Behavioral assays to assess the ability of G. marleyi to detect and preferentially differentiate between Caribbean reef fish hosts in John Brewers Bay, St Thomas, US Virgin Islands

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Project
» Beyond Cleaning and Symbiosis: Ecology of 'Ticks of the Sea' on Coral Reefs (Gnathiid isopod ecology)

Contributors	Affiliation	Role
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Abstract
Gnathiid isopods are common crustacean parasites that inhabit all oceans from shorelines to depths of over 3000 m and use chemical cues to find their marine fish hosts. While gnathiids are host-generalists, hosts vary in their susceptibility to infestation. However, the mechanisms that mediate differential susceptibility are unknown. Here we used laboratory experiments to investigate if the chemical attractiveness of hosts explains differences in susceptibility of Caribbean reef fishes to infestation by a common Caribbean gnathiid isopod, Gnathia marleyi. We showed that while G. marleyi can detect and locate hosts using only chemical cues, they do not exhibit a preference for chemical cues produced by more susceptible fish species. We conclude that species-specific chemical cues are not the main mechanism driving differences in host susceptibility to gnathiid isopod infestation and that visual or post-attachment factors such as ease of obtaining a blood meal are likely mediators.

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Coverage

Spatial Extent: **Lat**:18.333 **Lon**:-64.966
Temporal Extent: 2019-07-05 - 2020-03-10

Methods & Sampling

Host species:

Host fishes selected for testing were French grunt (*Haemulon flavolineatum*: Haemulidae), lane snapper (*Lutjanus synagris*: Lutjanidae), longspine squirrelfish (*Holocentrus rufus*: Holocentridae), and longfin damselfish (*Stegastes diencaeus*: Pomacentridae). French grunt and lane snapper are carnivorous, nocturnal fish that forage in seagrass and sandy areas at night and school on reefs during the day. Both species are highly and similarly susceptible to gnathiids. French grunts are locally abundant and are known to be able to attract gnathiids with only chemical cues and therefore were used as a standard for the experiments (see below). Longspine squirrelfish are nocturnal invertivores that forage at night and shelter in or near refuges on reefs during the day. Finally, longfin damselfish are diurnal herbivores that are highly territorial. Longspine squirrelfish and longfin damselfish represent moderate and low susceptibility to gnathiids, respectively.

Field collection:
Juvenile *Gnathia marleyi* and adult host fishes were collected from John Brewers Bay, St. Thomas, United States Virgin Islands (18°20’ N, 64°58’ W) from May to August 2019 and January to March 2020. Gnathiids were collected using zooplankton light traps similar to those described by Artim and Sikkel (2016). They were held at the University of the Virgin Islands’ MacLean Marine Science Center in small colonies in 150-milliliter (ml) containers filled with filtered seawater. All host fishes were caught by free divers using cast nets and were held in a 2800-Liter tank with continuously flowing seawater, at 28 degrees C. Fish were kept for no longer than 24 hours and all were returned to the original capture site.

Behavioral assays:
To assess the ability of *G. marleyi* to detect and preferentially differentiate between hosts of different susceptibility an Atema choice flume was used. The Atema flume allows two separate sources of water to flow through a choice arena without mixing. Within the choice arena, an organism is able to move freely between the two sources. Water was fed at 100 milliliters per minute into the choice flume. Preliminary trials indicated that this flow rate was high enough to provide a laminar flow within the flume but low enough to allow a gnathiid to swim freely without being impeded by the water flow. A single gnathiid was placed in the center of a ‘choice arena’, allowing it to move freely throughout the arena and/or swim towards a preferred water source. Immediately after placing in the flume, a two-minute habituation period began followed by a three-minute testing period. During the testing period, the position (left or right side of the flume) was recorded using a Canon EOS Rebel T5i digital camera under near-infrared lighting. Due to recording limitations, only the larger, third-stage unfed, juvenile gnathiid isopods were used in behavioral assays. Preliminary experiments showed that gnathiid isopods did not react to and exhibited typical behavior patterns under infrared or near-infrared lighting. All trials were conducted between dusk and dawn to assure they were done during peak gnathiid activity. After the testing period, the gnathiid was removed for one minute while the flume was flushed, and the sources were exchanged. The habituation and testing periods were then repeated with the same individual to ensure a side preference was not being displayed.

To determine behavioral preferences toward specific chemical cues, filtered seawater (200-µm) was conditioned using host fish. Before conditioning, all fish were inspected to ensure that there were no parasites currently infesting the fish. To condition seawater with host chemical cues, a single host fish (French grunt: n = 8, size range: 51–128 millimeters (mm); lane snapper: n = 5, size range: 80–120 mm; longspine squirrelfish: n = 8, size range: 80–130 mm; longfin damselfish: n = 12; size range: 56–92 mm) was held in an aerated closed 15-liter aquarium for 60 minutes. When comparing the chemical cues of two fish species simultaneously, individuals of a similar size were paired together. Before assays, water types were randomly assigned to either side of the flume.

To confirm the ability of gnathiid isopods to detect host chemical cues, gnathiids (n = 15) were offered the choice between water conditioned with the chemicals of French grunt against untreated filtered seawater. To determine if gnathiids have a preference for hosts of higher susceptibility, gnathiids were offered a choice between French grunt and another fish. Trials conducted included: 1) French grunt versus lane snapper (high vs high susceptibility); 2) French grunt versus longspine squirrelfish (high vs medium susceptibility); and 3) French grunt versus longfin damselfish (high vs low susceptibility). In total, 45 (15 each) behavioral assays were conducted between the three comparison species. Each assay consisted of two 180-second trials with 360 seconds worth of behavioral recordings for each gnathiid.

Data Processing Description

Statistical analysis: All statistical analyses were performed using RStudio (Version 1.2.1335). For behavioral assays, the time spent in the test species water within an Atema flume did not conform to a normal distribution and could not be transformed to fit a normal distribution, so a Kruskal-Wallis test was used to compare the time spent in test species water between treatment level. A Dunn’s Multiple Comparison Test was then used to

determine any differences between treatments (e.g. Control versus lane snapper, lane snapper versus longspine squirrelfish, etc.). Independently, Kolmogorov-Smirnov tests were used to evaluate the differences in chemical preference within each treatment level (e.g. French grunt versus each level of susceptibility).

Video data was collected using a DLSR camera. Video data was analyzed manually. Statistical analyses were conducted in RStudio (Version 1.2.1335).

BCO-DMO Processing Description:
- Missing data identifier 'NA' replaced with 'nd' (BCO-DMO's default missing data identifier)
- Adjusted field/parameter names to comply with BCO-DMO naming conventions
- Added a conventional header with dataset name, PI names, version date
- Added "date" column and converted dates to ISO date format (YYYY-MM-DDThh:mmZ)

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Data Files

File
lab_chemosensory.csv (Comma Separated Values (.csv), 1.86 KB) MD5:2e6cb8468c76738c7b71e6079e4fe39f Primary data file for dataset ID 887279

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Related Publications

Artim, J. M., & Sikkel, P. C. (2016). Comparison of sampling methodologies and estimation of population parameters for a temporary fish ectoparasite. International Journal for Parasitology: Parasites and Wildlife, 5(2), 145–157. https://doi.org/10.1016/j.ijppaw.2016.05.003
Methods

RStudio Team (2019). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. URL http://www.rstudio.com/.
Software

Santos, T. R. N., & Sikkel, P. C. (2017). Habitat associations of fish-parasitic gnathiid isopods in a shallow reef system in the central Philippines. Marine Biodiversity, 49(1), 83–96. https://doi.org/10.1007/s12526-017-0756-6
Methods

Sikkel, P. C., Sears, W. T., Weldon, B., & Tuttle, B. C. (2011). An experimental field test of host-finding mechanisms in a Caribbean gnathiid isopod. Marine Biology, 158(5), 1075–1083. https://doi.org/10.1007/s00227-011-1631-9
Methods

Vondriska, C., Dixson, D. L., Packard, A. J., & Sikkel, P. C. (2020). Differentially susceptible host fishes exhibit similar chemo-attractiveness to a common coral reef Ectoparasite. Symbiosis, 81(3), 247–253. https://doi.org/10.1007/s13199-020-00700-0
IsRelatedTo

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Parameters

Parameter	Description	Units
Trial	Trial number	unitless
Treatment	Host used to condition water with chemical cues in comparison (Control = unconditioned; Ls = Lutjanus synagris; Hr = Holocentrus rufus; Sd = Stegastes diencaeus)	unitless
Date	Date of laboratory experiment	unitless
Time_Hf	Time in seconds spent in water conditioned with the chemical cues of Haemulon flavolineatum	unitless
Time_Treatment	Time in seconds spent in water conditioned with the chemical cues of the treatment cell	unitless

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Instruments

Dataset-specific Instrument Name	Canon EOS Rebel T5i digital camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Host-attraction trap
Generic Instrument Name	Fish Cage
Dataset-specific Description	Host-attraction traps: PVC tubing with a reversed funnel on each side. An individual host is placed within the trap to test chemical attraction for gnathiids.
Generic Instrument Description	Used to catch fish.

Dataset-specific Instrument Name	Lighted plankton trap
Generic Instrument Name	Plankton Net
Dataset-specific Description	Lighted plankton traps: PVC tubing with a reversed funnel. A light is shined out of the funnel to attract plankton (including gnathiids) into the trap.
Generic Instrument Description	A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available.

Dataset-specific Instrument Name	
Generic Instrument Name	Swimming Flume
Dataset-specific Description	Two-channel choice flume: an instrument for performing behavioral assays related to chemical detection.
Generic Instrument Description	A tool used to analyze and quantify fish swimming behavior, physiology, and performance.

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Project Information

Beyond Cleaning and Symbiosis: Ecology of 'Ticks of the Sea' on Coral Reefs (Gnathiid isopod ecology)

Coverage: Eastern Caribbean, Philippines, Australia

NSF Award Abstract:
Most research on the complex biological interactions that inhabit coral reefs has focused on larger organisms that are easily observed by divers. However, marine scientists are increasingly aware of the importance of the tiny organisms that make up the "smaller majority." This includes parasites, organisms that feed on other organisms without killing them, which may make up as many as 80% of the species on coral reefs. Among the most important parasitic organisms on coral reefs are gnathiid isopods, so-called 'ticks of the sea', that share many similarities with blood-feeding ticks and other arthropods on land. Like ticks and mosquitoes, gnathiids transmit malaria-like blood parasites. In high numbers, they can remove enough blood to kill adult fish, but even a single gnathiid can kill a juvenile fish. Thus, gnathiids may have a significant effect on coral reef communities through their effects on coral reef fishes. This project will use an integrative interdisciplinary approach involving field and laboratory observations and experiments, and molecular tools. In addition to contributing to our understanding of life in our oceans, this research will provide continued support for U.S. Doctoral and Masters students and will create valuable research opportunities for undergraduates from multiple institutions. The project will further build on the investigators existing relationships with resource managers, local divers, fishers, and boat operators, as well as K-12 schools and environmental education programs, and will contribute to local economies. A major goal of our outreach efforts will include an exhibit featuring our research at Coral World Ocean Park on St. Thomas, participation in Virgin Islands radio programs, and hosting high school students from South Carolina Governor's School.

The overall goal this investigation is to understand the ecology of fish-parasite interactions on coral reef and associated ecosystems. This project focuses on fish-parasitic gnathiid isopods, the most common ectoparasites of coral reef fishes that are best known for their role in cleaning symbiosis, as the major food item of cleaner fishes. However, their abundance, host range, role as micropredator, disease vector, and potential prey item for other species, as well as their strong association with the benthos suggests the potential for much stronger community impacts. The goals for this project are to: 1) characterize the factors influencing local gnathiid isopod density by examining the role of fish-hosts, benthic cover, gnathiid predators including cleaners, and gnathiid conspecific attraction; 2) determine and quantify variation in host exploitation and the effects of gnathiid density on larval fish-host recruitment. To accomplish the first objective, the investigators will trap gnathiids from the substrate at sites in the Caribbean, Australia, and the Philippines. Variables associated with benthic habitat as well as local fish communities will be quantified and compared with local gnathiid abundance. Laboratory experiments will be conducted to determine the effects of different host species on gnathiid growth and reproduction and to determine the role of conspecific attraction in the formation of aggregations. Predators of gnathiids will be identified through examination of gut contents and through laboratory feeding studies. To accomplish the second objective, patterns of host-exploitation will be determined by DNA barcoding of blood meals from wild-caught gnathiids and results compared with the availability of different host species. To determine the effects of gnathiids on early life history stages of coral reef fishes, gnathiid abundance will be manipulated on small artificial patch reefs onto which newly-settled reef fishes will be transplanted.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536794

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