Running head: isotopic study of tropical ophiuroids symbiosis

Assessment of trophic relationships between symbiotic tropical ophiuroids using C and N stable isotope analysis.

Fourgon D.[1], Lepoint G.[2] and Eeckhaut I.[3]*
(1) Laboratoire de Biologie Marine, Université Libre de Bruxelles, 1050 Bruxelles, Belgium.
(2) Laboratoire d'Océanologie, Université de Liège, 4000, Liège, Belgium.
(3) Laboratoire de Biologie Marine, Université de Mons-Hainaut, 7000 Mons, Belgium.
*Corresponding author, e-mail : igor.eeckhaut@umh.ac.be

Ophiocoma scolopendrina and *Ophiomastix venosa* are two common Indo-West Pacific ophiuroids. They are encountered on the Great Reef of Toliara (Madagascar), where *O. venosa* juveniles are frequently associated symbiotically with *O. scolopendrina* adults. Analyses of the natural abundance of carbon and nitrogen stable isotopes were performed to investigate the feeding habits of the two ophiuroids, and to assess the potential trophic benefit obtained by the symbiotic *O. venosa* juveniles. A tracer experiment was also carried out to clarify the contribution of algae to the nitrogen uptake amongst the tested ophiuroids. Our results suggest that *O. scolopendrina* adults occupy a higher position in the food web than *O. venosa* and mainly feed on neuston. In contrast, *O. venosa* adults feed on the alga *Sargassum densifolium* and on organic matter associated with sediment. Free juveniles and symbiotic juveniles of *O. venosa* have intermediate $\delta^{13}C$ values between both adult species. The high proportion of ^{13}C in the symbiotic juveniles compared to the one in their conspecific adults indicates that their diet slightly differs from the latter and is closer to that of *O. scolopendrina*. This raises the hypothesis that symbiotic juveniles steal neuston from their associated host, *O. scolopendrina*.

INTRODUCTION

Ophiocoma scolopendrina and *Ophiomastix venosa* are tropical ophiuroids that co-occur on the Great Reef of Toliara located at the south-west of Madagascar. Although they both live on the boulder tract of the reef, *O. scolopendrina* adults inhabit parts of the reef -the domes- that emerge at low tide, while *O. venosa* adults are found in channels that are always immersed. These species are involved in a symbiosis where *O. venosa* juveniles live attached to *O. scolopendrina* adults (Fourgon 2006). This symbiosis seems facultative for both ophiuroids as some *O. venosa* juveniles, of the same size than symbiotic juveniles, live freely in the channels of the Great Reef. "Babbysitting" symbiosis has been reported in brittle stars from many localities in the Indo-Pacific Ocean. Such a symbiosis, that occurred between the subtidal *Ophiomastix annulosa* and the intertidal *O. scolopendrina,* was first described from Sesoko Island (Okinawa, Japan) by Hendler et al. (1999): *O. annulosa* juveniles were observed in the bursa and on the disk of *O. scolopendrina* adults. It would seem advantageous for these juveniles to take refuge in the intertidal on a large, abundant, widespread, mobile, calcified animals that occupied moist, sheltered crevices. Hendler et al. (1999) also suggested that these symbiotic juveniles would have at their disposal food from the host's arms. They considered this type of association as a brood parasitism.

O. scolopendrina has been described to employ several methods for food gathering depending on currents and tide levels: it can be deposit feeder or suspension feeder; it also feeds on the neuston (i.e., the microscopic organisms that inhabit the air-water interface)

(Magnus, 1967). Nothing has previously been published about the feeding habits of *O. venosa*.

Gut content analysis is the commonest and easiest method for determining the type of food ingested by an organism. However, when applied on invertebrates, it often fails to identify ingested particles. Yet, gut content analysis provides only "snapshots" of the diet and only takes into account the ingested food rather than the assimilated food. On the other hand, stable isotope methods are increasingly used as tracers to study food web structure (e.g., Lepoint et al., 2000; McCutchan et al., 2003). Stable isotopes of nitrogen and carbon of an animal tissue integrate dietary components that are assimilated over a much longer period of time. The strong enrichment in ^{15}N of a consumer related to its prey has been used in many studies as a tool to define the trophic levels of organisms (Post, 2002), the enrichment in ^{13}C is generally used to determine the relative contributions of different potential sources to the diet of a consumer (Phillips & Koch, 2002).

The gut content analysis of *O. venosa* and *O. scolopendrina* that we performed failed to give any information on their diet (unpublished observations). Therefore natural abundances of C and N stable isotopes in these ophiuroids were studied and are here presented in order to (*i*) investigate the feeding habits of *O. scolopendrina* and *O. venosa* on the Great Reef of Toliara and (*ii*) determine if *O. venosa* symbiotic juveniles could take a nutritional advantage in the symbiosis.

MATERIALS AND METHODS

Sampling location

All samplings were made at low tide on the boulder tract of the Great Reef of Toliara (Madagascar) in March 2002. The boulder tract is characterized by the presence of rocky domes measuring between 100 to 200 m long and ca. 10 m width. They consist in accumulations of dead corals emerging at low tides. Rocky domes are separated from each other by permanently immersed tidal channels (water height at low tide: ca. 50 cm) paved with living corals, dead corals and sand. The sampling area covered a rocky dome located at 23°25'00''S and 43°39'23''E, and its adjacent northern tidal channel.

Natural isotopic ratios

Four series of ophiuroids were collected: (*i*) *Ophicoma scolopendrina* adults and (*ii*) *Ophiomastix venosa* symbiotic juveniles (collected on the discs of their *O. scolopendrina* hosts) were sampled on the rocky dome; (*iii*) free juveniles and (*iv*) adults of *O. venosa* were sampled in the tidal channel. The free and symbiotic juveniles were approximately of the same size (disk diameter between 5 and 9 mm; $p>0.05$; Mann-Whitney U test). Free juveniles are *O.venosa* individuals that never entered in association with *O. scolopendrina*: they would metamorphose in the channels and not on the domes like the symbiotic juveniles (Fourgon 2006). We also collected four potential food sources for these ophiuroids. Amongst the algae present in the ecosystem, we sampled *Sargassum densifolium* that was the most abundant (fresh biomass of ca. 91.6 g/m^2), and *Ulva pertusa* (fresh biomass of ca. 0.24 g/m^2) that was frequently encountered near the ophiuroids. Though the investigated ophiuroids do not eat algae, they may ingest pieces of dying algae when they decompose in organic matter. The two other potential food sources sampled was the neuston, collected at the air-water interface at rising tide when it formed a dense organic film, and the organic matter associated with sediment, sampled in the tidal channel.

Five ophiuroid individuals of each category and five samples of each potential food sources were collected in order to measure their natural ratios of C and N stable isotope. The

ophiuroids were kept overnight in 60 l tanks filled with filtered sea water to facilitate the evacuation of their gut contents. The symbiotic juveniles were separated from their host the following day. All samples were dried for 48h at 50°C.

^{15}N Tracer experiment

Five ophiuroid individuals of each of the four categories (namely *O. scolopendrina* adults, *O. venosa* adults, free *O. venosa* juveniles and symbiotic *O. venosa* juveniles) were collected, brought to the laboratory and placed in four separated 60l tanks of filtered sea water, with bubbled air. Symbiotic juveniles were not separated from their hosts. Every other day, for one month, 10 g of ^{15}N enriched *Sargassum densifolium* and 30 g of freshly collected sediment were placed for 2h in each tank.

To obtain ^{15}N enriched food, one thallus of *S. densifolium* was kept for 6 days in an aerated 12 l tank filled with a ^{15}N labelled ammonium sulphate solution (75 µM; 99.0%^{15}N) (Eurisotop, France). This solution was changed every two days. The labelled alga was then thoroughly rinsed with filtered sea water, ground into pieces of ca. 1 mm^2 and kept for one week at 4°C. A sample of labelled food was dried for 48h at 50°C to verify the incorporation of ^{15}N in algal tissue.

After each feeding session, all tanks were emptied, thoroughly rinsed with sea water and filled with newly filtered sea water. After the entire experiment had ended, the ophiuroids were kept in the tanks for one night, without feeding, to allow evacuation of the gut contents, and dried the following day.

Isotope analysis

All dried samples were ground with a mortar and pestle into a homogeneous powder. They were divided in two equal subsamples. The subsample intended for C stable isotopes analysis, was slightly acidified in HCl 1M to remove inorganic carbonates, rinsed and oven-dried for 48h at 50°. The second non-acidified subsample was used to analyse N stable isotopes.

Isotopic and elemental measurements were performed in triplicates with an Optima mass spectrometer (Micromass, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). Natural stable isotope ratios were expressed in δ notation according to the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Carbon and nitrogen ratios are expressed relative to the Vienna Peedee Belemnite (VPDB) standard and to atmospheric nitrogen, respectively. Routine measurements are precise to within 0.3 ‰ for both ^{13}C and ^{15}N.

The abundance of ^{15}N in samples produced in the tracer experiment was expressed in atom %^{15}N. This represents the proportion of ^{15}N atoms relative to the total N atoms (^{14}N + ^{15}N).

Data treatment

ANOVA followed by post-hoc multiple comparison tests were used to compare the data among different categories of ophiuroids and among different food sources. Results were considered significant for $p < 0.05$.

RESULTS

Natural isotopic ratios

Amongst the ophiuroids tested, *Ophiomastix venosa* adults possess the lowest mean $\delta^{13}C$ value (-13.2‰) that is significantly different from the $\delta^{13}C$ value of *Ophiocoma scolopendrina* adults (-11.9‰) ($p<0.01$) (Fig. 1). *O. venosa* free and symbiotic juveniles have intermediate $\delta^{13}C$ signatures that are not significantly different from each other (-12.5 and –12.1, respectively) ($p>0.05$). However, while both juvenile categories do not significantly differ from *O. scolopendrina* adults in respect with their $\delta^{13}C$ values ($p>0.05$), only the symbiotic juveniles significantly differ from their conspecific adults ($p<0.02$).

Concerning the natural abundance of N stable isotopes, *O. scolopendrina* adults have the highest $\delta^{15}N$ signature (6.81‰) ($p<0.01$) (Fig. 1). The $\delta^{15}N$ values of adults, free and symbiotic juveniles of *O. venosa* do not differ significantly from each other (5.8, 5.7 and 5.9‰, respectively) ($p>0.05$).

Amongst the food sources, the organic matter associated with sediment and the neuston have the highest natural $\delta^{13}C$ and $\delta^{15}N$ values (-13.3 and –13.5‰ for $\delta^{13}C$, and 5.3 and 5.6 for $\delta^{15}N$; respectively) (significantly different from algae, $p<0.02$), and no significant difference was observed between them ($p>0.05$). The algae *U. pertusa* and *S. densifolium* do not differ from each other in their $\delta^{15}N$ mean values (3.2 and 3.4‰, respectively), but *U. pertusa* has by far lighter C isotope value than *S. densifolium* ($\delta^{13}C$ of –18.24 and –14.75‰, respectively).

^{15}N Tracer experiment

Isotopic analysis of labelled *S. densifolium* revealed that they are successfully enriched in ^{15}N: their mean ^{15}N abundance is 2.73 atom $\%^{15}N$ that is more than 7 times higher than their natural ^{15}N abundance (0.37 atom $\%^{15}N$). All ophiuroids are significantly enriched in ^{15}N comparing with their natural ^{15}N abundances ($p<0.05$). *O. venosa* free and symbiotic juveniles display the highest enrichments (0.033 and 0.032 atom $\%^{15}N$ in excess relatively to natural ^{15}N abundance, respectively) (Fig. 2). These enrichments are significantly higher than those of all adults ($p<0.03$) (Fig. 2). *O. scolopendrina* adults have the lowest enrichment (0.010 and atom $\%^{15}N$), although they do not differ significantly from those of *O. venosa* adults (0.017 atom $\%^{15}N$)($p>0.05$) (Fig. 2).

DISCUSSION

A very large difference is observed in the natural abundances of C and N stable isotopes between adults of *Ophiomastix venosa* and of *Ophiocoma scolopendrina*, the latter having the highest $\delta^{13}C$ and $\delta^{15}N$ values. *O. venosa* free and symbiotic juveniles have $\delta^{15}N$ values similar to their conspecific adults, but have intermediate values of $\delta^{13}C$.

Since $\delta^{15}N$ is related to the trophic level of organisms (Post, 2002), $\delta^{15}N$ values of *O. scolopendrina* would indicate that this species eats ^{15}N enriched food and occupies a higher position in the food web than the other sampled ophiuroids. From field observations, the neuston, which forms a dense film below the air-water interface at rising tide, seems to constitute the major component of the diet of *O. scolopendrina*. Indeed, it is abundantly caught by *O. scolopendrina*, which curls the tip of its arms (generally three at once), directing the oral side towards the air-water interface (Magnus, 1967; Chartock, 1983).

Isotope analyses support the importance of the neuston in *O. scolopendrina*'s diet. The analyses however indicate trophic shifts ($\Delta=\delta_{consumer} - \delta_{diet}$) of 1.21‰ in $\delta^{15}N$ and of 1.55‰ in $\delta^{13}C$, that strongly differ from the mean trophic shifts described in aquatic systems of 2.9 ‰ and 0.47 ‰, respectively (Vander Zanden & Rasmussen, 2001). Similarly low values for $\Delta^{15}N$ are known to occur amongst detritus feeders (Vanderklift & Ponsard, 2003), and

particularly in an unnamed ophiuroid (Fry, 1988). However, a large $\Delta^{13}C$ is known to occur in benthic detritus feeders because of the presence of bacteria in the substrates that raises the $^{13}C/^{12}C$ ratio of organic matter (McConnaughey & McRoy, 1979). It should however be pointed out that the small $\Delta^{15}N$ and the large $\Delta^{13}C$ observed for *O. scolopendrina* might also result from a selective feeding on the neuston, as suggested for other organisms that feed on particulate organic matter (Hsieh et al., 2000; Vanderklift & Ponsard, 2003).

O. venosa adults probably feed upon various nutrient sources, as indicated by the large variance associated to the $\delta^{13}C$ mean (see Bearhop et al., 2004 ; Matthews & Mazumder, 2004). The information on their $\delta^{13}C$ and $\delta^{15}N$ values suggests that *O. venosa* adults have a mixed diet composed of organic matter associated with sediment and algae. In the light of our results, *Sargassum densifolium* would constitute one of the algal component of their diet, *Ulva pertusa* $\delta^{13}C$ value being too far from the value recovered for *O. venosa* adults.

^{15}N enrichment analyses do not reveal significant difference between both adult species, which suggests that *O. scolopendrina* is also able to feed on algae when neuston is not available. The contribution of algae to the diet of *O. scolopendrina* is presumably less important than in *O. venosa*, as the former had the smallest enrichment in ^{15}N. Hence, in our tracer experiment, the low ^{15}N abundance in adults compared to juveniles might not only result from differences in their diet, but also from differences in the tissue turnover rate that would logically be higher in juveniles because of their faster growth, leading to higher ^{15}N assimilation.

The $\delta^{13}C$ values obtained on *O. venosa* juveniles (on both free juveniles and symbiotic juveniles) sampled in the field are significantly lower than those obtained on individuals that fed on *S. densifolium* during one month ($p<0.05$ for both categories). This result probably reflects that, in the field, free and symbiotic juveniles, if they feed on *S. densifolium*, have surely other carbon sources that would have a higher $\delta^{13}C$ than this alga. On their hand, the $\delta^{13}C$ values of adults (of both species) sampled in the field do not differ significantly from those of individuals that fed on *S. densifolium* during one month ($p>0.05$ for both categories).

The high $\delta^{13}C$ value in symbiotic juveniles, compared to that in their conspecific adults, indicates that their diet is slightly different. It would be closer to the diet of their *O. scolopendrina* hosts. Symbiotic juveniles might obtain neuston by stealing it from their hosts. Indeed, juveniles cling to the host in such a way that the oral side of their arms cross the oral side of *O.* scolopendrina's arms. This would allow them to pick up food particles that move toward *O. scolopendrina*'s mouth. Such similar stealing behaviour has also been proposed for other ophiuroids, for instance juveniles *Ophiothrix fragilis* are thought to intercept the food from conspecific adults, *Ophiomaza cacaotica* from crinoids, and *Ophiomastix annulosa* from *O. scolopendrina* (Warner, 1969; Clark, 1976; Hendler et al., 1999; respectively). Our results support the proposal (Hendler et al.,1999) that juveniles *Ophiomastix* that are associated with *O. scolopendrina* may steal food from the host. That appears to be the case for juvenile *O. venosa*. Furthermore, the results indicate that *O. venosa* juveniles ingest the neuston collected by *O. scolopendrina*, and perhaps do so selectively in preference to other potential food items.

ACKNOWLEDGMENTS

This work was supported by the Belgian National Fund for Scientific Research (FRFC contract 2.4.583.05). We acknowledge the assistance of J.M. Ouin, F. Mamitiana, J. Ralainirina, P. Manohitsara, N. Fohy, and Prosper. We thank G. Hendler and D. Vaitilingon for insightful comments that improved the quality of this manuscript. This paper is a

contribution from the “Centre Interuniversitaire de Biologie Marine” (CIBIM) and from the MARE centre (University of Liège).

REFERENCES

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of animal ecology*, **73**, 1007-1012.

Chartock, M.A., 1983. Habitat and feeding observations on species of *Ophiocoma* (Ophiocomidae) at Enewetak. *Micronesica*, **19(1-2)**, 131-149.

Clark, A.M., 1976. Tropical epizoic echinoderms and their distribution. *Micronesica*, **12**, 111-117.

Fourgon, D. 2006. Etude intégrée (écologique, éthologique et morphologique) d’une symbiose interophiuroidéenne dans l’écosystème corallien à Madagascar. PhD thesis. Free University of Brussels.

Fry, B., 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnology and Oceanography*, **33(5)**, 1182-1190.

Hendler, G., Grygier, M.J., Maldonado, E. & Denton, J., 1999. Babysitting brittle stars: heterospecific symbiosis between ophiuroids (Echinodermata). *Invertebrate Biology*, **118(2)**, 190-201.

Hsieh, H.L., Kao, W.Y., Chen, C.P. & Liu, P.J., 2000. Detrital flows through the feeding pathway of the oyster (*Crassostrea gigas*) in a tropical shallow lagoon: $\delta^{13}C$ signals. *Marine Biology*, **136**, 677-684.

Lepoint, G., Nyssen, F., Gobert, S., Dauby, P. & Bouquegneau, J.M., 2000. Relative impact of a *Posidonia* seagrass bed and its adjacent epilithic algal community in consumers diet. *Marine Biology*, **136**, 513-518.

Magnus, D.B., 1967. Ecological and ethological studies and experiments on the echinoderms of the Red Sea. *Studies in Tropical Oceanography*, **5**, 635-664.

Matthews, B. & Mazumder, A., 2004. A critical evaluation of intrapopulation variation of $\delta^{13}C$ and isotopic evidence of individual specialization. *Oecologia*, **140**, 361-371.

McConnaughey, T. & McRoy, C.P., 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology*, **53**, 257-262.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.

Phillips, D.L. & Koch, P.L., 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, **130**, 114-125.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology*, **83(3)**, 703-718.

Vanderklift, M.A. & Ponsard, S., 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. *Oecologia*, **136**, 169-182.

Vander Zanden, M.J and Rasmussen, J.B., 2001. Variation of $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implication for aquatic food web studies. *Limnology and Oceanography*, **46(8)**, 2061-2066.

Warner, G.F., 1969. Brittle-star beds in Torbay, Devon. *Underwater Association Report*, 81-85.

FIGURE LEGENDS

Figure 1 : Natural abundances of C and N stable isotope in four ophiuroids categories and four potential food sources. Osa, *Ophiocoma scolopendrina* adults; Ova, *Ophiomastix venosa* adults; Ovfj; *O. venosa* free juveniles; Ovsj, *O. venosa* symbiotic juveniles; ns, neuston; Sd, *Sargassum densifolium*; sdm, organic matter associated with sediment; Up, *Ulva pertusa*.

Figure 2: Enrichment in atom $\%^{15}N$ in ophiuroids after the tracer experiment compared with natural values, and their abundances of C stable isotopes. Osa, *Ophiocoma scolopendrina* adults; Ova, *Ophiomastix venosa* adults; Ovfj; *O. venosa* free juveniles; Ovsj, *O. venosa* symbiotic juveniles.

FIGURE 1

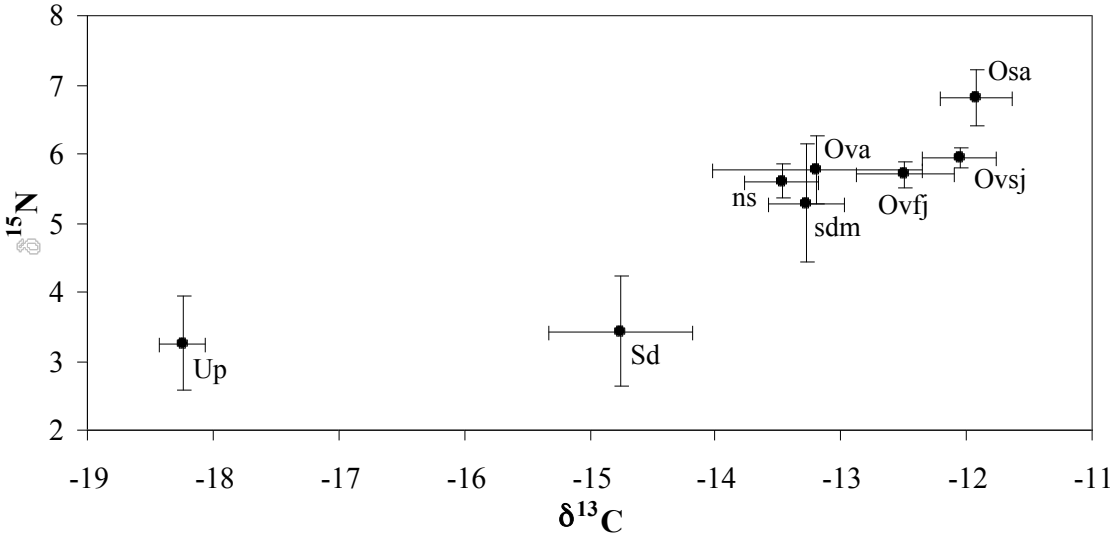

FIGURE 2