs	Useful to sample branching corals
Core sampler	Useful to sample massive corals. I use a wood core chisel (ca. 4-5 cm diameter) and a chuck.
Cold chisel	Same as above
Tool bag	Holds rongeurs, core sampler, chisel
Sledge hammer	Needed for core sampler and chisel. Find one that is at least 2 kg as this will facilitate hammering underwater
Plastic collecting bags	Hold coral samples. Medium whirlpaks or equivalent work really well.
Underwater camera	Used to photograph gross lesions. Know your camera and how to operate it.
Scale	Included in photograph to aid in any measurements. I use a 10 cm long piece of ¾ inch white PVC and clip this to my camera.
Fixative*	Preserves coral samples for histopathology
Mixing bottle	To mix and dilute fixative

Plastic gloves	To protect your hands when working with fixatives
Plastic Jars	Hold coral samples for fixation in the field. 100 ml urine collection jars are cheap and work well.
Sharpie	Label plastic bags and jars
Plastic bucket	Holds field gear in one place in boat or field
Clipboard	Used to hold underwater data sheet. Make sure your data sheets are well secured to the clipboard. I attach a regular No. 2 pencil to the clipboard using cable ties and rubber tubing.
Underwater paper	Used to record data on underwater data sheet
Decalcifying solution	Used to decalcify coral prior to trimming tissues for histopath. We use CalEx II from Fisher Scientific but other commercially available decalcification solutions will also work.



WORKING WITH FIXATIVES: The optimal fixative for corals is Zinc Formalin available from [Anatech](#) (Z-fix concentrate Cat. 171). If this is not available, then 10% neutral buffered formalin is the next best practical and available reagent. This can be made by mixing 15 parts of 37% formaldehyde with 85 parts of seawater. Fixatives are HAZARDOUS and must be manipulated in well ventilated areas using rubber gloves.

The process at a glance



1) Pre-dive: Prepare collection bags, gear, data sheets, and camera.



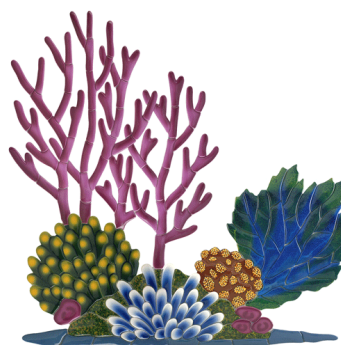
2) Dive: Photograph lesions, collect samples, record data



3) Post-Dive: Photograph fragments, fix samples, record data.



4) Histology prep: Curate and label samples, decalcify, trim for histopathology.



PRE DIVE

- 1) **Prepare collection bags:** Pre-number bags 1-20 (or more if you plan on collecting more corals during a particular dive). In my experience, 20 bags is usually sufficient for a single dive. If using whirlpaks, make sure to remove the plastic tabs.
- 2) **Clipboard:** Ensure your underwater paper and pencil are properly secured onto your clipboard and that you have an extra pencil with you.
- 3) **Tools:** Rongeurs, corer, chisel should all be in a single tool bag clipped to your BC. Hammer can be clipped separately on BC.
- 4) **Camera:** Ensure batteries are charged, memory card is installed, housing is watertight, and settings are adequate. Ensure you have a scale with you. A 10 cm length of white PVC pipe is what I use.



DIVE

- 1) **Underwater:** White balance your camera at the working depth (see manual white balance in camera instructions). Doing this will greatly improve the color fidelity and quality of photos.



Photo with no white balance. Note how everything is blue and washed out.



Photo with white balance. Note greater variety and fidelity of colors.

- 2) **Photographing corals:** When encountering a diseased coral, pull out a pre numbered plastic bag (order does not matter), photograph the colony with the bag, and record the bag number. This will help you sort out later what samples go with what photos. I like to take photos of the entire colony and depending on size of the lesion, medium and close up photos. Use of a scale bar in the photo is useful as it serves as a reference if you want to do further analyses regarding size of lesion or colony. Ensure you take multiple photographs as some will inevitably not turn out. The photos are **EXTREMELY IMPORTANT** as they provide a documentation of gross lesions and are essentially analogous to gross necropsy observations.



Photo with numbered bag to help keep track of samples

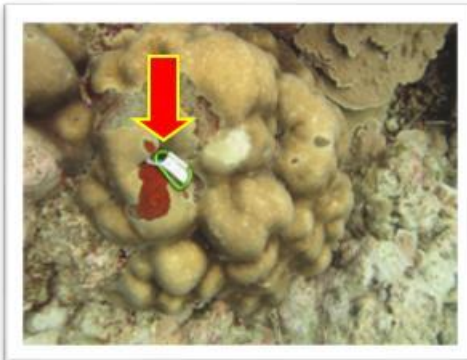
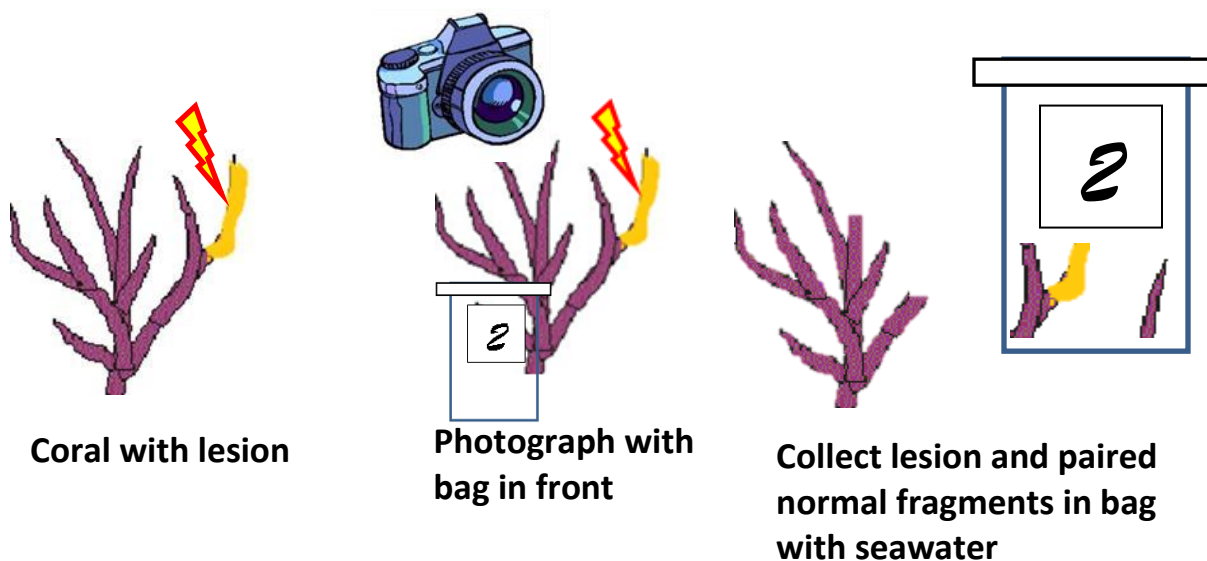


Photo of colony with scale bar (arrow)

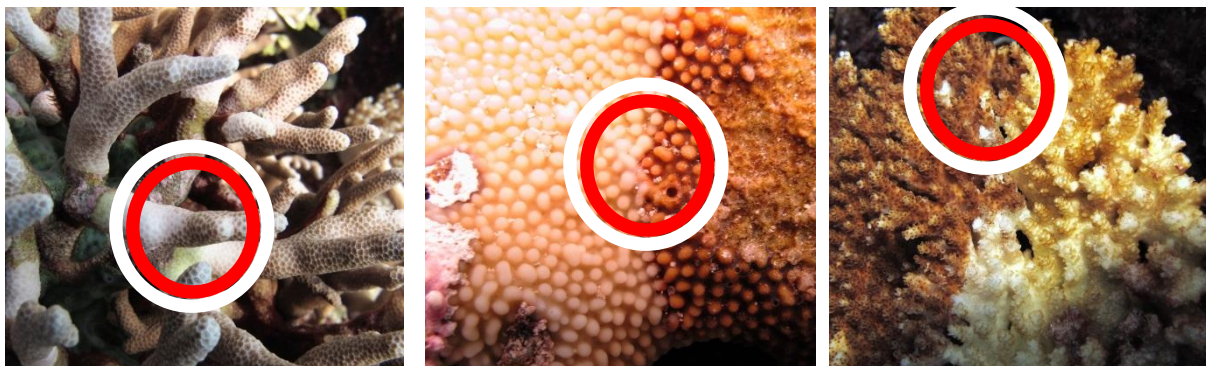


Close up of lesion

- 3) **Sample corals:** Corals have various morphologies ranging from delicate branches to massive stone-like structures. The challenge in sampling is to ensure you can get adequate samples with minimal damage to the colony. As such, it is important to use good judgment, and if a lesion is positioned so that sampling would severely damage the colony, don't sample it. Use the tool appropriate to the morphology of the colony. Rongeurs are ideal for sampling coral branches whereas chisels and corer are better for massive colonies. When sampling lesions, ensure you have both normal tissue and lesion tissues in your fragment. It is also important to take a paired "normal" or non-lesion sample from the same colony as this will aid histopathology interpretation.



! **CAUTION:** Many times, folks think they are sampling live tissues but actually it is all dead and there is nothing to process for histology. This is particularly a problem with tissue loss. Pay attention to where the margin of live tissue is located. Some examples photos of where you want to actually sample (circled).



- 4) **Place samples in bags:** The numbered bag you used to take your photograph is the one where the paired normal and diseased samples will go. Ensure that the collection bag is sealed with plenty of seawater to shield the samples as they are transported in the goody bag.



Bag should be sealed with plenty of sea water to protect coral fragments during the dive

- 5) **Record data:** Record the dive number, date, bag number, a brief (1-3 word) description of lesion, and colony genus/species on your data sheet.

Date: 3 June 2013

Location: Woleai

Dive 1

Bag 6-A. cytherea acute tissue loss

Bag 1-P. compressa discoloration

Bag 3-P. compressa subacute tissue loss

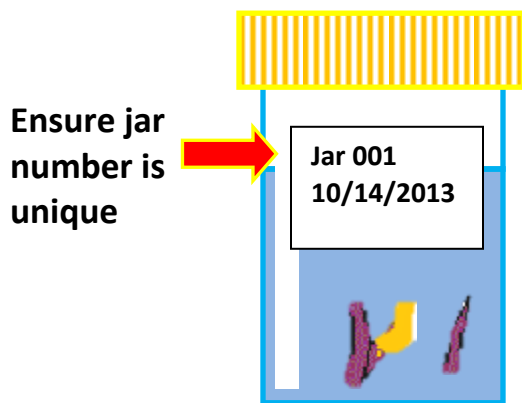
POST DIVE

Once back in the dive boat, it is critical that coral fragments get placed in fixative as quickly as possible. Do this in the following steps:

- 1) **Photograph the fragments:** This is important because corals will lose their color on fixation. Having a photograph of the actual fragments and lesion will greatly aid you when you are decalcifying and trimming corals for histopathology to guide how to trim the tissues. I generally have a standardized way of photographing fragments with the normal on the left and lesion on the right.



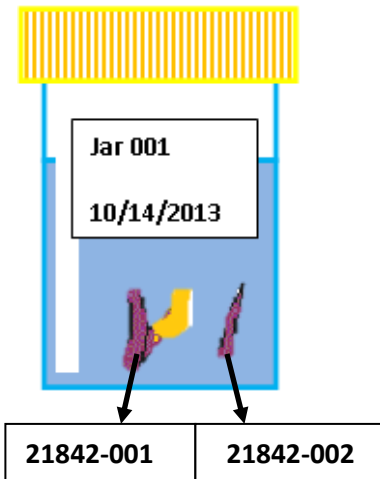
- 2) **Fix the fragments:** Place the fragments in a jar and fill the jar with fixative (at least 2 times or more of volume of coral fragments). I assign a unique number to each jar that serves as an identifier for those samples. I then record this number next to the bag number so I can reconcile samples to photos at the end of the day. DO NOT use the bag number for the jar unless you plan not to re-use the plastic bags (I do).



Date:	<u>3 June 2013</u>
Location:	<u>Woleai</u>
<i>Dive 1</i>	
<i>Bag 6-A. <u>cytherea</u> acute tissue loss (Jar 001)</i>	
<i>Bag 1-P. <u>compressa</u> discoloration (Jar 002)</i>	
<i>Bag 3-P. <u>compressa</u> subacute tissue loss (Jar 003)</i>	

Record jar data next to bag on data sheet

- 3) **Label and curate photos:** At the end of the day, download your photos, label your photos, and record information that accompanies samples on spreadsheet (see Data Handling). I assign a unique identifier to each sample.



Assign unique numbers to fragments



Download and Label photos

Enter data in spreadsheet

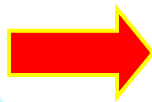
	A	B	C	D	E	F	G	H	I
1	Case	Acc	Date	Bag	Jar	Species	Lesion	Dive	Location
2	21824	1	6/23/2013	6	1	A. cytherea	Normal	1	Woleai
3	21824	2	6/23/2013	6	1	A. cytherea	Tissue loss	1	Woleai
4									

- 4) **Shipping fragments:** If you plan to mail or ship fragments, drain all fixative from the jar and pack the jar with paper towels moistened with fixative. This prevents them from jostling around during transport and getting damaged.

HISTOLOGY PREP



Decalcify




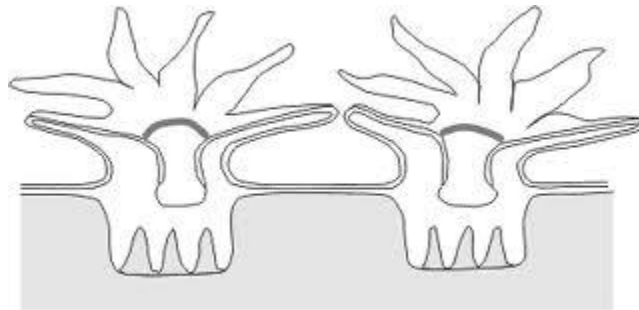
Process for routine paraffin embedding and H&E staining



Examine under microscope

- 1) **Decalcification:** We use Cal-Ex II from Fisher Scientific that is a mix of formic acid and formaldehyde. This normally decalcifies within 2-3 days. If you can't get it, then any other commercially available tissue decalcifying solution will work. EDTA also works but takes a lot of time (days to weeks).
- 2) **Trimming:** Coral tissues are trimmed like any other animal tissues. You may want to refer back to the photos of the fragments you took above to ensure you are trimming across the actual lesion in the section. Tissues are placed in cassettes, and we send ours to a commercial histology lab that embeds them in paraffin, sections them, and stains them with hematoxylin and eosin.

 **NOTE:** For some genera (Acroporidae), tissues will collapse completely after decal, so you trim as best you can looking at the photos of the fresh fragments (see above). In other genera (*Porites*, *Pavona*, *Astreopora*), tissues retain their architecture. In those cases, we trim sagittal sections to visualize epidermis to skeleton.



Sagittal section of corals

- 3) **Examine under the microscope:** This is where the real work begins. You can only learn to properly interpret lesions by “pushing glass”, but in the end, you will find the effort worthwhile. Many papers on coral histology exist in the literature, and a good place to start learning about coral histology is available by clicking [here](#).

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