

Collecting Marine Gnathiid Isopod Fish Parasites with Light Traps

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Abstract

A method to collect marine gnathiid isopod fish parasites with the use of light traps is presented. Gnathiid isopods are a major group of marine fish parasites that feed on blood and fluid from host fishes, mostly at night. Like ticks and mosquitos on land, they associate only temporarily with their host and spend most of their life free-living in the benthos. Given their high mobility and transient and predominantly nocturnal association with hosts, they cannot easily be collected by capturing free-living hosts. However, they are readily attracted to underwater light sources, creating the opportunity to collect them in light traps. Here the design and individual steps involved in the deployment and processing of specially adapted light traps for collecting free-living stages of gnathiid isopods are outlined. Sample results and possible modifications of the basic protocol for a variety of different sampling needs are presented and discussed.

Introduction

Parasitic crustaceans are important in the ecology and life histories of reef fishes. The biomass and energy they remove from their hosts are considerable and influence behavior, physiology, and survivorship¹. Gnathiid isopod crustaceans represent the most prominent group of fish parasites in tropical and subtropical reef systems, where they are both abundant and diverse^{2,3} and are the primary food item of cleaner fishes^{4,5}. Gnathiids are generally 1-3 mm in size. They have unusual life histories in which only the three

juvenile stages feed on the blood and body fluids of fishes^{6,7}. They are most active at night^{8,9}, and while vision appears to play some role, host-finding¹⁰ relies heavily on olfactory cues to find hosts^{11,12}. Each of the three juvenile feeding stages feeds on a single host fish, with each feed separated by a molting phase. After the final feed, third-stage larvae metamorphose into non-feeding adults, which reproduce and then die. Given that feeding requires only brief association

with the host, while each inter-feeding interval lasts days, gnathiids spend most of their life free-living in the benthos.

Gnathiids impact hosts in multiple ways¹. Aside from their role as drivers of interactions between cleaner fish and clients^{13,14,15}, gnathiids can increase cortisol levels and decrease hematocrit in adult fish hosts¹⁶ and in high numbers, can even cause death¹⁷. For juvenile fish, even a single gnathiid can be fatal^{18,19,20}, and even if the fish survives, its ability to compete for space and escape predators is compromised^{20,21,22}. Avoiding gnathiids may even constitute one of the benefits of nocturnal migration in some reef fishes²³.

In addition to cleaner fishes, gnathiid populations can be impacted by other micro carnivorous fishes²⁴, as well as corals^{25,26}. Ocean warming and the associated loss of live corals appear to have opposing impacts on gnathiids^{27,28,29}.

Given their clear ecological importance and the likely influence of anthropogenic environmental change on their populations, there are compelling reasons to include them in ecological studies of coral reefs. However, their unique life history and the small number of researchers who study them create a barrier to the development, implementation, and dissemination of reliable, reproducible sampling methods to collect them for research.

Light traps have long been used to collect small marine organisms at night^{30,31}. They take advantage of and are based on the fact that many nocturnally active organisms, including arthropods, are attracted to light. Traditionally they have been used to collect planktonic organisms in the water column³⁰. However, the basic principles can be applied to collecting free-swimming organisms that are active near the benthos. Here we present a light trapping method adapted for collecting free-living stages of gnathiid isopods near the ocean bottom in remote coral reef environments such as the Philippines. For collecting in remote areas, these light

traps (**Figure 1**) offer some advantages over other methods developed for collecting these organisms³². They are highly portable and durable, requiring only three parts, which are easily obtainable and inexpensive. They are also negatively buoyant, as when deployed, they are completely filled with seawater. Because they depend on light for attraction, they are only effective at night for collecting nocturnally active species. They also attract more than the target species, requiring sorting of the samples under a dissecting scope to obtain the target organisms. Three methods have thus far been used by our team and collaborators to collect gnathiids in coral reef systems throughout the world³². These include emergence traps, live fish-baited traps, and light traps, each with advantages and limitations.

Protocol

Collection of samples was permitted by the Department of Agriculture-Bureau of Fisheries and Aquatic Resources (0154-18 DA-BFAR) in accordance with Philippine laws and regulations (RA 9147; FAO 233) and approved by the Silliman University (SU) animal ethics committee.

1. Light Traps

1. Construction

1. Construct light traps from commercial polyvinyl chloride (PVC) tubes originally designed for plumbing. Use 10-15 cm diameter PVC cut to 30-40 cm length (**Figure 1**).
2. To both ends of the tubes, add PVC "caps" with a transparent acrylic funnel inserted in the center of the opening and glue in place with transparent epoxy glue (**Figure 1**). Let it dry.

3. Ensure that one end of the tube has a screw-on or otherwise removable lid and that both ends are water-tight when the trap is "closed" (e.g., with the addition of an O-ring).
2. Light source
 1. Before deployment, turn on an underwater light/torch (see **Table of Materials**) and place it in the tube, facing one of the transparent funnels, such that the light from the underwater torch illuminates the area in front of one side of the tube. If necessary, chemical glow sticks can be used in place of underwater torches, although their light intensity is lower.

NOTE: The light attracts a variety of small nocturnal organisms³¹, including gnathiids, and drives them to swim into the tube through the clear funnel. Once they have entered the tube, they are unable to escape due to the geometry of the light trap (small funnel opening) and the continued presence of a light source.
3. Placement
 1. When in the water at the deployment site, fill light traps, with the light turned on, with seawater, and secure both ends. To ensure that the torch is not below or blocking the funnel tip, tilt the "front" of the tube upward to allow the torch to slide back away from the funnel.
 2. Place traps on the seafloor, in the sand or rubble, next to coral heads or other complex structures known to attract fish. Focus the light cone "inwards", towards areas where fish aggregate.

NOTE: In shallow water, traps can be placed by breath-hold diving. Deeper deployment requires scuba.

4. Retrieval

1. Immediately prior to retrieving the trap, seal the openings of both the funnels (on either end of the tube) with a piece of modeling clay or rubber stopper sealing, keeping all the seawater and the contained organisms inside.

NOTE: The organisms will remain in the trap once the batteries of the lights have expired and the light is no longer illuminated. This provides flexibility when the traps are retrieved ("soak time"). Factors to consider when deciding on soak time are presented below (see Discussion).

5. Transport

1. Once the traps have been retrieved from the bottom, carry them to a boat, or swim ashore.
2. Maintain traps close to ambient seawater temperature once removed from the ocean.
3. Transport them to the laboratory for processing as soon as possible since no gas or water exchange will take place once removed from the ocean.

2 Laboratory processing

1. Storing and filtering of the samples

1. Once the light traps are removed from the ocean and brought back to the lab, empty their contents into buckets with fresh seawater.
2. Add aeration to keep organisms alive until filtering.
3. Filter the contents of the bucket by pouring through a funnel lined with 50-100 μ m plankton mesh, then

empty the contents into a 100 mL container of fresh seawater.

4. Use a pipette to draw from this smaller container to place aliquots of the sample into a Petri dish for microscopy. Repeat until the entire sample has been processed.

2. Gnathiid isopod identification and rearing

1. Because light trap samples attract multiple species of small invertebrates, carefully screen the samples to identify and remove gnathiid isopods. 10-20x magnification is the best for this task (**Figure 2**).

NOTE: Identifying gnathiids at the family level does not require living specimens. However, adult gnathiids, which are seldom caught in light traps, are needed for morphological species identification and breeding (see reference^{1,3,9} for a methodology for breeding and rearing gnathiids in captivity).

2. In cases where gnathiids need to be kept alive for rearing, gently remove them with a pipette and place them in small plastic containers of fresh seawater.

Representative Results

For sampling in the central Philippines, the outlined trap design (**Figure 1**) was used. When 36 traps were set

overnight (in one site), 1 to 1343 gnathiids per trap (275 ± 54) were collected. These included both fed and unfed juvenile stages (**Figure 2; Table 1, 2**). These results demonstrate the effectiveness of light traps for collecting gnathiid isopods under the study conditions. **Figure 3** shows the placement of the trap underwater.

This method for gnathiid collection is effective and sufficiently flexible for various field sites and scientific questions. For example, reference²⁹ used light traps to quantify the effects of coral cover and fish biomass on gnathiid abundance (**Figure 4**), and reference³³ used similar traps to quantify the effects of hurricanes on genetic haplotypes (**Figure 5**). While other techniques have been developed and used for collecting gnathiids³², this technique is particularly efficient (**Table 3**). Emergence traps, "tents" made of plankton mesh³² require large platforms to transport, are difficult to deploy, and tear easily, requiring continual repair. Moreover, they rarely collect fed juvenile stages. Traps baited with live fish³² (necessary because gnathiids will not feed on dead fish), require capture and housing of live fish. This makes their use more difficult in remote places. Moreover, their effectiveness relies on olfactory cues^{11,12}, which are influenced by the size of the fish, the size of the openings of the trap, and water currents. They tend to collect fewer gnathiids, are more difficult to retrieve than light traps, and only collect fed stages.

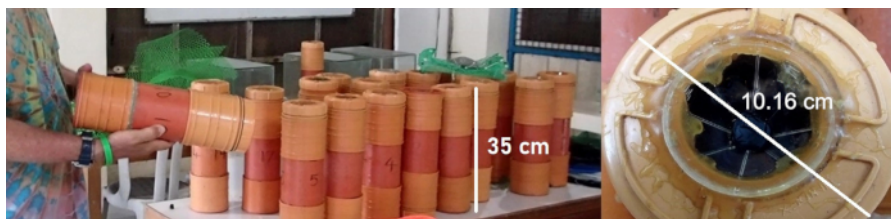


Figure 1: Light trap PVC tubes. Right: top view, with the translucent funnel glued into the tube with acrylic glue, visible.

[Please click here to view a larger version of this figure.](#)



Figure 2: A gnathiid isopod in a Petri dish after capture. Note the transparent body fluid of the fish and brownish red fish blood in the gnathiid's gut. [Please click here to view a larger version of this figure.](#)



Figure 3: A modified light trap (foreground) in which the light is directed down. This design can be used in calm sea conditions to sample more directly the substrate below the trap. Emergence traps are shown in the background. This figure is reprinted with permission from reference³². [Please click here to view a larger version of this figure.](#)

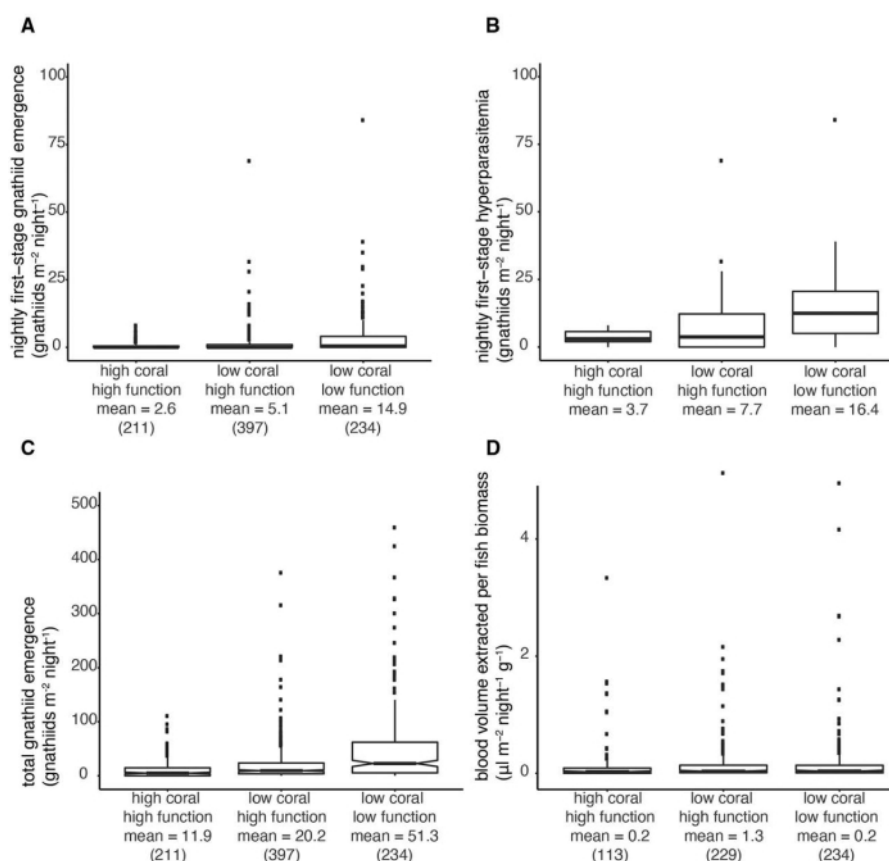


Figure 4: Boxplots showing differences in the distribution. Boxplots showing differences in the distribution (A) of first-stage gnathiid abundance, (B) first-stage hyperabundance, (C) total abundance, and (D) blood volume extracted per fish biomass. The sample size for each subgroup is shown in parentheses under each subgroup mean. Boxes display first- and third-quartile edges, while whiskers show the third quartile plus 1.5 times the interquartile range. For (C), hinges are an approximation of the 95% confidence interval. Differences seen at (A), (B), and (C) are significant; see Appendix S1: Table S7 in reference²⁹. This figure is reprinted with permission from reference²⁹. [Please click here to view a larger version of this figure.](#)

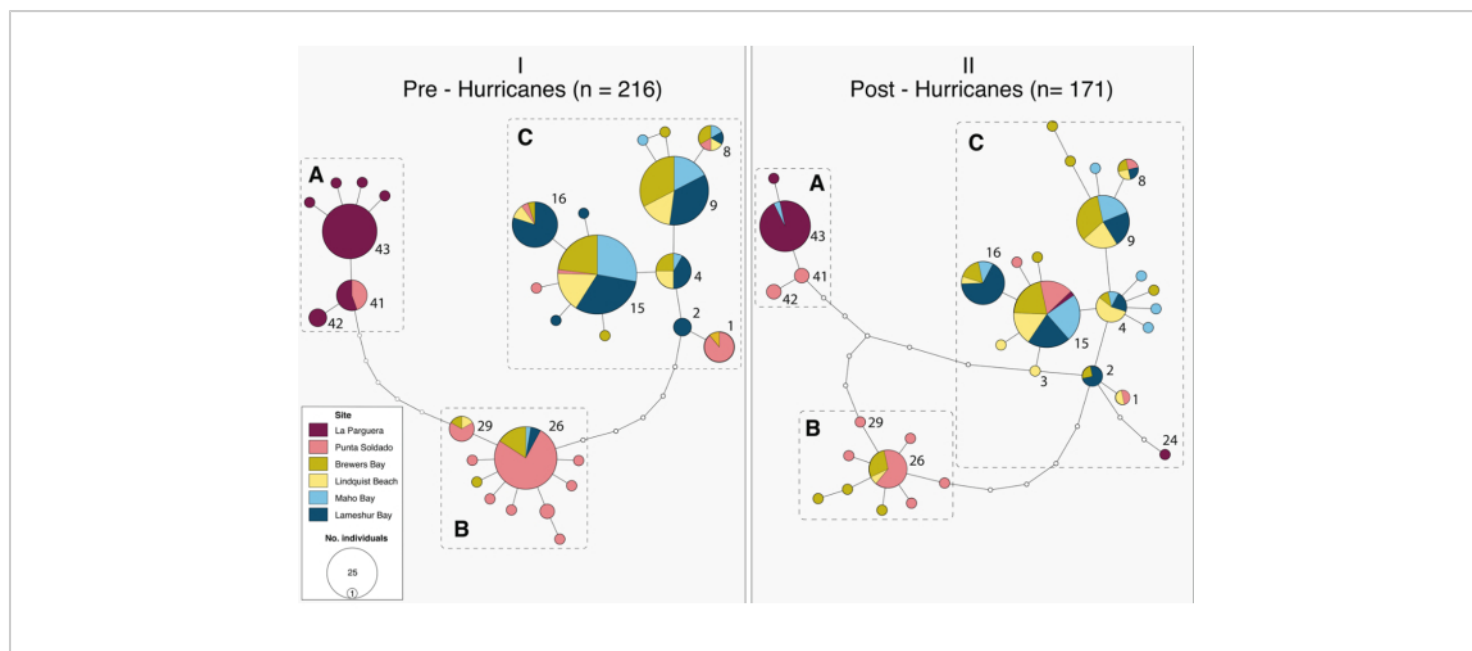


Figure 5: Haplotype networks representing the genetic diversity of *Gnathia marleyi* and its spatial distribution before and after the 2017 hurricanes. Pre- and post-hurricane datasets are indicated in panels I and II, respectively. The haplotypes found in both pre- and post-hurricane datasets are indicated by haplotype number. Haplogroups A, B, and C are indicated by dashed boxes. This figure is reprinted with permission from reference³³. [Please click here to view a larger version of this figure.](#)

Gnathiid count	Light traps (n)	Total count	Mean	Median	Minimum (per trap)	Maximum (per trap)	Standard Error (+-)
Total	34	9336	275	191	1	1343	54
Live	34	6605	206	114	4	1226	46
Dead	34	2667	86	42	1	659	24

Table 1: Summary statistics for gnathiid counts from 36 light traps deployed by breath-hold diving overnight in the Bantayan coral reef, Philippines, from July - September 2017. The numerical value in the *Live* column refers to gnathiids from the light trap that were alive at the time of counting, the *Dead* column refers to gnathiids that were dead, and the *Total* column is the sum total of the dead and live gnathiid counts.

Gnathiid count	N	Total count	Mean
Total	10	434	43

Table 2: Summary statistics for gnathiid counts from light traps deployed for 3 hours in Bantayan coral reef, Negros Oriental province, Philippines, from July-August 2022.

Trap type	Median count	95% CI	
		Lower	Upper
Un-baited Emergence	0.31	0.04	0.81
Fish-baited Emergence	0.42	0.19	0.69
Fish-baited Tripod	0.92	0.46	1.46
Open-mesh Fish-baited	1.5	0.35	3.54
Lighted Plankton Trap	5.69	2.69	9.58

Table 3: The performance of different trap designs, including light traps, in the Caribbean were compared. Estimates of median count per sample and 95% confidence intervals for each trap design were evaluated in the multi-trap comparison. Estimates were derived from 10,000 bootstrapping iterations drawing with replacement from the 26 sample counts for each trap type. This table is adapted with permission from reference³².

Discussion

Traditional light traps, such as those used for collecting larval fishes, are large and are suspended in water column³⁴. In contrast, the light traps described here are small and deployed on the sea bottom. These traps can be easily transported and rapidly deployed. They can be placed by breath-hold (free)-diving in shallow sites (as in this study) or on scuba in deeper sites, and attract both fed and unfed juvenile stages.

Variations of the benthic light traps described here for studying gnathiids have been used for a variety of purposes by our team and collaborators, and modifications can be

made in the design or protocol, depending on the specific purpose (**Figure 3**). These are described below.

Some studies require live gnathiids. These include studies identifying the source (fish species) of blood meals^{35,36}, or studies that require rearing. The light traps in this study were 30 cm long, with a 10.16 cm diameter, and hence a volume of ~1.3 L. When they become overly crowded with organisms, the oxygen demand inside the tube can surpass the delivery of oxygen via the ports, causing organisms to die and exacerbating the problem. To avoid this, the trap can be deployed for shorter periods or modified by increasing its volume (larger diameter PVC) or adding additional holes covered with plankton mesh^{9,29,32}. Note that while larger diameter PVC can be used to increase the volume, this is

done at the cost of lower portability, resulting in the transport of fewer traps to the field site. This is of particular concern when the transport involves extended surface swims and/or the use of small boats with limited storage space.

Alternatively, or in addition, the trap can be deployed for a shorter duration. A major advantage of this design is that while the trap will only collect organisms at night (when the light is effective), it can be set at any time and retrieved at any time. To collect as many gnathiids as possible, we set traps before sunset and retrieved them shortly after sunrise the following day. However, to maximize the number of live gnathiids, we limit deployment to 3-4 hours of darkness. A short set time also reduces the amount of "bycatch" that must be sorted through to extract the gnathiids.

The traps described here lie horizontally, with the beam of the light pointing in one direction. Thus, the stimulus signal extends as far as the light will shine, usually traversing multiple substrate types, and some light is emitted from the rear funnel as well. In order to focus sampling on a particular substrate at a short distance from the trap, the trap can be modified to sit vertically, with the light pointing downward (**Figure 3**). This is accomplished by adding "legs" to form a tripod^{29,32}. However, this type of light trap is useful only in low-current situations.

The torch we used (see materials table) takes four (4) AAA batteries. We use rechargeable batteries to reduce cost and waste. The burn time for these lights is approximately 12 hours, which allows them to function overnight. However, a suitable substitute is "glowsticks". These are particularly useful when setting in deeper water, such as mesophotic reefs.

While the light traps described here attract a wide range of small, mobile invertebrates, the composition of this bycatch is highly variable. However, when set in a habitat that includes rubble and fish, gnathiids are caught consistently. Although this demonstration study focused on sites in the Philippines, traps of similar design have also been used successfully for studies at sites in the Caribbean region and the Great Barrier Reef.

Disclosures

The authors declare no disclosures to make.

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