International Journal for Parasitology: Parasites and Wildlife 4 (2015) 125–134

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Enhanced understanding of ectoparasite–host trophic linkages on coral reefs through stable isotope analysis

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ARTICLE INFO

Article history:
Received 3 November 2014
Revised 7 January 2015
Accepted 8 January 2015

Keywords:
Ectoparasites
Reef fish
Fish parasitic isopods
Cleaning symbiosis
Food webs
Stable carbon isotopes
Stable nitrogen isotopes

ABSTRACT

Parasitism, although the most common type of ecological interaction, is usually ignored in food web models and studies of trophic connectivity. Stable isotope analysis is widely used in assessing the flow of energy in ecological communities and thus is a potentially valuable tool in understanding the cryptic trophic relationships mediated by parasites. In an effort to assess the utility of stable isotope analysis in understanding the role of parasites in complex coral-reef trophic systems, we performed stable isotope analysis on three common Caribbean reef fish hosts and two kinds of ectoparasitic isopods: temporarily parasitic gnathiids (*Gnathia marleyi*) and permanently parasitic cymothoids (*Anilocra*). To further track the transfer of fish-derived carbon (energy) from parasites to parasite consumers, gnathiids from host fish were also fed to captive Pederson shrimp (*Ancylomenes pedersoni*) for at least 1 month. Parasitic isopods had $\delta^{13}C$ and $\delta^{15}N$ values similar to their host, comparable with results from the small number of other host–parasite studies that have employed stable isotopes. Adult gnathiids were enriched in ^{15}N and depleted in ^{13}C relative to juvenile gnathiids, providing insights into the potential isotopic fractionation associated with blood-meal assimilation and subsequent metamorphosis. Gnathiid-fed Pedersen shrimp also had $\delta^{13}C$ values consistent with their food source and enriched in ^{15}N as predicted due to trophic fractionation. These results further indicate that stable isotopes can be an effective tool in deciphering cryptic feeding relationships involving parasites and their consumers, and the role of parasites and cleaners in carbon transfer in coral-reef ecosystems specifically.

1. Introduction

Parasitism, whereby smaller organisms derive energy from larger ones (hosts), usually without killing them, is the most common animal lifestyle and thus the most common consumer strategy (Hudson et al., 2006). It is therefore surprising that, with few exceptions (Price et al., 1986), parasites have historically been ignored in both theoretical and empirical analyses of ecological food webs (Morand and Arias-Gonzalez, 1997; Arias-Gonzalez and Morand, 2006; Dunne et al., 2013). Ecologically-minded parasitologists have called attention to this gap (Marcogliese and Cone, 1997; Wood et al., 2007; Byers, 2009; Johnson et al., 2010), drawing comparisons between parasites and "micropredators" (Raffel et al., 2008) and proposing them as the "ultimate missing link" in our understanding of trophic ecology (Lafferty et al., 2008), with the potential to dominate food-web links. In spite of this, fewer than 10 food-web studies within the past two decades have incorporated parasites (Morand and Arias-Gonzalez, 1997; Arias-Gonzalez and Morand, 2006; Lafferty et al., 2006, 2008; Kuris et al., 2008; Amundsen et al., 2009; Johnson et al., 2010; Hatcher and Dunn, 2011; Sato et al., 2012; Dunne et al., 2013).

Our understanding of food-web ecology, host–parasite interactions, and especially the interface between the two in marine ecosystems lags far behind terrestrial and freshwater systems (Hatcher and Dunn, 2011). A major challenge is that current food-web models are insufficient for inclusion of parasites (Petchey et al., 2008; Sukhdeo, 2010). This gap is particularly apparent for coral-reef systems, where the majority of the biodiversity is comprised of parasites (Rohde, 2002). Parasites can have substantial biomass in marine ecosystems (Kuris et al., 2008) and influence food-web linkages by increasing trophic efficiency (Arias-Gonzalez and Morand, 2006), link density, and connectance (Amundsen et al., 2009). By consuming host tissue, they represent a direct means of host carbon transfer. Indirectly, parasites may influence food-web linkages by altering host movement and other behavioural patterns (Huebner and Chadwick, 2012a, 2012b; Sato et al., 2012; Welicky and Sikkel, 2014).

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http://dx.doi.org/10.1016/j.ijppaw.2015.01.002

Two of the most common external parasites of coral-reef fishes are gnathiid and cymothoid isopods. Gnathiids are small (1–3 mm) protelean parasites that feed on fish blood during each of three larval stages, each of which lives in the substratum between feedings. Thus, they are ecologically similar to terrestrial haematophagous arthropods such as ticks and fleas. After the third feeding, they metamorphose into non-feeding adults, where females produce a single brood and then die (Smit and Davies, 2004). Gnathiid larvae represent a major component of the diet of cleaner fishes, and they are also eaten by cleaner shrimps (Grutter, 1996; Arnal et al., 2000), who remove the parasites from fish hosts. High gnathiid activity even appears to influence the tendency of host fish to visit cleaners (Grutter, 2001; Sikkel et al., 2004). During free-living stages, gnathiids may also be consumed by other microcarnivores (Alldredge and King, 1977; Holzman et al., 2005; Motro et al., 2005), including corals (Artim and Sikkel, 2013). Thus, their potential contribution to food webs is self-evident. Because gnathiids feed during and grow between each of their three larval stages, there is an ontogenetic shift in size with each stage and with each fish host. Consequently, as they grow, their potential role in carbon transport increases because bigger gnathiids can consume, and require, more blood to grow and metamorphose than smaller ones.

Cymothoid isopods of the genus *Anilocra* attach to hosts as juveniles, feed on blood and possibly mucus, epithelium, and subcutaneous tissue (Bunkley-Williams and Williams, 1998). In contrast to gnathiids, *Anilocra* reach large body sizes (up to 25% of host body size – Smit et al., 2014) and remain attached to the host for their entire life, unless they are dislodged or eaten (Ostlund-Nilsson et al., 2005). *Anilocra* females feed on host blood, and likely produce multiple broods during a life span of 12–14 months (Adlard and Lester, 1995). Cleaner species are known to dislodge and consume juvenile cymothoid isopods, which can also be consumed by microcarnivores during free-living stages, but predation on adults is unknown (Bunkley-Williams and Williams, 1998; Ostlund-Nilsson et al., 2005).

Ectoparasites such as these offer a promising starting point for the integration of parasites into food webs. Unlike internal parasites, ectoparasites can often be seen with the naked eye and collected without sacrificing the host. Perhaps most importantly, they can impact food webs through direct consumption by other organisms (Johnson et al., 2010). Stable isotope analysis can provide an indirect measure of parasite trophic ecology, providing an advanced tool in the analysis of host–parasite food-webs (Gómez-Díaz and González-Solís, 2010). Stable carbon isotopes ($\delta^{13}C$) are used to discern carbon or food sources and indicate relative contributions of primary sources to local food webs, with a typical trophic shift of 0–1‰ (DeNiro and Epstein, 1981; McCutchan et al., 2003). In contrast, stable nitrogen isotope ($\delta^{15}N$) values increase with trophic level, typically 2–3‰ (Minagawa and Wada, 1984; Post, 2002; McCutchan et al., 2003), and are useful when estimating trophic level (Post, 2002; McCutchan et al., 2003). Smaller trophic shifts in $\delta^{15}N$ are associated with animals raised on invertebrate diets (1.4 ± 0.2‰, McCutchan et al., 2003). Thus, stable isotope analysis could assist with understanding the complexity of the cryptic trophic relationships involving parasites and other symbionts in biologically complex systems, such as coral reefs.

If parasites function as predators, we would predict a stepwise enrichment in ^{15}N of parasites relative to hosts, on the order of 2–3‰ (DeNiro and Epstein, 1981; Post, 2002). However, applications of stable isotope analysis and published trophic fractionation values to examine parasite–host isotopic relationships have yielded variable results (Lafferty et al., 2008; Doi et al., 2010; Gómez-Díaz and González-Solís, 2010). Isotope patterns are influenced by the feeding strategy or life history stage of the parasite (Iken et al., 2001; Pinnegar et al., 2001; O'Grady and Dearing, 2006), and the level of enrichment can vary in a parasite species found among multiple hosts (Deudero et al., 2002) or among different parasite taxa within hosts (Boag et al., 1997; Neilson et al., 2005; Gómez-Díaz and González-Solís, 2010). In addition, interpretation of results from parasite isotope studies is often limited by the selection of tissue analysed for isotopic comparison with parasites (Power and Klein, 2004; Stapp and Salkeld, 2009). For haematophagous parasites (e.g., gnathiids and *Anilocra*), estimates of trophic shift should be based on isotopic differences between fluids (blood) and consumers (ectoparasites) rather than differences between muscle or bulk tissue and consumers (e.g., McCutchan et al., 2003; Doi et al., 2010), because blood may differ isotopically from muscle tissue or whole organisms (Pinnegar et al., 2001). To date, few studies have examined multiple parasites and hosts simultaneously (Gómez-Díaz and González-Solís, 2010), and no studies, to our knowledge, have incorporated parasite consumers in an isotopic study of food webs.

As a first step in assessing the utility of stable isotope analysis in elucidating the cryptic trophic relationships and carbon transfer mediated by parasites in marine reef systems, we conducted stable carbon and nitrogen isotope analysis of ectoparasitic gnathiid and cymothoid isopods, three fish host species, and one cleaner shrimp species. We hypothesized that host fish, parasite, and parasite consumer will have similar $\delta^{13}C$ values, with little change (0–1‰) in $\delta^{13}C$ with each trophic level. We predicted that parasites would be enriched in ^{15}N by approximately 2–3‰ relative to host heart and blood tissues, due to trophic fractionation of $\delta^{15}N$ (Post, 2002; Stapp and Salkeld, 2009; Schmidt et al., 2011). We calculated the magnitude and direction of fractionation between parasites (gnathiid and *Anilocra*) and host tissues, and between juvenile and adult gnathiid life stages. Lastly, we expected that parasite consumers (cleaner shrimp) will be enriched in ^{15}N relative to gnathiids, consistent with the trophic shift of ~ 1.4‰ reported for invertebrate consumers (McCutchan et al., 2003).

2. Materials and methods

2.1. Study site and organisms

The field portion of the study was conducted during May–August of 2009 and 2010. The primary base of operation was the Virgin Islands Environmental Resource Station in Lameshur Bay, St. John, U.S. Virgin Islands (18°19 N, 65°44 W) and all specimens were collected within Lameshur Bay. Fish species used in the study included longfin damselfish (*Stegastes diencaeus*, Pomacentridae), French grunt (*Haemulon flavolineatum*, Haemulidae), and squirrelfish (*Holocentrus adscenscionis*, Holocentridae). These fish species were chosen based on their distinct differences in habitat use and behaviour, as well as their abundance at our study site. Longfin damselfish are diurnal herbivores that defend year-round territories in shallow reef habitat (Robertson, 1984). French grunts live in shoals or individually in reef habitat during the day and typically migrate to seagrass beds at night where they feed on benthic and demersal invertebrates (Nagelkerken et al., 2008; Appeldoorn et al., 2009). Like French grunt, squirrelfish also refuge in reef structure during the day and are active at night, feeding on demersal and benthic invertebrates and even small fishes. However, in contrast to French grunt, they remain on the reef at night (Randall, 1967; Gladfelter and Johnson, 1983; Menard et al., 2008). Thus, all three fish species demonstrate high site fidelity to resting sites (Nagelkerken et al., 2008) and French grunt in particular may play a significant role in the transfer of parasites among habitats. All three species are infected by the gnathiid isopod *Gnathia marleyi* (Farquharson et al., 2012) at our study sites, although their susceptibility varies (Coile and Sikkel, 2013). In addition, French grunt are infected by the cymothoid isopod, *Anilocra haemuli*, and squirrelfish by *A. holocentric* (Bunkley-Williams and Williams, 1981; Bunkley-Williams, 1984;