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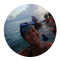
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FISH-PARASITIC GNATHIID ISOPODS METAMORPHOSE FOLLOWING INVERTEBRATE-DERIVED MEAL

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KEY WORDS

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Lettuce Sea Slug
Hermodice carunculata
Bearded Fireworm

ABSTRACT

Organisms with a parasitic lifestyle comprise a high proportion of biodiversity in aquatic and terrestrial environments. However, there is considerable variation in the ways in which they acquire nutrients. Hematophagy is a common consumption strategy utilized by some terrestrial, aquatic, and marine organisms whereby the parasite removes and digests blood from a host. Gnathiid isopods are marine hematophagous parasites that live in benthic substrates from the intertidal to the abyss. Although ecologically similar to ticks and mosquitoes, they feed only during each of 3 juvenile stages and adults do not feed. They have long been considered as generalist fish parasites and to date, there have been no reports of their successfully feeding on invertebrates. Based on observations of gnathiids attached to soft-bodied invertebrates collected from light traps, we conducted a laboratory experiment in which we collected and individually housed various common Caribbean invertebrates and placed them in containers with gnathiids to see if the gnathiids would feed on them. All fed gnathiids were subsequently removed from containers and given the opportunity to metamorphose to the next developmental stage. In total, 10 out of the 260 gnathiids that were presented with 1 of 4 species of potential invertebrate hosts had fed by the next morning. Specifically, 9 of a possible 120 gnathiids fed on lettuce sea slugs (*Elysia crispata*), and 1 of a possible 20 fed on a bearded fireworm (*Hermodice carunculata*). Eight of these 10 fed gnathiids metamorphosed to the next stage (5 to adult male, 2 to adult female, and 1 to third-stage juvenile). Even though feeding rates on invertebrates were considerably lower than observed for laboratory studies on fishes, this study provides the first documented case of gnathiids' feeding on and metamorphosing from invertebrate meals. These findings suggest that when fish hosts are not readily available, gnathiids could switch to soft-bodied invertebrates. They further provide insights into the evolution of feeding on fluids from live hosts in members of this family.

Parasitism as a consumer-resource strategy has arisen independently multiple times (Poulin, 2011) and may comprise over 40% of known species (Dobson et al., 2008; Hatcher and Dunn, 2011). Many species that have adopted this lifestyle have evolved to use similar consumptive pathways as traditional predators (sometimes including predators themselves), but through different mechanisms because parasites "eat prey in units less than one" (Wilson, 2014). There is a wide diversity of life-history strategies that parasites use to infest both vertebrate and invertebrate hosts (Lafferty and Kuris, 2002). These relationships can vary in the amount of time spent with hosts (temporary vs. permanent), location of exploitation (ecto- or endoparasites), mode of infestation (direct vs. complex), as well as which host tissues they consume. Within this suite of specialized parasites are parasitic arthropods that have evolved to consume blood (hematophagous; Lehane, 2005).

Over 14,000 species of arthropods are hematophagous (Adams, 1999; Lehane, 2005). This includes well-known terrestrial groups such as ticks (Order: Ixodida), fleas (Order: Siphonaptera), mosquitos (Order: Diptera), and midges (Order: Diptera). These species vary in host specialization, with some infesting a wide range of species and others being restricted to 1 specific host species (Smit and Davies, 2004; Lehane, 2005; Coile and Sikkel, 2013). Permanent parasite species are more often host specialists, and those that are temporary (and more mobile) are more often generalists. For example, there are various louse species that are host specialists (e.g., elephants [Godara et al., 2009]; humans [Nuttall, 1917]; hogs [Clay, 1963]), whereas mosquito *Culex salinarius* bloodmeals are obtained from multiple vertebrate hosts that span orders or even classes (Hayes, 1961; Murphey et al., 1967).

Table I. Summary of gnathiid isopod feeding trials completed per invertebrate species, including the number of successfully fed gnathiids and the result of any subsequent metamorphosis.

Species	Trials completed	Gnathiids added per trial	Fed gnathiids	Molted to			
				Male	Female	Juvenile	Died prior to molt
Hermodice carunculata	2	10	1	0	0	0	1
Elysia crispata	6	20	9	5	2	1	1
Aplysia dactylomela	5	20	0	–*	–	–	–
Octopus briareus	1	20	0	–	–	–	–

* Dash = no data available because no gnathiids fed.

Although these terrestrial hematophagous arthropods are best known and studied, their ecological marine equivalent, the gnathiid isopods (Isopoda: Gnathiidae), are likely more abundant. They are the most common ectoparasites of bottom-associated fishes and occur in marine habitats worldwide, from estuaries and shallow tide pools to the depths of the continental margins (2,900–3,300 m; Smit and Davies, 2004; Tanaka, 2007; Quattrini and Demopolous, 2016). They are only parasitic in each of their 3 larval instars, emerging from the substrate to attach to and remove a blood or body fluid meal from a host. After feeding, they return to the benthos to digest the meal (Smit and Davies, 2004; Tanaka, 2007). After the third and final feeding, they metamorphose into nonfeeding adults. Because of their temporary association with hosts (and thus extensive time spent free-living), and the fact that they feed on up to 3 hosts during their entire larval life, they have been referred to variously as protelian parasites, temporary ectoparasites, and micropredators (Smit and Davies, 2004; Sikkel et al., 2004; Artim et al., 2015).

Gnathiids have been long-regarded as generalist fish parasites, and there are no documented cases of their feeding on aquatic invertebrates, let alone successfully molting after feeding on them. Here, we report observations of gnathiids feeding on soft-bodied invertebrates, and provide the first documented case of gnathiids developing after feeding on an invertebrate host.

MATERIALS AND METHODS

Field collection of gnathiids

Gnathiid isopods (*Gnathia marleyi*; Farquharson et al., 2012) were collected from May 2015 to August 2017 in Brewer's Bay, St. Thomas (18°20′37″N, 064°58′38″W), Lameshure Bay, St. John (18°19′08″N, 064°43′36″W), White Bay, Guana Island (18°28′30″N, 64°34′15″W), Tamarindo Beach, Isla Culebra (18°17′13″N, 65°17′04″W), and Cayo Enrique, Puerto Rico (17°57′11.9″N, 67°2′47.7″W) using benthic light traps (see Artim and Sikkel, 2016 for design) as part of a broader study on factors influencing the abundance and distribution of gnathiids on shallow Caribbean reefs. Light traps were placed on the reef prior to sunset and were retrieved just after sunrise the next morning. In addition to gnathiids, the traps collected a wide range of small mobile invertebrate species, as well as settlement-stage fishes (Artim et al., 2015). After retrieval, samples were sorted under a dissecting microscope and gnathiids were removed.

Laboratory experiments

To assess whether gnathiids would opportunistically feed and could metamorphose after feeding on soft-bodied invertebrates, four commonly occurring soft-bodied invertebrates were collected with hand nets in Brewer's Bay. These included the Caribbean reef octopus (*Octopus briareus*), lettuce sea slug (*Elysia crispata*), spotted sea hare (*Aplysia dactylomela*), and the bearded fireworm (*Hermodice carunculata*). All specimens were placed in individual aerated plastic containers. The octopus was kept in a larger container so that the ratio of body size to container size was similar to that of the other species in the study. Unfed *G. marleyi* were then added at dusk and were checked the next morning. The experiment did not extend through daytime hours because gnathiid activity at this site peaks from dusk to dawn (Sikkel et al., 2009). The trials with *H. carunculata* were conducted with 10 gnathiids each, and the remaining trials were conducted with 20 gnathiids each (Table I). This was because more gnathiids were available for the experiment after the initial trials were already completed. We attempted to rerun the *H. carunculata* trials with 20 gnathiids per trial but we were unable to find any fireworms on the reefs later in the field season. Invertebrates were returned to their habitats and fed gnathiids were maintained in the lab until they molted to the next stage. All procedures followed guidelines and regulations of the government of the Virgin Islands and the University of the Virgin Islands.

RESULTS

During the course of sorting light trap samples, we occasionally observed that gnathiids were attached to soft-bodied invertebrates, particularly polychaete worms (Fig. 1). Although we did not record the frequency of such events, all members of the research team observed this independently.

Out of the 260 gnathiids placed in tanks with invertebrates, 10 were fed the next morning (9 on *E. crispata* and 1 on *H. carunculata*; Table I). No gnathiids fed on *O. briareus* or *A. dactylomela*. The lone gnathiid to feed on *H. carunculata* was dead the next morning but had a partially distended and opaque-colored gut indicative of a fluid meal. Of the 9 gnathiids to feed on *E. crispata*, 5 molted to males (Fig. 2), 1 to female, 1 to third-stage juvenile (P3, the final juvenile instar), and 2 died prior to molting.

DISCUSSION

Although individual species of gnathiid isopods are known to feed on a wide range of fish hosts, they have thus far not been

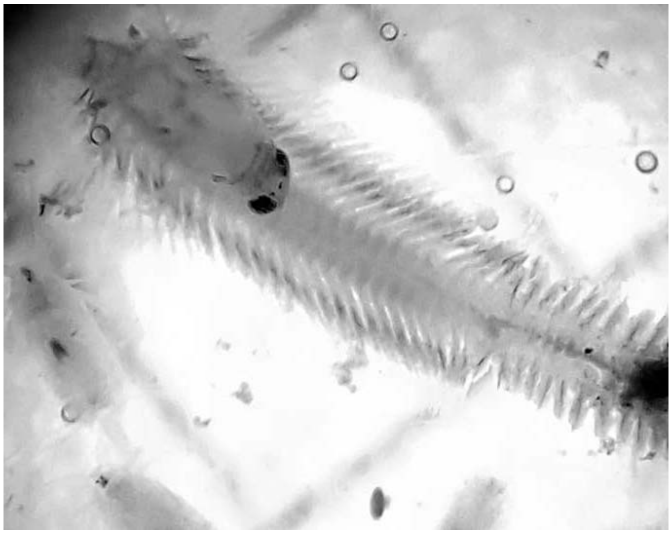

Figure 1. Gnathiid recovered from lighted plankton trap attached to a polychaete worm. Photograph by Gina C. Hendrick.

Figure 2. Male gnathiid that had fed and metamorphosed off of an invertebrate derived blood meal. Photograph by J. Andres Pagan.

reported to feed on and metamorphose from invertebrates successfully. Thus, aside from Monod (1926), which reports a gnathiid being induced to feed on a frog, this is the first documented case of gnathiids feeding on something other than a fish host and certainly the first to show the potential to feed on an invertebrate host. However, other species of hematophagous arthropods have shown plasticity in diet. These include omnivorous mosquitos that consume plant sugars in addition to vertebrate blood (Foster, 1995), as well as adult ticks (*Antricola delacruzi*), which feed on cave-dwelling bats as larvae but appear to feed on bat guano as adults (Ribiero et al., 2012)

There are numerous confounding variables that may result in a gnathiid attaching to an invertebrate in a densely packed environment such as a light trap. Light traps are often retrieved with a high density and diverse assemblage of species. Gnathiids may simply attach to larger organisms to use in lieu of substrate. Further, they may probe surfaces if there are chemical cues from fishes in the water, which is often the case, because our light traps attract settlement stage fishes (Artim et al., 2015). However, our experimental conditions eliminated these potential confounding effects of a light trap environment, demonstrating that at least some gnathiids will opportunistically feed on invertebrates and are capable of completing metamorphosis with invertebrate-derived blood meals.

Gnathiids fed on *H. carunculata* and *E. crispata*, but not on *O. briareus* or *A. dactylomela*. Because we were only able to perform a single trial with *O. briareus*, we cannot confirm with certainty that gnathiids will not feed on them. *Aplysia dactylomela* have thick, "leathery" skin and produce a toxic ink and opaline fluids when irritated, which can be lethal to brine shrimp (Melo et al., 1998) and are effective at deterring predators (Kicklighter et al., 2005; Kicklighter and Derby, 2006). Thus, these secretions, which were present in our experiments, may explain why no gnathiids successfully fed on them. It is unclear whether the *A. dactylomela* specimens released their secretions in response to gnathiid activity or the stress of being out of their natural environment.

Although it is now clear that at least some gnathiids can feed on soft-bodied invertebrates, it is not clear how many species can do so, or whether they also do so in situ. The dominant species at all of our study sites where gnathiids were collected while attached to invertebrates is *Gnathia marleyi*. Moreover, this is the only species found in Brewer's Bay, where all gnathiids used in our experiment were collected. Thus, our observations do not appear to reflect species differences in host preference. *Gnathia marleyi* has been confirmed to feed on at least 37 fish species (Farquharson et al., 2012; Coile and Sikkel, 2013; G. C. Hendrick and colleagues, unpubl. data) and, even though individual light traps yielded hundreds of gnathiids and soft-bodied invertebrates, most unfed gnathiids were not attached to invertebrates. Although gnathiids did successfully feed in the lab experiments, it was a relatively small percentage (*E. crispata* at 7.5% was the highest of the 5 species; Table I). Comparatively, in experiments similar to those reported here but using juvenile fishes, the majority of gnathiids (>90%) successfully fed on the fish host (Artim et al., 2015; Sellers et al., 2019). Another intriguing observation suggesting that gnathiids indeed feed on invertebrates (rather than simply attaching to them) in the wild is that DNA sequences from up to 25% of light-trap caught gnathiids may have come from invertebrates (G. C. Hendrick and colleagues, unpubl. data).

Prior to specializing on fishes, gnathiids may have had a broader diet that included a wider range of hosts. Gnathiids are currently regarded as "generalists with preferences," because although they will indeed infest a range of fish hosts, there are differences in susceptibility among hosts (Coile and Sikkel, 2013), and in the consequences of feeding on different hosts (Coile et al., 2014). The factors that determine the susceptibility of a potential host include abundance (of both parasite and host), spatiotemporal overlaps in activity, physiological responses to infestation, nutritive quality, and the ability of a parasite to locate and successfully infest a host species. *Hermodice carunculata* are most active during daytime hours and are typically hidden when not feeding (Marsden, 1960). Activity patterns of *E. crispata* are less well-known, but phylogenetically similar chloroplast-symbiotic sarcoglossans typically situate themselves on or within algal mats during the day (presumably where they can take advantage of their photosynthetic capabilities) and move to the upper portions of the algae to feed at night (Weaver and Clark, 1981). The cryptic nature of those species, including *O. briareus*, may make it harder to locate them. *Aplysia dactylomela* are nocturnally active, but the previously mentioned ink and opaline secretions may deter both micropredators and predators alike (Kicklighter et al., 2005; Kicklighter and Derby, 2006). Indeed, further experiments are needed to determine whether gnathiids will infest these species on the reef.

For ectoparasites, which are unable to rely on trophic transmission to reach their host, the ability to detect and locate hosts is critical. Gnathiids, especially nocturnal ones, are known to be able to use chemical cues alone to detect hosts in situ (Sikkel et al., 2011; Santos and Sikkel, 2017), and injured hosts are more likely to be infested than noninjured hosts (Jenkins et al., 2018). However, nothing is known about the specific chemical compounds involved in host finding. Our observations, along with those of Shodipo et al. (2019), who reported unfed gnathiids from the Philippines "stealing" bloodmeals from other fed gnathiids, suggest that cues from the blood or fluids themselves are involved in host finding and that there is at least some overlap between the cues produced by fishes and those produced by some soft-bodied invertebrates.

For nearly all vertebrates, including the fishes that comprise the majority of the gnathiid diet, hemoglobin (which is iron-based) is the respiratory pigment. Although it has been previously noted that gnathiids will sometimes get partial or even full meals of lymph rather than blood from fishes (Smit and Davies, 2004; Tanaka, 2007), it was previously unknown whether gnathiids were capable of digesting blood or other body fluids from invertebrates. Blood from many molluscs (including *E. crispata*) have hemocyanin, which is copper-based (Barrington, 1967). This is also the respiratory pigment that *H. carunculata* (an annelid) uses. Thus, we can now confirm that the digestive capability of gnathiid isopods extends beyond iron-based blood or fish lymph and that the olfactory signals produced by fish and soft-bodied invertebrates may overlap. It is perhaps noteworthy that *A. dactyloma* lack respiratory pigments, which may somehow prevent a primarily blood-feeding parasite from recognizing it as a potential host.

Gnathiid isopods have for decades been regarded as "fish parasites" or micropredators of fishes. Our findings indicate that although fishes may be their primary hosts, soft-bodied invertebrates can serve as hosts in some circumstances. Our laboratory study sets the stage for more ambitious field studies aimed at determining the extent to and conditions under which this occurs in nature and how widespread it is among gnathiid species.

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