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Symbiotic relationship between the carapid fish *Onuxodon fowleri* (Ophidiiformes: Carapidae) and the pearl oyster *Pinctada margaritifera* (Mollusca: Bivalvia: Pteriidae)

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Summary: At Makemo Atoll (French Polynesia), the carapid fish *Onuxodon fowleri* lives in symbiosis with the black-lip pearl oyster *Pinctada margaritifera*. Although the symbiont seems to live inside its host bivalve by using it as a shelter, additional data are still needed to better understand the exact nature of this association. For this purpose, we implemented an approach using stable isotope ratios of carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N). The δ¹³C and δ¹⁵N values were measured in tissues of the pearl oyster (gonads, gills, mantle and muscles), white muscle tissue from the fish and other food sources. This stable isotope approach was also complemented by the analysis of stomach contents in the carapid fish. Overall, the isotopic compositions measured in the present study support a commensal relationship between *O. fowleri* and *P. margaritifera*. In addition, our isotopic data bring new information about another guest living inside *P. margaritifera*, namely the palaemonid shrimp *Conchodytes meleagrinae*. Based on the δ¹³C and δ¹⁵N values, it appears that the shrimp might feed on the bivalve gonads.

Keywords: symbiosis; stable isotopes, diet, pearl oyster, Carapidae.

Relación simbiótica entre el pez carápido *Onuxodon fowleri* (Ophidiiformes: Carapidae) y la ostra perlífera *Pinctada margaritifera* (Mollusca: Bivalvia: Pteriidae)

Resumen: En el atolón Makemo (Polinesia Francesa), el pez carápido *Onuxodon fowleri* vive en simbiosis con la ostra perlífera *Pinctada margaritifera*. Aunque el simbiote se aloja en el interior del bivalvo utilizándolo aparentemente como refugio, se necesitan datos adicionales para comprender mejor la naturaleza exacta de esta asociación. Para ello, hemos empleado las relaciones de los isótopos estables de carbono (¹³C/¹²C) y nitrógeno (¹⁵N/¹⁴N). Los valores de δ¹³C y δ¹⁵N fueron medidos en diferentes tejidos de la ostra perlífera (gónadas, branquias, manto y músculo), en el músculo blanco del pez y en otras fuentes de alimentación. El estudio de isótopos estables se complementa con el análisis del contenido estomacal del pez carápido. Globalmente, las composiciones isotópicas medidas en este estudio apoyan una relación de comensalismo entre *O. fowleri* y *P. margaritifera*. Además, nuestros datos isotópicos aportan información nueva sobre otro huésped que vive dentro de *P. margaritifera*, concretamente el camarón palaemónido *Conchodytes meleagrinae*. En base a los valores de δ¹³C y δ¹⁵N parece que el camarón podría alimentarse de las gónadas del bivalvo.

Palabras clave: simbiosis; isótopos estables; dieta, ostra perlífera, Carapidae.

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INTRODUCTION

Classically, symbiosis refers to the close association of two different species living together, with organisms being involved as hosts or symbionts (de Bary 1879). A symbiotic relationship can have different forms (parasitism, mutualism and commensalism), but they are part of a broad continuum and these associations cannot always be arranged in adjacent drawers (Parmentier and Michel 2013). The black lip pearl oyster *Pinctada margaritifera* (Linnaeus, 1758) (Mollusca: Bivalvia: Pteriidae) is widely distributed in tropical Indo-West Pacific regions, living in coral reef areas (Gervis and Sims 1992, Southgate and Lucas 2008). This species occurs as large populations in many atolls of French Polynesia, where it is one of the most characteristic benthic bivalve molluscs due to its economic importance for the pearl farming industry (Salvat 2009). Some cases of symbiotic organisms living in association with *P. margaritifera* have been reported in the past, and these include both vertebrate and invertebrate symbionts.

Pearlfishes (Ophidiiformes: Carapidae) are eel-like fishes that mainly occur in shallow to moderately deep waters of tropical seas (Markle and Olney 1990). Within this family, several genera (*Onuxodon* spp., *Carapus* spp. and *Encheliophis* spp.) share a remarkable peculiarity: they are able to penetrate and live inside different invertebrate hosts such as echinoderms (holothurians, starfish) and bivalves (Fowler 1927, Tyler 1970, Trott and Trott 1972). Based on stomach content analysis (Trott 1970, Vanden Spiegel and Jangoux 1989), morphological descriptions of the buccal and pharyngeal jaw apparatus (Parmentier et al. 1999, 2000) and stable isotope analysis (Parmentier and Das 2004), some *Carapus* spp. and *Encheliophis* spp. living inside echinoderms have been considered commensal or parasite, depending on the species. Basically, commensal species use their host as a shelter and leave it for foraging whereas parasitic species are known to feed on the internal tissues of their host (Smith 1964, Trott 1970, Parmentier et al. 2000). Among this fish family, members of a third genus (*Onuxodon*) are also known to live inside bivalves, being located between the mantle and the shell (Fowler 1927, Trott 1970, Tyler 1970). Fowler's pearlfish, *Onuxodon fowleri* (Smith, 1955) (Carapinae: Echiodontini), lives inside representatives of the pearl oyster *P. margaritifera* (Fowler 1927, Parmentier et al. 2000, Kéver et al. 2014). *Onuxodon fowleri* is considered a commensal species that uses its host as a shelter and leaves it to feed on small benthic preys such as annelids and small crustaceans (Trott 1981, Parmentier et al. 2000). According to scientific evidence, no apparent harm caused by the fish to its host has ever been reported. However, the exact nature of this host/symbiont association has not yet been experimentally demonstrated and additional data are needed to gain further insight into the type of symbiosis taking place.

Stable isotope analysis has become a powerful tool for tracing dietary sources by providing an integrated measure of the dietary components over a long period

of time. This method clearly shows that the isotope ratios of a consumer are related to those of its food (De Niro and Epstein 1978, 1981, Peterson and Fry 1987). Stable isotope analysis gives an average estimate of the dietary preferences of an organism that is less subject to temporal bias (Pinnegar and Polunin 1999), but it does not provide a detailed picture of the food ingested by this organism. For this purpose, stomach content analysis can be used as a complementary tool (Frédérich et al. 2009). The combination of the two methods has the advantage of compensating for the inaccuracy of each one (Frédérich et al. 2012). Interestingly, an approach that combines stomach content analysis and the use of stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) has proved to be a valuable tool to get more information on the symbiotic relationship (i.e. commensal or parasite) between carapids and their hosts (Parmentier and Das 2004).

The present study aimed to establish the symbiotic relationship between the carapid fish *O. fowleri* and the pearl oyster *P. margaritifera* through an approach using stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$). This stable isotope approach was also complemented by the analysis of stomach contents in the carapid fish.

MATERIALS AND METHODS

Sampling site and data collection

The present study was carried out in two separate phases: from November to December 2011 and from October to November 2013. Sampling was conducted during daytime (10:00 AM to 3:00 PM) near Arikita-mori Pass located on the northeastern part of Makemo Atoll ($16^{\circ}38'S$, $143^{\circ}42'W$; Tuamotu Archipelago, French Polynesia; Fig. 1). Over the two sampling campaigns, 209 wild pearl oysters (*P. margaritifera*) were collected by scuba diving on 13 isolated reef pinnacles (Fig. 1) at depths ranging from 5 to 30 m. During the first sampling phase, only the overall number of fish found inside the collected pearl oysters was counted. The second field campaign was conducted differently in order to determine precisely the number of fish observed inside the pearl oysters collected on each of the reef pinnacles (see details in Table 1).

Once in the laboratory, each pearl oyster was opened using a shell speculum in order to keep them open while looking for individuals of *O. fowleri*. Immediately after their capture, 16 specimens of *O. fowleri* (60-85 mm in total length) randomly selected among all the collected fish were euthanized with an overdose of MS-222 (500 mg l^{-1}). Their entire digestive tracts were removed and conserved in 70% alcohol for stomach content analysis. Small pieces ($\pm 0.5\text{ cm}^3$) of lateral white muscle of these fish were used for stable isotope analysis. In addition, tissues (gonads, gills, mantle and adductor muscles) from 15 specimens of *P. margaritifera* were sampled for stable isotope analysis. Ten individuals (five males and five females) of *Conchodytes meleagrinae* Peters, 1852 were also collected. These palaemonid shrimps are typically found as one small

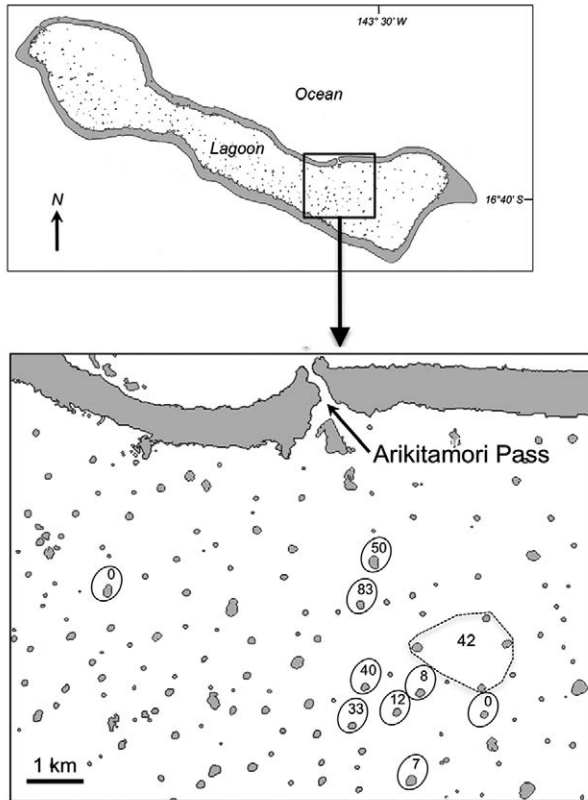


Fig. 1. – Location of the experimental site in the northeastern part of Makemo Atoll (Tuamotu Archipelago, French Polynesia). The dashed line encompasses the four reef pinnacles sampled during the first field campaign (November-December 2011). Full circles indicate the nine other reef pinnacles sampled during the second field campaign (October-November 2013). Numbers refer to the occupation rates (i.e. percentage of the collected bivalves *Pinctada margaritifera* being occupied by carapid fish *Onuxodon fowleri*) observed on the reef pinnacles. A detailed listing of the collected samples is given in Table 1.

Table 1. – Summary of the occupation rates (i.e. percentage of the collected bivalves *Pinctada margaritifera* being occupied by carapid fish *Onuxodon fowleri*) observed on the 13 reef pinnacles sampled during the two field campaigns (November-December 2011 and October-November 2013). Note: During the 2011 field campaign, we only determined the overall occupation rate related to the four reef pinnacles sampled. Figure 1 gives the exact geographical location of the 13 reef pinnacles.

Sampling campaign	Pinnacle	Number of bivalves collected	Number of fish collected	Occupation rate
2011	1-4	73	31	42 %
2013	5	16	8	50 %
	6	12	10	83 %
	7	5	2	40 %
	8	3	1	33 %
	9	17	2	12 %
	10	13	1	8 %
	11	30	2	7 %
	12	15	0	0 %
	13	25	0	0 %

Table 2. – $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm standard deviation) in pearl oysters, carapid fish and selected invertebrates (shrimp and zoobenthos) from Makemo Atoll (French Polynesia).

Species	Tissue	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	
Zoobenthos Mollusk	<i>Pinctada margaritifera</i>	whole body	3	-15.4 \pm 0.3	11.2 \pm 0.2
		gills	15	-17.2 \pm 0.1	10.6 \pm 0.3
		gonads	15	-17.8 \pm 0.8	10.1 \pm 0.3
		mantle	15	-17.1 \pm 0.2	10.1 \pm 1.1
Fish	<i>Onuxodon fowleri</i>	muscle	15	-17.3 \pm 0.2	9.8 \pm 0.3
		muscle	16	-17.8 \pm 0.2	14.7 \pm 0.8
Shrimp	<i>Conchodytes meleagrinae</i>	whole body	10	-16.8 \pm 0.6	13.1 \pm 1.0

male accompanied by one large female in the mantle cavity of the pearl oyster *P. margaritifera* (Bruce 1976, Poupin 1998). The shrimps were killed by immersion in ice-cold water and their entire body was used for stable isotope analysis. Other potential food sources were also taken from the fish collection site: small benthic invertebrates (amphipods and decapods) found in the vicinity of the bivalves were collected using small light traps made of plastic bottles containing glow sticks (Frédérich et al. 2009). They were pooled together, considered as zoobenthos and used for stable isotope analysis. All these food sources (fish muscle tissues, oyster tissues, palaemonid shrimps and zoobenthos) were dehydrated for 24 h at 50°C and then stored in glass flasks until stable isotope analysis. Sample sizes of these food sources and their mean isotopic values are summarized in Table 2.

Stomach content analysis

After dissection, the fish stomachs were opened and all dietary constituents were placed into a Petri dish. All food items were identified using a Leica MS5 binocular microscope (Leica, Solms, Germany). Preys were identified to the lowest taxonomic level possible (Ruppert et al. 2004), and amorphous material (i.e. items lacking any identifiable features) was considered as unidentifiable.

Stable isotope analysis

All dehydrated samples were ground into a homogeneous powder using mortar and pestle. Prior to running the stable isotope analysis, samples containing carbonates (zoobenthos and shrimps) were placed for 24 h under a glass bell with fuming HCl (37%) (Merck, Darmstadt, Germany, for analysis quality) in order to

eliminate calcareous material, the presence of inorganic carbon being a source of bias for C stable isotope ratio analysis. Then, carbon and nitrogen gas contained in all samples were analysed with an Isoprime 100 isotope ratio mass spectrometer (Isoprime, UK) coupled to an N–C–S elemental analyser (Vario Micro, Elementar, Germany). Stable isotope ratios were expressed in δ notation according to the following equation:

$$\delta X = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ for samples or standards.

Carbon and nitrogen ratios are expressed relative to the vPDB (Vienna Peedee Belemnite) standard and to the atmospheric nitrogen standard, respectively. Certified materials were IAEA-N1 ($\delta^{15}\text{N}=+0.4\pm 0.2\text{‰}$) and IAEA CH-6 (sucrose) ($\delta^{13}\text{C}=-10.4\pm 0.2\text{‰}$). Routine measurements were repeatable to within 0.3‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Statistical analyses

A Shapiro-Wilk test was used to test the normality of the data. As the assumption of normal distribution was met, one-way ANOVA with a subsequent post hoc multiple comparisons test (Tukey test) was used to compare isotopic data among the bivalve tissues (gonads, gills, mantle and muscles). Then, another one-way ANOVA with a subsequent post hoc multiple comparisons test (Tukey test) was used to compare isotopic data among the different species (fish, shrimp and bivalve tissues). All statistical analyses were carried out with GrafPad Prism 5 (GrafPad Software, Inc. USA). Results are expressed as means \pm standard deviation (sd). Significance level was determined at $P < 0.05$.

RESULTS

Over the two sampling campaigns, we found a total of 57 *O. fowleri* individuals sheltered inside the 209 *P. margaritifera* that were collected on the 13 reef pinnacles. Therefore, the overall ratio between the number of occupied hosts and the number of collected hosts was about 1:4, with an overall occupation rate of 27.3%. A more detailed listing of the occupation rates observed on the different reef pinnacles is presented in Table 1. All fish were encountered in pearl oysters collected in front of Arikitamori Pass. The highest numbers of fish (occupation rate $\geq 50\%$) were observed inside bivalves collected close to the Pass, whereas very few fish (occupation rate $< 15\%$) were identified in pearl oysters collected at some of the reef pinnacles located further away from the Pass (Fig. 1). Moreover, no fish (occupation rate 0%) were observed inside bivalves collected on two reef pinnacles: one of these pinnacles was located far from the Pass and the other one was not in its alignment (Fig. 1).

In addition, a pair of palaemonid shrimp (*C. meleagrinae*) was observed in almost each of the collected pearl oysters (pers. obs.), implying that the co-

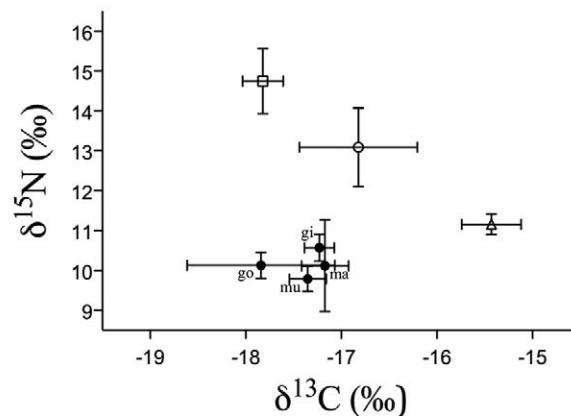


Fig. 2. – Mean (\pm sd) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the carapid fish *Onuxodon fowleri* (white square), the palaemonid shrimp *Conchodytes meleagrinae* (white circle), zoobenthos (white triangle), and tissues of the black-lip pearl oyster *Pinctada margaritifera* (black circles) from Makemo Atoll (Tuamotu Archipelago, French Polynesia); gi, gills; go, gonads; ma, mantle; mu, muscles.

occurrence of both symbionts within the same host was frequent.

Stomach contents

Out of the 16 digestive tracts of *O. fowleri* examined, 10 were empty. Two stomachs contained remains of annelid worms, two contained conical eggs of invertebrates and two contained unidentifiable soft tissues that appeared to be shredded preys.

Stable isotopes

Isotopic values in bivalve tissues ranged from -18.6‰ to -16.9‰ for $\delta^{13}\text{C}$ and from 9.0‰ to 11.2‰ for $\delta^{15}\text{N}$ (Fig. 2, Table 2). Statistical analyses revealed significant differences among the pearl oyster tissues (ANOVA, $F_{3,56}=7.497$, $P < 0.0001$ for $\delta^{13}\text{C}$; ANOVA, $F_{3,56}=2.557$, $P=0.0659$ for $\delta^{15}\text{N}$). According to the $\delta^{13}\text{C}$ values, gonads were depleted compared with the muscles (Tukey test, $P < 0.01$), gills (Tukey test, $P < 0.001$) and mantle (Tukey test, $P < 0.0001$), but no significant difference was observed between the $\delta^{15}\text{N}$ values of the bivalve tissues (Tukey test, $P > 0.05$; Fig. 2, Table 2).

Both symbionts (carapid fish and palaemonid shrimp) showed isotopic values ranging from -18.0‰ to -16.2‰ for $\delta^{13}\text{C}$ and from 12.1‰ to 15.5‰ for $\delta^{15}\text{N}$ (Fig. 2, Table 2). There were significant isotopic differences between the host tissues and symbionts (ANOVA, $F_{5,80}=12.63$, $P < 0.0001$ for $\delta^{13}\text{C}$; ANOVA, $F_{5,80}=109.1$, $P < 0.0001$ for $\delta^{15}\text{N}$). *Onuxodon fowleri* displayed a mean $\delta^{15}\text{N}$ value higher than all the different *P. margaritifera* tissues (Tukey test, $P < 0.0001$; Fig. 2, Table 2), while palaemonid shrimps were significantly ^{15}N -enriched compared with the muscles, gills, mantle and gonads of their bivalve host (Tukey test, $P < 0.0001$; Fig. 2). For example, shrimps displayed a mean $\delta^{15}\text{N}$ enrichment of 3.0‰ compared with *P. margaritifera* gonads (Table 2). Regarding the $\delta^{13}\text{C}$ values, *O. fowleri*

muscle tissues were significantly depleted compared with the muscles (Tukey test, $P < 0.01$), gills (Tukey test, $P < 0.001$) and mantle (Tukey test, $P < 0.001$) of their bivalve host. However, the $\delta^{13}\text{C}$ values of bivalve gonads were similar to those of fish tissues (Tukey test, $P > 0.05$; Fig. 2, Table 2). In addition, the $\delta^{13}\text{C}$ values did not differ between the palaemonid shrimp and the tissues of the bivalve host (Tukey test, $P > 0.05$), except that shrimps were significantly enriched in $\delta^{13}\text{C}$ compared with the host gonads (Tukey test, $P < 0.0001$; Fig. 2, Table 2), with a mean increase of 1.0‰ (Table 2).

Regarding the two symbionts, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly different (Tukey test, $P < 0.0001$; Fig. 2, Table 2), with shrimps displaying higher $\delta^{13}\text{C}$ but lower $\delta^{15}\text{N}$ values than fish (Fig. 2, Table 2). For the other food sources, zoobenthos had higher $\delta^{13}\text{C}$ values than both symbiont and bivalve tissues, whereas they had lower and higher $\delta^{15}\text{N}$ values than symbiont and bivalve tissues, respectively (Table 2).

DISCUSSION

All *O. fowleri* individuals were found in host bivalves collected in the axis of Arikitamori Pass. Overall, the percentages of pearl oysters being occupied by fish were very low, or zero, at some reef pinnacles located far from the Pass, while the highest numbers of fish were observed inside bivalves collected close to the Pass (Fig. 1, Table 1). Moreover, no fish were observed among bivalves collected at the only reef pinnacle that was not in the alignment of the Pass (Fig. 1). These differences in the occupation rates observed at the different locations of capture might be explained by the way of life of carapids. Like most coral reef fishes, carapids have a complex life history divided into two stages: a dispersive pelagic larval stage followed by sedentary demersal juvenile and adult stages associated with the coral reef environment (Leis 1991, Leis and McCormick 2002). At the end of the pelagic stage, larvae settle on the patch reef within the lagoon and rapidly try to enter a benthic host (Smith 1964, Smith et al. 1981, Colley et al. 2008). This behaviour appears to be essential for the growth and survival of carapids (Parmentier et al. 2004a,b, Parmentier 2016). Therefore, it is likely that the greater amount of fish found inside pearl oysters collected close to Arikitamori Pass results from the fact that *O. fowleri* directly seek to enter a bivalve host shortly after settlement.

A large proportion (60%) of the stomach contents were empty and very little prey material was found in the non-empty stomachs. This high percentage of empty stomachs could have been misleading: the remains of host tissues, if any, could not have been detected during stomach content analysis since these soft tissues would have been digested very fast. Nonetheless, the way of life of symbiotic carapids would not require a great amount of energy since they are quite inactive inside their host. This proportion of empty stomachs might simply reflect the infrequency of feeding due to the low metabolism of species that spend a great part of their adult life within their host (Parmentier et al. 2002). In *C. bermudensis* living inside holothurians,

the periodicity of feeding ranges from 15 to 24 days on average but it can last up to 60 days (Smith et al. 1981). In addition, the high percentage of empty stomachs observed in *O. fowleri* might be related to the time of sampling (10:00 am – 3:00 pm). Recently, Kéver et al. (2014) reported that the majority of sounds produced by *O. fowleri* in the field were recorded between 5:00 PM and 12:00 AM, which implies a nocturnal activity in this species. Assuming that *O. fowleri* forages for food mainly at night, it is thus likely that most of the stomach contents were empty because they were opened during daytime.

Typically, the $\delta^{15}\text{N}$ values increase by approximately 2 to 5‰ with each trophic transfer between a consumer and its diet, while the $\delta^{13}\text{C}$ values of an animal are close to that of its diet or slightly enriched by 1‰ (DeNiro and Epstein 1978, 1981, Vander Zanden and Hulshof 1998). Instead of being used as a reliable indicator of trophic level, $\delta^{13}\text{C}$ values are generally used to indicate the relative contribution of different primary food sources (Parmentier and Das 2004, Frédéricich et al. 2009, Cabanellas-Reboredo et al. 2010). Regarding carapid fishes, Parmentier and Das (2004) observed that tissues of commensal species such as *C. homei* and *C. boraborensis* were strongly ^{13}C -depleted and ^{15}N -enriched compared with the respiratory trees and gonads of their holothurian host *B. argus* (e.g. the mean decrease in $\delta^{13}\text{C}$ ranged from 4.5‰ to 9‰, and the mean increase in $\delta^{15}\text{N}$ ranged from 6‰ to 9‰, depending on host tissues). A similar ^{13}C depletion and ^{15}N enrichment was observed for the muscles of the commensal species *C. mourlani* compared with the gonads of its starfish host *C. novaeguineae* (e.g. the mean $\delta^{13}\text{C}$ decreased by about 8‰ and the mean $\delta^{15}\text{N}$ increased by about 7‰; Parmentier and Das 2004). On the other hand, Parmentier and Das (2004) noticed a mean increase in $\delta^{13}\text{C}$ of about 1.5‰ and a mean increase in $\delta^{15}\text{N}$ of 2.5‰ between *B. argus* gonads and *Encheliophis gracilis* muscles. These isotopic values indicated that *E. gracilis* could feed on its host gonads (Parmentier and Das 2004). All these observations were also confirmed by stomach content analysis and morphological characteristics (Smith 1964, Trott 1970, Parmentier et al. 1998), which supported the commensal and parasitic relationship attributed to these carapid species. In the present study, a mean increase in $\delta^{15}\text{N}$ ranging from 4‰ to 5‰ was observed between the tissues of *P. margaritifera* and *O. fowleri* muscles. Moreover, fish muscles were significantly ^{13}C -depleted compared with their host tissues. From an ecological point of view, the ^{13}C depletion of *O. fowleri* muscles compared with some of the bivalve tissues seems to indicate that these are not the main source of food for the fish. Given that the bivalve gonads showed the same $\delta^{13}\text{C}$ values as the fish muscles (Table 2), they should also be excluded from the fish diet. We also found that the $\delta^{13}\text{C}$ values of *O. fowleri* did not match the isotopic composition of small benthic invertebrates (zoobenthos; see Fig. 2, Table 2) collected in the vicinity of the pearl oysters. Similarly, Parmentier and Das (2004) observed that adults of commensal *Carapus* species are ^{13}C -depleted compared with the lagoon benthic inver-

tebrates. Stable isotope ratios of carbon are known to be typically higher in species from coastal or benthic food webs than those from offshore food webs (Guo et al. 2002, Frédérich et al. 2009, 2012). It is thus likely that the $\delta^{13}\text{C}$ values displayed by *O. fowleri* reflect a diet including pelagic preys or settling larvae entering the lagoon, which might also explain why most of the *O. fowleri* specimens were observed inside pearl oysters collected close to Arikitamori Pass. Regarding the $\delta^{15}\text{N}$ difference between the carapid fish and its bivalve host, it seems very unlikely that *O. fowleri* specimens might feed on their host tissues, considering their strong ^{15}N enrichment compared with *P. margaritifera*. Parmentier and Das (2004) measured a mean increase in $\delta^{15}\text{N}$ of 2.5‰ between *B. argus* gonads and the parasite carapid *E. gracilis*, which is about two times less than the ^{15}N enrichment observed between *P. margaritifera* tissues and *O. fowleri* muscles (Fig. 2). In a similar coral reef ecosystem, it is also interesting to note that fish considered as pelagic and benthic feeders showed an enrichment ranging from 2‰ to 3‰ in ^{15}N relative to their assimilated food (Frédérich et al. 2009, 2012, Wyatt et al. 2010). In this context, *O. fowleri* would occupy two trophic levels higher than its bivalve host (Fig. 2).

Our isotopic analysis showed that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values increased between the bivalve tissues and the palaemonid shrimp *C. meleagrinae* (Fig. 2, Table 2). Due to the mean increase in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, it appears that the shrimp occupies a higher trophic level than its host. More interestingly, *C. meleagrinae* showed a mean increase in $\delta^{13}\text{C}$ of 1‰ and a mean $\delta^{15}\text{N}$ enrichment of 3.0‰ compared with its host gonads. Considering these isotopic compositions, it cannot be totally ruled out that the shrimp might feed on its host gonads. From the evolutionary point of view, this assumption could explain why the palaemonid shrimp became morphologically adapted to living inside its host and why it adopted a sedentary way of life (Bruce 1976). Feeding on its host gonads would provide the shrimp with easy access to a food source rich in lipids, especially during the sexual maturation of the bivalve (Vahirua-Lechat et al. 2008). Moreover, it is not uncommon to encounter a crustacean guest parasitizing a bivalve host in nature. Although the association had regularly been considered commensalism, it was reported that the crab *Zaops ostreus* was parasitic on the American oyster *Ostrea virginica* (Stauber 1945). Likewise, the pea crab *P. pisum* is known to cause stress and lesions to its host bivalve, the common mussel *Mytilus edulis* (Bierbaum and Ferson 1986, Haines et al. 1994). At this point, further analysis of the shrimp diet using stomach contents should provide additional data in order to confirm this parasitical behaviour.

Finally, it is interesting to note that both symbionts may co-occur within the same host bivalve, which suggests a potential trophic competition between the two guests. *Onuxodon fowleri* seems to occupy a higher trophic level by being significantly ^{15}N -enriched compared with *C. meleagrinae* (Table 2), but the carapid also showed a mean decrease in $\delta^{13}\text{C}$ of 1‰ compared with the palaemonid shrimp (Table 2). As a result,

these isotopic compositions indicated that the shrimp should not be part of the fish diet, a finding which was also supported by the fact that no fragments of cuticular remains were found in the stomach contents of *O. fowleri*.

CONCLUSION

Our results provide new data on the symbiotic relationship between Fowler's pearlfish and the black lip pearl oyster. On the basis of the isotopic compositions measured in the present study, the commensal relationship usually attributed to *P. margaritifera* and its guest *O. fowleri* is supported. The carapid fish seems indeed to use its bivalve host as a shelter. In addition, our $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements suggest that the palaemonid shrimp *C. meleagrinae* might feed on the bivalve tissues, especially considering the enrichment in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared with its host gonads. Ultimately, further isotopic measurements of other food sources (sessile invertebrates, zooplankton and algae) as well as stomach content analysis of the palaemonid shrimp are needed to better characterize the diet of both symbionts.

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REFERENCES

- Bierbaum R.M., Ferson S. 1986. Do symbiotic pea crabs decrease growth rate in mussels? Biol. Bull. 170: 51-61.
<https://doi.org/10.2307/1541380>
- Bruce A.J. 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. In: Jones O.A., Dean R.M. (eds), Biology and geology of coral reefs. Academic Press, New York, pp. 37-94.
<https://doi.org/10.1016/B978-0-12-395527-2.50009-3>
- Cabanellas-Reboredo M., Blanco A., Deudero S., et al. 2010. Effects of the invasive macroalga *Lophocladia lallemantii* on the diet and trophism of *Pinna nobilis* (Mollusca: Bivalvia) and its guests *Potonia pinnophylax* and *Nepinnotheres pinnotheres* (Crustacea: Decapoda). Sci. Mar. 74: 101-110.
<https://doi.org/10.3989/scimar.2010.74n1101>
- Colleye O., Brié C., Malpot E., et al. 2008. Temporal variability of settlement in Carapidae larvae at Rangiroa atoll. Env. Biol. Fish. 81: 277-285.
<https://doi.org/10.1007/s10641-007-9199-4>
- de Bary A. 1879. Die erscheinung der symbiose. Verlag von Karl J. Trubner, Strassburg.
- DeNiro M.J., Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42: 495-506.
[https://doi.org/10.1016/0016-7037\(78\)90199-0](https://doi.org/10.1016/0016-7037(78)90199-0)
- DeNiro M.J., Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45: 341-351.
[https://doi.org/10.1016/0016-7037\(81\)90244-1](https://doi.org/10.1016/0016-7037(81)90244-1)
- Fowler H.W. 1927. Fishes of the tropical central Pacific. B. P. Bishop. Mus. Bull. 38: 1-32.
- Frédérich B., Fabry G., Lepoint G., et al. 2009. Trophic niches of thirteen damselfishes (Pomacentridae) at the Grand Récif of Toliara, Madagascar. Ichthyol. Res. 56: 10-17.

- <https://doi.org/10.1007/s10228-008-0053-2>
- Frédérich B., Colleye O., Lepoint G., et al. 2012. Mismatch between shape changes and ecological shifts during the post-settlement growth of the surgeonfish, *Acanthurus triostegus*. *Front. Zool.* 9: 8. <https://doi.org/10.1186/1742-9994-9-8>
- Gervis M.H., Sims N.A. 1992. The Biology and Culture of Pearl Oysters (Bivalvia: Pteriidae). *ICLARM Stud. Rev.* 21: 1-56.
- Guo W., Yang Y., Wu L., et al. 2002. Carbon isotope study on trophic relationships of Zhubi reef ecosystem in Nansha Islands. *J. Oceanogr. Taiwan* 21: 94-101.
- Haines C.M.C., Edmunds M., Pewsey A.R. 1994. The pea crab, *Pinnotheres pisum* (Linnaeus, 1767), and its association with the common mussel, *Mytilus edulis* (Linnaeus, 1758), in the solent (UK). *J. Shellfish. Res.* 13: 5-10.
- Kéver L., Colleye O., Lugli M., et al. 2014. Sound production in *Onuxodon fowleri* (Carapidae) and its amplification by the host shell. *J. Exp. Biol.* 217: 4283-4294. <https://doi.org/10.1242/jeb.109363>
- Leis J.M. 1991. The pelagic stage of reef fishes: the larval biology of coral reef fishes. In: Sale P.F. (ed.), *The ecology of fishes on coral reefs*. Academic Press, San Diego, pp. 183-230. <https://doi.org/10.1016/B978-0-08-092551-6.50013-1>
- Leis J.M., McCormick M.I. 2002. The Biology, Behavior, and Ecology of the Pelagic, Larval Stage of Coral Reef Fishes. In: Sale P.F. (ed.), *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem*. Academic Press, San Diego, pp. 171-200. <https://doi.org/10.1016/B978-012615185-5/50011-6>
- Markle D.F., Olney J.E. 1990. Systematics of the pearlfishes (Pisces: Carapidae). *Bull. Mar. Sci.* 47: 269-410.
- Parmentier E. 2016. Further insights into the metamorphosis process of a carapid fish. *J. Zool.* 298: 249-256. <https://doi.org/10.1111/jzo.12314>
- Parmentier E., Das K. 2004. Commensal vs. parasitic relationship between Carapini fish and their hosts: Some further insight through $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. *J. Exp. Mar. Biol. Ecol.* 310: 47-58. <https://doi.org/10.1016/j.jembe.2004.03.019>
- Parmentier E., Michel L. 2013. Boundary lines in symbiosis forms. *Symbiosis* 60: 1-5. <https://doi.org/10.1007/s13199-013-0236-0>
- Parmentier E., Chardon M., Poulicek M., et al. 1998. Morphology of the buccal apparatus and related structures in four species of Carapidae. *Aust. J. Zool.* 46: 391-404. <https://doi.org/10.1071/ZO97035>
- Parmentier E., Chardon M., Poulicek M., et al. 1999. Morphological particularities of the head in four Carapidae (Ophidiiformes). In: Séret B., Sire J.Y. (eds), *Proc. 5th Indo-Pacific Conf, Nouméa, 1997*. Soc. Fr. Ichthyol., pp. 135-146.
- Parmentier E., Castro-Aguirre J.L., Vandewalle P. 2000. Morphological comparison of the buccal apparatus in two bivalve commensal Teleostei, *Encheliophis dubius* and *Onuxodon fowleri* (Ophidiiformes, Carapidae). *Zoomorphology* 120: 29-37. <https://doi.org/10.1007/s004359900020>
- Parmentier E., Lagardère F., Vandewalle P. 2002. Relationships between inner ear and sagitta growth during ontogenesis of three Carapini species and consequences of life-history events on the otolith microstructure. *Mar. Biol.* 141: 491-501. <https://doi.org/10.1007/s00227-002-0853-2>
- Parmentier E., Lecchini D., Vandewalle P. 2004a. Remodelling of the vertebral axis during metamorphic shrinkage in the pearlfish. *J. Fish. Biol.* 64: 159-169. <https://doi.org/10.1111/j.1095-8649.2004.00294.x>
- Parmentier E., Lecchini D., Lagardère F., et al. 2004b. Ontogenic and ecological control of metamorphosis onset in a carapid fish, *Carapus homei*: experimental evidence from vertebra and otolith comparisons. *J. Exp. Zool. Part A* 301: 617-628. <https://doi.org/10.1002/jez.a.50>
- Peterson B.J., Fry B. 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18: 293-320. <https://doi.org/10.1146/annurev.es.18.110187.001453>
- Pinnegar J.K., Polunin N.V.C. 1999. Differential of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among fish tissues: implications for the study of trophic interactions. *Funct. Ecol.* 13: 225-231. <https://doi.org/10.1046/j.1365-2435.1999.00301.x>
- Poupin J. 1998. Crustacea Decapoda and Stromatopoda of French Polynesia. *Atoll Res. Bull.* 451: 1-62. <https://doi.org/10.5479/si.00775630.451.1>
- Ruppert E.E., Fox R.S., Barnes R.D. 2004. *Invertebrate Zoology, A functional Evolutionary Approach*. Brooks/Cole – Thomson Learning Inc., Belmont, CA, USA, 963 pp.
- Salvat B. 2009. Dominant benthic mollusks in closed atolls, French Polynesia. *J. Coral Reef Stud.* 11: 197-206. <https://doi.org/10.3755/galaxea.11.197>
- Smith C.L. 1964. Some Pearlfishes from Guam, with Notes on Their Ecology. *Pac. Sci.* 18: 34-40.
- Smith C.L., Tyler J.C., Feinberg M.N. 1981. Population ecology and biology of the pearlfish (*Carapus bermudensis*) in the lagoon at Bimini, Bahamas. *Bull. Mar. Sci.* 31: 876-902.
- Southgate P.C., Lucas J.S. 2008. *The Pearl Oyster*. Elsevier, Amsterdam, The Netherlands.
- Stauber L.A. 1945. *Pinnotheres ostreum*, parasitic on the American oyster, *Ostrea (Gryphaea) virginica*. *Biol. Bull.* 88: 269-291. <https://doi.org/10.2307/1538315>
- Trott L.B. 1970. Contribution of the biology of carapid fishes (Paracanthopterygian: Gadiformes). *Univ. Calif. Publ. Zool.* 89: 1-41.
- Trott L.B. 1981. A general review of the pearlfishes (Pisces, Carapidae). *Bull. Mar. Sci.* 31: 623-629.
- Trott L.B., Trott E.B. 1972. Pearlfishes (Carapidae: Gadiformes) collected from Puerto Galera, Minobra, Philippines. *Copeia* 1972: 839-843. <https://doi.org/10.2307/1442743>
- Tyler J.C. 1970. A redescription of the inquiline carapid fish *Onuxodon parvibrachium*, with a discussion of the skull structure and the host. *Bull. Mar. Sci.* 29: 148-164.
- Vahirua-Lechat I., Laure F., LeCoz J.R., et al. 2008. Changes in fatty acid and sterol composition during oogenesis in the pearl oyster *Pinctada margaritifera*. *Aquacult. Res.* 39: 1739-1746. <https://doi.org/10.1111/j.1365-2109.2008.02050.x>
- Vanden Spiegel D., Jangoux M. 1989. La symbiose entre poissons Carapidae et holothuries autour de l'île de Laing (Mer de Bismarck, Papouasie Nouvelle-Guinée). *Indo-Malayan Zool.* 6: 223-228.
- Vander Zanden M.J., Hulshof M. 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. *Trans. Am. Fish. Soc.* 127: 729-739. [https://doi.org/10.1577/1548-8659\(1998\)127<0729:AOSITT>2.0.CO;2](https://doi.org/10.1577/1548-8659(1998)127<0729:AOSITT>2.0.CO;2)
- Wyatt A.S.J., Waite A.M., Humphries S. 2010. Variability in Isotope Discrimination Factor in Coral Reef Fishes: Implications for Diet and Food Web Reconstruction. *PLoS ONE* 5: e13682. <https://doi.org/10.1371/journal.pone.0013682>