NOTES AND NEWS

PRELIMINARY STUDY INTO THE TROPHIC POSITION OF SYMBIOTIC PALAEMONID SHRIMPS (DECAPODA, PALAEMONIDAE) USING STABLE ISOTOPES

BY

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The species-rich shrimp family Palaemonidae is well known for the majority of species forming a symbiotic relationship with a large variety of invertebrate hosts, such as cnidarians, sponges, molluscs, echinoderms and ascidians (Horká et al., 2016). Although these associations have often been termed as commensal in systematic and taxonomic literature, their true relationship remains unknown for the majority of species, largely due to a general dearth of ecological observations on them, and a broad lack of dietary studies in small-sized shrimp species.

Direct, microscope based, observations of the diet of symbiotic shrimps are largely lacking in the literature. Johnson & Liang (1966) demonstrated that the bivalve associate, *Anchistus custos* (Forskål, 1775) scrapes the gill lamellae of its host, with the stomach content largely composed of diatoms and filamentous algae, as well as host mucus. Patton (1972) equally demonstrated that the stomach content of *Neopontonides beaufortensis* (Borradaile, 1920), a gorgonian associate, comprised of spicules, mucus and other material gathered from the gorgonian surface, although he called it a scavenger rather than a symbiont. Bruce (1976) speculated that the coral-dwelling species of *Coralliocaris* feed on coral mucus with entrapped detritus, whilst echinoderm associates, such as *Stegopontonia* equally feed on their hosts' mucus, but provided no direct evidence to support

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this claim. Patton et al. (1985) demonstrated through direct aquarium observations of feeding, that *Gnathophylloides mineri* Schmitt, 1933, a sea-urchin associate, primarily derives its food from its host's epithelium, with the mouthparts and the dactyli of the pereiopods exhibiting special adaptations to abrade the sea urchin spines. Patton (1994) found that the stomach content of several dissected coral-associated genera largely contained sand grains, spicule fragments and undetermined soft material, from which it was deduced that coral mucus was indeed the prime food source of these species. Ďuriš et al. (2011) provided direct evidence from the analysis of stomach content, that several genera of sponge endosymbionts largely feed on host tissue, and drew attention to the distinctive claw morphology of these genera, being adapted to cutting into host tissue, a common morphological adaptation across numerous sponge symbionts. The majority of these sponge associated taxa do not appear to offer benefits to their host, as they live deep inside them, with limited access to other food sources, and thus were considered to be parasites rather than commensals.

Based on a combination of field and experimental observations, Suzuki & Hayashi (1977) also demonstrated that the anemone-associated species, *Hamopontonia corallicola* Bruce, 1970, *Actinimenes ornatus* (Bruce, 1969) and *Ancylocaris brevicarpalis* Schenkel, 1902 tear off tentacles from their host, and thus refer to these species as parasites. In further laboratory experiments on *A. brevicarpalis* by Fautin et al. (1995), it was noted that during periods of low food availability, the shrimp may indeed be parasitic and clip the anemone's tentacles, but could equally be maintained in aquaria, fed on *Artemia* nauplii. These authors stress that classifying this particular shrimp species as parasitic largely depends on how parasitism is defined and that net lifetime effects, rather than short term effects should be taken into account to untangle the various aspects of symbiosis. Olliff (2013) used host separation experiments in *Zenopontonia soror* (Nobili, 1904) to demonstrate complete mortality when shrimps (directly fed or not) are kept away from hosts, inferring a direct feeding relationship, but did not investigate actual stomach contents or conducted observational feeding, and was unable to conclude if the shrimp fed on the host, its ectoparasites or accumulated detritus.

Based on the meagre evidence available in the literature, it is thus postulated that shrimp symbionts largely feed on the mucus excreted by their hosts or their hosts' epithelium or tissue, although their exact trophic position is unclear and they have been variously referred to as parasites and symbionts, likely depending on host taxon. Stable isotope analysis is particularly useful to infer trophic relationships of species where identification of stomach content may be difficult or impossible by eye due to size or nature of the diet (Michener & Kaufman, 2007).

In line with the suggestion of Fautin et al. (1995) that net lifetime effects should be used to investigate the dietary spectrum rather than short term observation, we

herein use stable isotope techniques to investigate the trophic position of symbiotic palaemonid shrimps.

Despite the general utility of stable isotopes to reveal the trophic position of symbionts, only three prior studies have used this technique on Palaemonidae. Kennedy et al. (2001) revealed that *Pontonia pinnophylax* (Otto, 1821), a symbiont of *Pinna nobilis* Linnaeus, 1758 (bivalve mollusc) assimilates carbon from similar sources as their host and should thus be considered to be commensal, which corroborates the finding of Johnson & Liang (1966) for a different genus of bivalve symbiont. Terrana et al. (2019) resolved that *Pontonides unciger* Calman, 1939 did not use its host (*Cirrhipathes* spp.) as its primary food source, but instead consumed diurnal plankton. Brasseur et al. (2018) conclusively showed that the echinoid associate *Tuleariocaris holthuisi* Hipeau-Jacquotte, 1965 fed exclusively on its host *Echinometra mathaei* (Blainville, 1825), but with the co-occurring alpheid shrimp species, *Arete indicus* Coutière, 1903 having a broader diet.

These studies are labour intensive, solely focused on trophic interactions, and sofar only focus on a single host-symbiont system each. In contrast, biodiversity assessments for taxonomic purposes of caridean shrimps are much broader in scope, but often only result in a limited amount of shrimp species per unique host species, with additionally small pieces of the host frequently preserved or collected. The purpose of the present study is to evaluate the usage of stable isotopes to determine the trophic position and nature of the symbiotic relationship (1) on the basis of samples obtained during general survey work, as opposed to targeted trophic studies, and (2) preserved in ethanol for several years under standard museum collection conditions. We use ten randomly selected species of Palaemonidae from a general survey, including representatives of the four main host taxa of Palaemonidae, as well as two free-living species.

Specimens of symbiotic Palaemonidae and their host were collected from reefs around Iriomote-jima, Ryukyu Islands, Japan during June 2014. Eight species of symbiotic shrimps (table I) were collected by SCUBA diving in depths of 5-30 m, together with small tissue pieces of their hosts, based on a single collecting event per host (as is routine in general surveys). Additionally, two species of free-living shrimp were also collected (table I). For each species, 3–5 specimens were collected and placed underwater in ziplock bags or vials, with the host pieces in separate bags. For Coelenterata and Echinodermata tissue was scraped, avoiding skeletal material; for *Hippopus* a piece of muscle tissue was clipped, whilst for the Porifera tissue clips included spicules. In the lab, specimens and host tissue were cleaned of debris, washed in distilled water and preserved in 75% ethanol. Specimens were further rinsed in distilled water and cleaned of debris under a microscope prior to stable isotope analysis. For each species, 3 specimens were mixed prior to analysis. For the larger species tissue was obtained from the major

TABLE I

Commensal Palaemonidae and their host species, as well as free-living species sampled for stable isotope analysis from Iriomote-jima

Host	Shrimp
Porifera	
Haliclona sp.	*Typtonychus bruceorum* Bruce, 2011
Stylissa sp.	*Periclimenaeus rastrifer* Bruce, 1980
Coelenterata	
Cirrhipathes sp.	*Pontonides loloata* Bruce, 2005
Cryptodendron adhaesivum (Klunzinger, 1877)	*Actinimenes ornatus* (Bruce, 1969)
Heliofungia actiniformis (Quoy & Gaimard, 1833	*Hamopontonia fungiacola* Marin, 2012
	Ancylomenes venustus (Bruce, 1969)
Mollusca	
Hippopus hippopus (Linnaeus, 1758)	*Anchistus miersi* (De Man, 1888)
Echinodermata	
Protoreaster nodosus (Linnaeus, 1758)	*Zenopontonia soror* (Nobili, 1904)
Free-living	*Cuapetes tenuipes* (Borradaile, 1898)
	Periclimenella spinifera (De Man, 1902)

claws, although for smaller species whole bodies were used. For the host taxa, tissue used was from a single specimen or colony. Shrimp species were identified by the first author, host taxa (except sponges) were identified from underwater photos, sponges were identified by Prof. N. de Voogd (Naturalis, Leiden, The Netherlands) based on spicule preparations. Voucher specimens of all shrimp species are deposited in the Zoological Collections of the Oxford University Museum of Natural History (OUMNH.ZC.2015-07). Samples were dried at 70°C for 48 hours, treated with HCl to remove carbonates and re-dried prior to analysis.

Stable isotope analysis and concentration measurements of nitrogen and carbon were performed simultaneously with a Thermo/Finnigan MAT V isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.), coupled to a Thermo Flash EA 1112 elemental analyser via a Thermo/Finnigan Conflo IV-interface in the stable isotope laboratory of the Museum für Naturkunde, Berlin. Stable isotope ratios are expressed in the conventional delta notation ($\delta^{13}C/\delta^{15}N$) relative to atmospheric nitrogen (Mariotti, 1983) and VPDB (Vienna PeeDee Belemnite standard). Standard deviation for repeated measurements of lab standard material (peptone) is generally better than 0.15 per mill (‰) for nitrogen and carbon, respectively. Standard deviations of concentration measurements of replicates of the lab standard are <3% of the concentration analysed.

Samples were analysed in June 2018, having thus spent 4 years in 75% ethanol under standard museum collection conditions. This is perhaps not ideal to fully disentangle isotopic metrics of the symbiont-host relationship, as ethanol storage affects carbon and nitrogen values (e.g., Le Bourg et al., 2019). However, the

reported value shifts in the literature do not affect gross trophic levels (Sarakinos et al., 2002).

Based on the isotopic composition (fig. 1), the sampled shrimp community and their host taxa, broadly speaking, resolves at three trophic levels: primary producers, primary and secondary consumers. Three host taxa can be considered as primary producers, the anemone *Cryptodendron adhaesivum* (Klunzinger, 1877), the solitary coral *Heliofungia actiniformis* (Quoy & Gaimard, 1833) and the bivalve *Hippopus hippopus* (Linnaeus, 1758), all of which are known to harbour zooxanthellae (Dunn, 1981; Klump & Griffiths, 1994), although filter feeding for particulate organic matter (POM) has also been documented for the bivalve, with the relative proportion of both sources of carbon being size-dependent (Klump & Griffiths, 1994). Both sponge hosts (*Haliclona* sp., *Stylissa* sp.), the black coral *Cirripathes* sp., as well as the seastar *Protoreaster nodosus* (Linnaeus, 1758), resolve as primary consumers in the present analysis. Although black corals are generally considered to be suspension feeding zooplanktivores (Terrana et al., 2019; Wagner et al., 2012), other studies of allied reef-dwelling genera (Williams & Grottoli, 2010), have demonstrated that the primary food source is POM, perhaps more in line with the present result. *Protoreaster nodosus* is known to feed on meiobenthos, as well as microbial and macroalgal films, which it derives through extraoral digestion of plant detritus (Scheibling & Metaxas, 2008). The diet of sponges in general consists of largely coral-derived POM (Rix et al, 2016), as well as dissolved carbon and smaller fractions of the phytoplankton (Reiswig, 1971). In general, the herein resolved trophic position of the host taxa for Palaemonidae are congruent with literature, and by extension provide some confidence in the herein resolved trophic position of the commensal and free-living Palaemonidae.

All symbiotic Palaemonidae (with one exception) and both free-living shrimp species resolve as primary consumers. The free-living species studied herein belong to a basal, phylogenetically distinct clade (Horká et al., 2016) from which it is assumed the symbiotic clades evolved, with the majority of species in this clade roaming across coral reefs and associated habitats. Although there is a prevailing notion in taxonomic and evolutionary literature that non-symbiotic species in the genera *Cuapetes*, *Palaemonella*, *Periclimenes*, *Periclimenella* and allied genera are largely micro-predators (e.g., Bruce, 1977; Dobson et al., 2016), their isotopic composition reveal both *Cuapetes tenuipes* (Borradaile, 1898) and *Periclimenella spinifera* (De Man, 1902) to be primary consumers, and thus presumably herbivores and/or detrivores, rather than more active predators; a feeding mode likely more widespread amongst members of these genera.

Based on their isotopic composition, the symbiotic shrimp species fall into two indicative ecological groupings, one in which their isotopic composition is similar
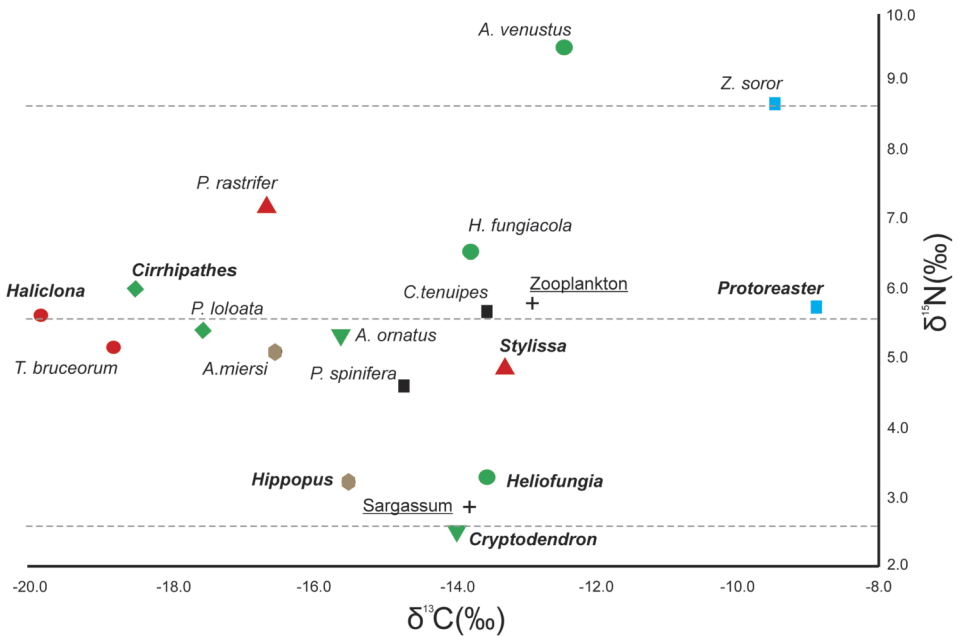
Fig. 1. Carbon and nitrogen isotopic composition of Palaemonidae and their host taxa from Iriomote-jima. Host and symbiont are denoted by identical coloured symbols (host names at genus level and bold), free-living taxa are denoted by filled squares. Dashed lines indicate general trophic levels. Zooplankton and *Sargassum* values are from Yamamuro et al. (1995), from nearby Ishigaki-jima.

to their host taxon and one with an elevated carbon assimilation. It can thus be postulated that the sponge dwelling, *Typtonychus bruceorum* Bruce, 2011 and the black coral symbiont, *Pontonides loloata* Bruce, 2005 largely consume the same food sources as their host (fig. 1), although a minor amount of host consumption cannot be excluded, and are thus best described as commensal or mutualistic species. It is noted here that in the more focused study by Terrana et al. (2019), *P. unciger* consumes diurnal plankton, in contrast to its host black coral, which largely consumes nocturnal plankton. In contrast, the bivalve dwelling, *Anchistus miersi* (De Man, 1888), the solitary coral-dwelling *Hamopontia fungiacola* Marin, 2012 and the seastar inhabiting *Z. soror*, derive much of their carbon intake from their host taxa (fig. 1) and are therefore best described as obligate or facultative parasites. The position of the sponge dwelling, *Periclimenaeus rastrifer* Bruce, 1980 is somewhat anomalous in this respect. Although there is a suggestion, based on its isotopic composition, that the species largely feeds on its host. No dietary studies are available for these taxa, but the isotopic composition of *Z. soror* is congruent with Olliff (2013) inferring a direct feeding relationship between shrimp and host. Interestingly, the isotopic composition of *A. miersi*, is in contrast to that of the East Atlantic, bivalve dwelling, *P. pinnophylax*, which was shown to be a

commensal species (Kennedy et al., 2001). This effectively demonstrates that no inference about trophic grouping can be deduced from simple host records. The isotopic composition of *Ancylomenes venustus* (Bruce, 1969) provides evidence for this species to be classed as a secondary consumer, in line with the species being known as a facultative fish cleaner (Horká et al., 2018), with a diet likely composed of fish parasites, such as flatworms and gnathiid isopods (Reiswig, 2005).

The present analysis can only be considered as preliminary in scope, given the methodology employed and the low number of species analysed, i.e., 10 species of Palaemonidae out of a currently total known biodiversity of more than 400 symbiotic species, as well as more than 50 free-living coral reef-associated species. In view of the methodology employed, the results are only truly comparable within the context of this study, and the herein obtained values should only be extrapolated to future studies with caution. For example, the single value obtained herein for *P. loloata* is lower than the median value obtained for *P. unciger* in Terrana et al. (2019), albeit at the same trophic level. Although regional differences in diet composition may exist, these two species are synonyms (SDG, pers. obs.) and should thus be much more similar in isotopic composition. Despite the limitations of the study, it does provide a partial insight into the variety of trophic relationships of tropical Palaemonidae across the four main host groups. Based on their isotopic composition, some species are evidently better defined as being commensal or mutualistic in the relationship with their host. The exact lifestyle of these species cannot be discerned by stable isotope analysis, and must await further studies combing behavioural observation and a combination of DNA and stable isotope analysis (Carrean-Martinez & Heath, 2010). Other species are better characterized as parasites, although broad brush suggestions, such that many (or most) sponge dwelling shrimp species are parasites (Ďuriš et al., 2011) need to be more refined. Many taxa inhabiting the same group of host taxa (i.e., sponges) do exhibit common morphological adaptations in their mouthparts and pereiopods to their host environment. Nevertheless, the sponge dwelling, *T. bruceorum* exhibits similar morphological adaptations and is herein suggested to be a commensal or mutualistic species, although it cannot be excluded that it may partly feed on its host. Equally, the bivalve dwelling *A. miersi* is shown herein to be more parasitic in nature than *P. pinnophylax*. Although host switching of symbiotic Palaemonidae has been demonstrated as a dominant driver in the evolution of the group (Horká et al., 2016), the present results suggest that far more nuanced pathways are at play. This is in line with the concept, that the boundary lines between the various forms of symbiosis are rather vague, potentially changing throughout the life-cycle of the symbiont, as well as ambient food supply (Parmentier & Michel, 2013).

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