CARBON AND NITROGEN ASSIMILATION BY THE SUSPENSION-FEEDING BRITTLE-STAR *OPHIOTHRIX FRAGILIS* FROM TWO LOCALITIES IN THE ENGLISH CHANNEL

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OPHIUROIDEA
ORGANIC MATTER
METABOLIC BUDGET
STABLE ISOTOPE
C:N RATIO

ABSTRACT. – Carbon and nitrogen metabolic budgets and stable isotope analysis were used to determine the fate of the matter assimilated by the suspension-feeding brittle-star *Ophiothrix fragilis* in summer conditions. Metabolic budgets were established based on the experimental measurements of excretion and egestion rates and on previously published respiration and production rates. Two populations, from sites differing for the availability of macroalgae in the near shores, were investigated to test whether the assimilated matter reflected the local features of available matter. However, C:N (atomic ratio < 6) as well as $\delta^{13}C$ values (as negative as -25.05 ‰) of the suspended particulate matter indicated a dominance of living microalgae over detrital material at both sites. Furthermore, $\delta^{13}C$ and $\delta^{15}N$ values of *Ophiothrix fragilis* were close for the two sites (respectively -19.56 and -18.62 ‰ for $\delta^{13}C$; 8.29 and 7.40 ‰ for $\delta^{15}N$), supporting the hypothesis of a similar dietary composition for the two populations. Both metabolic budget and stable isotope analysis showed a discrepancy between the assimilated matter and the available matter composition. Some hypotheses are proposed to explain this discrepancy, as well as potential complementary studies needed to test for them.

INTRODUCTION

The common brittle-star *Ophiothrix fragilis* (Abildgaard, in O.F. Müller, 1789) is known to form very dense aggregations in areas of European waters submitted to high tidal currents (Brun 1969). Its suspension feeding has been described in detail (Warner & Woodley 1975) and the importance of dense beds in removing and recycling suspended organic material has been recognized (Hily 1991). The species harbours symbionts in arm tissues, sub-cuticular bacteria which role is unknown but that could contribute to the nutrition of their host (McKenzie & Kelly 1994). Knowledge of the seston-removal rates by *O. fragilis* in areas of dense aggregations, as well as of the type and fate of seston removed, are essential to understanding of processes like energy transfer, food web dynamics or nutrient cycling. Some experimental measurements of the filtering efficiency and the filtration rate have been made on this species (Olscher & Fedra 1977, Davoult *et al*. 1994, Allen 1998). Few examinations of stomach content showed that either phytoplankton (Brun 1969, Gounin 1993) or unidentifiable detritus (Warner & Woodley 1975) were the most frequent food items. Stable isotope ratios for carbon and fatty acid profiles also suggested a diet rich in phytoplankton as well as the potential

use of dissolved organic matter by the bacterial symbionts and its transfer to their host (McKenzie *et al*. 2000). *O. fragilis* seems then capable of digesting a variety of different forms of organic material and the composition of its diet could vary according to the available matter.

As the isotopic composition of consumers is closely related to that of their diet (DeNiro & Epstein 1978), stable isotope analysis can provide information on the origins and pathways of the assimilated food. In particular, $\delta^{13}C$ and $\delta^{15}N$ have been frequently used in coastal habitats to discriminate which primary producers support the food web and to construct trophic relationships (*e.g*. Kaehler *et al*. 2000). The quality of the matter assimilated by a consumer can also be assessed as the ratio of organic carbon to nitrogen content, which can be estimated from metabolic budgets. The balance between carbon and nitrogen supply and demand has such been examined to explore relationships between variability in the food supply and the metabolic responses of suspension-feeders (*e.g*. Bayne 2009).

Metabolic budget and stable isotope analysis are two complementary approaches and both were used in the present study in the aim to characterize the fate of matter assimilated by the suspension feeder *Ophiothrix fragilis* in summer conditions. First, metabolic budgets were

established in terms of carbon and nitrogen in order to calculate the C:N ratio of the assimilated matter. Second, $\delta^{13}C$ and $\delta^{15}N$ have been used to determine the origin of the assimilated matter. The study has been conducted in parallel on two dense populations in the English Channel to test whether the fate of the matter assimilated by each brittle-star population reflected the features of the matter locally available. In particular, the two sites differ for the availability of macroalgae in the near shores (either spare or abundant) and it was hypothesized that the macroalgal-derived matter could be a major dietary item for the brittle-star population in the site with abundant macroalgae in the near shores.

MATERIALS AND METHODS

Sampling: The brittle-stars were sampled in two already well-studied dense populations of the English Channel (*e.g.* Lefebvre *et al*. 1999, Muths *et al*. 2010). The two sites differed for the availability of macroalgae in the near shores, spare in Boulogne in the eastern part of the English Channel (50°43'N-1°27'E) and abundant in Roscoff in the western part of the English Channel (48°47'N-3°59'W). The sampling was done in Boulogne on the 28th of June and in Roscoff on the 6th of July 2005. Individuals of *Ophiothrix fragilis* were collected at slack water using a "Rallier du Baty" dredge.

Bottom sea water (about 30 m depth in Boulogne and 70 m in Roscoff) was sampled at the same moment using a Niskin bottle and immediately filtered on pre-combusted (6 h at 520°C) Whatman GF/F filters in order to collect the suspended particulate matter (SPM). One liter was sampled for CHN analysis and 5 l for carbon- and nitrogen- stable isotope analyses.

Some stranded macroalgae were sampled in order to test if the possible difference observed in the isotopic values of SPM from the two sites could be explained by a different contribution of macroalgae-derived matter. Stranded macroalgae are easy to sample and their $\delta^{13}C$ values are supposed to show no or only slight changes through the degradation process (Stephenson *et al*. 1986, Fellerhoff *et al*. 2003, Hill & McQuaid 2009). Stranded macroalgae were collected by hand at low tide on the shores near each of the brittle-stars sampling sites. Three species were collected at the Boulogne site (*Ulva* sp., *Fucus vesiculosus* and *Chondrus crispus*) and seven at the Roscoff site (*Ulva* sp., *Fucus vesiculosus*, *Fucus serratus*, *Ascophyllum nodosum*, *Himanthalia elongata*, *Sargassum* sp. and *Laminaria digitata*).

Excretion and egestion experiments: The excretion and egestion experiments were performed on board, immediately after the samplings and at the measured temperature (17.6 °C in Boulogne and 16.8 °C in Roscoff). The feeding state of the animals was supposed to be the same for the two sites as they were sampled under comparable tide conditions.

The *O. fragilis* adults (254 and 100 in Boulogne and Roscoff experiments respectively, corresponding to about the same total biomass for both experiments) were placed for four hours in a plastic tank filled with 8 liters sea water which was sampled (3 replicates of 100 cm³) every 30 min for the ammonium concentration measurement (according to the colorimetric indophenol method, Koroleff 1970). The ammonium excretion rate was calculated as the slope of the regression between the ammonium concentration, corrected for the removing of water during sampling, and the incubation time. To take into account a potential water column effect, ammonium measurements were also performed in control tanks (*i.e.* without any brittle-star) at both sites. The excretion rate was expressed in $\mu gN\ g_{afdw}^{-1}\ h^{-1}$ after the individuals have been weighed in ash free dry weight (afdw). The ash free dry weight was the difference between the dry weight (dw) obtained after 48 h at 60 °C and the weight of ashes obtained after 6 h at 520 °C. A Student's t-test was used to check for difference between excretion rates of brittle-stars from the two sites.

Some other individuals (256 and 175 in Boulogne and Roscoff experiments respectively) were placed on a net into a plastic tank filled with 7 l sea water which was filtered after 6 hours (on pre-combusted Whatman GF/F filters) to collect the faecal pellets. The egestion rate was expressed in $mg_{dw}\ g_{afdw}^{-1}\ h^{-1}$ after the faecal pellets and the individuals have been weighed in dry weight and ash free dry weight respectively. To take into account a potential water column effect, the water from control tank (*i.e.* without any brittle-star) was also filtered after 6 hours at both sites.

Organic carbon and nitrogen analyses: The organic carbon and nitrogen contents of faecal pellets (collected at the end of the egestion experiments) and suspended particulate matter (from the water collected at the bottom just prior the experiments) were determined on a CHN-analyser (NA 2100-CE Instruments) after acidification (1M HCl).

Stable isotope analyses: The suspended particulate matter, stranded macroalgae and brittle-stars were prepared in view of stable isotope analyses. The filters with SPM were quickly acidified (1 M HCl) to remove the carbonates (the remaining matter being organic) and rinsed with distilled water. The stranded macroalgae and brittle-stars were acidified with 10% HCl and rinsed with distilled water. These samples were dried (60°C) for 48 h and ground to a fine powder. The carbon and nitrogen isotope ratios were determined using a Flash EA CN analyser coupled with a Finnigan Delta Plus mass spectrometer, via a Finnigan Con-Flo III interface (4 replicated measures for the POM, 3 for the stranded macroalgae from Boulogne, 12 for the stranded macroalgae from Roscoff and 10 for the brittle-stars). The data were expressed in the conventional δ unit notation, where $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$, with $R_{sample} = ^{13}C/^{12}C$ ratio for carbon or $^{15}N/^{14}N$ ratio for nitrogen. $R_{standard}$ values were determined according to the Vienna Pee Dee Belemnite standard (PDB) for carbon and to N_2 atmospheric for nitrogen. A laboratory working standard (Peptone) was run every 10 samples. Average variations based on replicate measurements, using peptone standard, for $\delta^{13}C$ and $\delta^{15}N$ were less than ± 0.10‰. The stable carbon- and nitrogen-isotope signatures of the theoretical

resources of *O. fragilis* were estimated by taking into account a trophic enrichment (*i.e.* an increase in heavy isotopes) factor of 1 ‰ in $\delta^{13}C$ and 2.5 ‰ in $\delta^{15}N$ (DeNiro & Epstein 1978, Vander Zanden & Rasmussen 2001). A Student's t-test was used (after data have been log-transformed) to check for difference between mean $\delta^{13}C$ and $\delta^{15}N$ values of brittle-stars from the two sites.

Metabolic budget calculation: The ingestion rates of carbon and nitrogen by *O. fragilis* individuals from the two populations were calculated applying the equation of conservation of matter: I = P + R + Ex + Eg (with I: Ingestion, P: Production (somatic and gonadal), R: Respiration, Ex: Excretion and Eg: Egestion). The rate of assimilated matter was calculated as ingestion minus egestion (I – Eg). C:N mass ratio of ingested matter was calculated as the ratio of ingested carbon (R + P + Eg) on ingested nitrogen (Ex + P + Eg). C:N mass ratio of assimilated matter was calculated as the ratio of assimilated carbon (R + P) on assimilated nitrogen (Ex + P).

The excretion (in $\mu gN\ g_{afdw}^{-1}\ h^{-1}$) and egestion (either in $\mu gC\ g_{afdw}^{-1}\ h^{-1}$ or in $\mu gN\ g_{afdw}^{-1}\ h^{-1}$) rates were determined experimentally (see above). The respiration rate was estimated as a function of the day of measurement according to the relationship published by Migné and Davoult (1997):

$$y = 0.310 - 0.218 \sin[(2\pi/365)\,x + 1.094],$$
$$(n = 22, r^2 = 0.977)\ (1)$$

where x is the day number and y is the O_2-consumption rate in mg $g_{afdw}^{-1}\ h^{-1}$.

This relationship resulted from a two year survey and integrated the response of the species to seasonal variations of environmental conditions such as temperature and food availability.

The respiration rate was expressed in $\mu gC\ g_{afdw}^{-1}\ h^{-1}$ using a respiratory quotient (atomic ratio of CO_2 released to O_2 consumed) of 0.69 (Migné & Davoult 1997). The production rate was calculated according to the size distribution and cohort survey made on each population in April, June and August 2005 (Muths *et al*. 2010). The calculation was applied to a theoretical individual which weight was the mean weight of the individuals

used for the excretion and egestion experiments at each site. The production rate was expressed either in $\mu gC\ g_{afdw}^{-1}\ h^{-1}$ or in $\mu gN\ g_{afdw}^{-1}\ h^{-1}$ according to the conversion relationships established for the species by Davoult *et al*. (1992) between the disc diameter and the biomass expressed either in ash free dry weight, carbon or nitrogen:

$$\log(y) = -0.377 + 2.740\log(x),\ (n = 198, r = 0.982)\ (2)$$

where x is the disc diameter in mm and y is the biomass in mg of ash free dry weight;

$$\log(y) = -1.180 + 3.070\log(x),\ (n = 74, r = 0.991)\ (3)$$

where x is the disc diameter in mm and y is the biomass in mg of organic carbon;

$$\log(y) = -1.495 + 2.830\log(x),\ (n = 71, r = 0.991)\ (4)$$

where x is the disc diameter in mm and y is the biomass in mg of nitrogen.

RESULTS

The quantity and quality of the suspended particulate matter (SPM) measured in the bottom water at the moment of the brittle-stars sampling varied between the two sites. The concentration of SPM was 34.44 $mg_{dw}\ l^{-1}$ in Boulogne and 20.81 $mg_{dw}\ l^{-1}$ in Roscoff. The concentration in organic carbon was 0.52 mg l^{-1} in Boulogne and 0.42 mg l^{-1} in Roscoff. The concentration in nitrogen was 0.12 mg l^{-1} in Boulogne and 0.08 mg l^{-1} in Roscoff. The C:N mass ratio of the particulate organic matter (POM) was 4.26 in Boulogne and 5.11 in Roscoff.

The isotopic signatures of POM were close for the two sites (Table I) and then did not seem to be influenced by macroalgae-derived matter in Roscoff. A significant difference was found between *O. fragilis* from the two sites for either $\delta^{13}C$ (p = 0.033, n = 10) or $\delta^{15}N$ (p < 0.001, n = 10). However, common to the two sites was a $\delta^{13}C$ signal which was intermediate between POM and macroalgae (Fig. 1). The stable carbon- and nitrogen-isotope
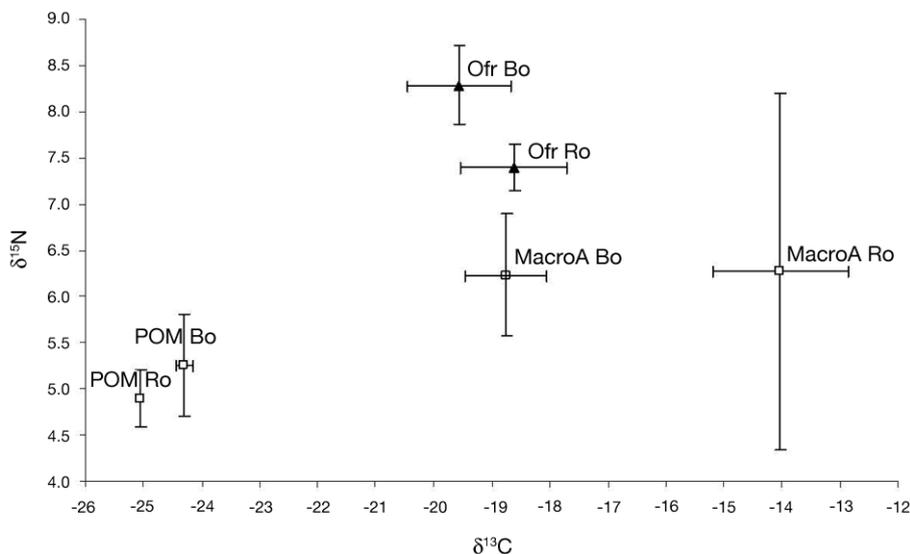

Fig. 1. – Stable carbon- and nitrogen-isotope signatures (± SD) of suspended particulate organic matter (POM), stranded macroalgae (MacroA) and *O. fragilis* (Ofr) from Boulogne (Bo) and Roscoff (Ro) sites.

Table I. – $\delta^{13}C$ and $\delta^{15}N$ (‰) values of suspended particulate organic matter (POM), various stranded macroalgae species and *O. fragilis* (Ofr) from Boulogne (Bo) and Roscoff (Ro) sites.

	n	Average $\delta^{13}C$	SD	Average $\delta^{15}N$	SD
POM Bo	4	-24.30	0.14	5.25	0.55
POM Ro	4	-25.05	0.00	4.89	0.31
Macroalgae Bo					
Ulva sp.	1	-19.00		5.70	
Fucus vesiculosus	1	-17.97		6.01	
Chondrus crispus	1	-19.31		6.97	
Macroalgae Ro					
Ulva sp.	1	-14.64		9.28	
Fucus vesiculosus	2	-13.33	0.02	6.85	0.33
Fucus serratus	1	-14.35		3.53	
Ascophyllum nodosum	1	-15.29		6.27	
Himanthalia elongata	1	-12.56		8.15	
Sargassum sp.	1	-15.96		7.17	
Laminaria digitata	5	-13.78	1.17	5.42	1.97
Ofr Bo	10	-19.56	0.89	8.29	0.42
Ofr Ro	10	-18.62	0.91	7.40	0.26

Table II. – Summer metabolism rates (in $\mu gC\ g^{-1}\ h^{-1}$ or $\mu gN\ g^{-1}$ h^{-1}) of *Ophiothrix fragilis* individuals from the Boulogne and Roscoff populations. The excretion (Ex) and egestion (Eg) rates were measured experimentally in summer 2005 (present study), the production (P) and respiration (R) rates were estimated according to previous published surveys (Migné & Davoult 1997, Muths *et al.* 2010).

	Boulogne		Roscoff	
	$\mu gC\ g^{-1}\ h^{-1}$	$\mu gN\ g^{-1}\ h^{-1}$	$\mu gC\ g^{-1}\ h^{-1}$	$\mu gN\ g^{-1}\ h^{-1}$
Ex		15.98		15.10
Eg	243.21	34.84	298.89	51.23
P	55.06	15.28	128.88	33.55
R	108.51		110.57	

tion rates, calculated for the two sites as the slopes of the regression lines were not significantly different (15.98 and 15.10 $\mu gN\ g_{afdw}^{-1}.h^{-1}$ respectively, t = 0.49, p = 0.34).

The egestion rates were calculated after correcting for water column effect detected in the control tanks (which was less than 15 %). The egestion rate was about the same for the two populations when expressed as total dry weight (2.60 and 2.71 $mg_{dw}\ g_{afdw}^{-1}$ h^{-1}). Nevertheless, the carbon and nitrogen content of the faeces varied between the two populations leading to different carbon and nitrogen egestion rates (Table II).

Ingestion rate calculation

To calculate the ingestion rate from the equation of conservation of matter, the respiration and production rates had to be estimated. The respiration rate of each population was estimated according to the sampling date (day number 179 for Boulogne and 187 for Roscoff) using the relationship (1). As the same relationship was used for the two populations, the difference in respiration rates (Table II) reflected only the 8 days delay between the two sampling dates.

According to the relationship between the disc diameter and the biomass in ash free dry weight (2), the individuals used for excretion and egestion experiments were considered to have a mean disc diameter of 6.3 mm at Boulogne and 8.6 mm at Roscoff. Their production rate (Table II) was then calculated as the increase in weight expected for individuals belonging to the cohorts including these disc diameters in spring and summer 2005. The calculation was performed for individuals from Boulogne belonging to the cohort with a mean diameter of 6.9 mm the 25th of April and to the cohort with a mean diameter of 7.7 mm the 26th of June (Muths *et al.* 2010). The calculation was performed for individuals from Roscoff belonging to the cohort with a mean diameter of 8.6 mm the 18th of June and to the cohort with a mean diameter of 10.3 mm the 16th of August (Muths *et al.* 2010).

The ingestion rates, calculated from the equation of conservation of matter using summer metabolic rates of *O. fragilis* given in Table II, were lower in the Boulogne than the Roscoff population (0.41 mgC g^{-1} h^{-1}, 0.07 mgN g^{-1} h^{-1} and 0.54 mgC g^{-1} h^{-1}, 0.10 mgN g^{-1} h^{-1} respectively). The C:N mass ratio of ingested matter was 6.15 in Boulogne and 5.39 in Roscoff and the C:N mass ratio of

signatures of the theoretical resources of *O. fragilis* were estimated to be -20.56 in $\delta^{13}C$ and 5.79 in $\delta^{15}N$ at Boulogne and -19.62 in $\delta^{13}C$ and 4.90 in $\delta^{15}N$ at Roscoff, by taking into account a trophic enrichment factor of 1 ‰ in $\delta^{13}C$ and 2.5 ‰ in $\delta^{15}N$.

Excretion and egestion experiments

The individuals of *Ophiothrix fragilis* used for the excretion and egestion experiments were smaller in Boulogne than in Roscoff (mean individual biomass of 64.71 and 152.76 mg_{afdw} in Boulogne and Roscoff experiments respectively). The Roscoff individuals were characterized by their swollen gonads. No increase in ammonium concentration was observed for any control experiment, and the regression between ammonium concentration and incubation time was highly significant in each experiment with brittle-stars (n = 9, R^2 = 0.968, p < 0.001 at Boulogne and n = 9, R^2 = 0.952, p < 0.001 at Roscoff). The excre-

assimilated matter (I - Eg) was 5.23 in Boulogne and 4.92 in Roscoff.

DISCUSSION

In general terms, seston C:N atomic ratios < 6 (*i.e.* mass ratio < 5.14 as observed for the two sites) are indicative of a dominance of living microalgae over detrital material (Parsons *et al*. 1977). δ^{13}C values of particulate organic matter of both sites, very depleted as compared to macroalgae detritus, are also typical of phytoplankton-dominated marine POM as previously observed in the Roscoff area (Riera & Hubas 2003, Riera *et al*. 2009) or in the Norwegian Sea (Fredriksen 2003). This prevents the conclusion that the diet of brittle-stars was subsidized by macroalgae-derived organic matter at Roscoff. However, the phytoplankton production is highly seasonal in the English Channel. During winter months, phytoplankton abundances are falling to very low levels (Hoch & Ménesguen 1997) while many macroalgae are senescing. The production of detritus is favoured by storms and the macroalgae-derived matter might reach its highest levels in winter. The analyses should then be repeated in winter conditions to test if, under low phytoplankton availability, the fate of suspended particulate matter could vary between the two sites and be influenced by the important macroalgae standing-stock in Roscoff.

When considering the δ^{15}N values of POM, the δ^{15}N values of *Ophiothrix fragilis* are consistent with the trophic level of primary consumer (Post 2002) generally admitted for this species (Roushdy & Hansen 1960, Warner & Woodley 1975). The δ^{13}C values of *O. fragilis* were intermediate between phytoplankton-dominated marine POM and stranded macroalgae for the two sites. They were more enriched compared to POM values that what would be expected of organisms forming part of a phytoplankton-dominated food chain. Since benthic algae have a heavier carbon isotopic ratio than phytoplankton, feeding on benthic primary producers is often proposed to explain the enriched carbon ratios in nearshore benthic consumers. However, reviewing 20 studies which provided 88 measurements of δ^{13}C for different groups of suspension and deposit feeders (including Ophiuroidea), Nadon & Himmelman (2006) observed a δ^{13}C enrichment of about 4 ‰ compared to POM, and the degree of enrichment did not appear to be related to the proximity of benthic primary producers. This was confirmed by their δ^{13}C analyses of scallops placed in cages at various depths and distances from shore and led them to conclude that factors other than the feeding on ^{13}C-rich benthic primary producers may explain the enriched carbon ratios in benthic consumers. Enriched δ^{13}C values for *O. fragilis* compared to POM values could be caused by a selective feeding within enriched POM as previously reported for the oyster *Crassostrea gigas* (Riera & Richard 1996) or

for sponges (Kaehler *et al*. 2000, Riera *et al*. 2009, Topcu *et al*. 2010). Selective feeding within enriched POM could correspond to a differential utilisation between living and detritic fractions and/or among different components of the phytoplankton. Another explanation for the discrepancy between isotope signature of POM and theoretical source for *O. fragilis* might be that the sampled POM did not correspond to the matter available for suspension-feeder at the bottom. This should be checked by the analyses of complementary sediment trap samples as done by Fredriksen (2003) in the Norwegian Sea.

The *O. fragilis* excretion and egestion rates measured in the present survey are in accordance with previously published summer rates for populations of the eastern English Channel (Davoult & Gounin 1995, Migné & Davoult 1998, Davoult & Migné 2001). Furthermore, the very similar rates measured on individuals from Boulogne and Roscoff showed a similar metabolic activity for the two populations though individuals from Boulogne and Roscoff obviously differed in their size and reproductive status. This agreement in excretion rates supports the use of a single relationship previously established for an eastern English Channel population (Migné & Davoult 1997) in estimating the respiration rate of both populations as a function of the day of sampling. The production rates (including both somatic and gonadal production) were about two fold lower at Boulogne than at Roscoff. That could be due to a discrepancy in the gonad growth in the two populations. Previous studies based on gonad growth (Lefebvre *et al*. 1999) and larval distribution (Lefebvre & Davoult 2000) showed that sexual maturity is likely to occur in June-July at both sites and main spawning in July-August, with some inter-annual variability. In 2005, there was an obvious difference in gonad development of individuals collected at the end of June at Boulogne and at the beginning of July at Roscoff. This is in accordance with the observations made during this summer by Muths *et al*. (2010): the major recruitment event occurred before the sampling at Boulogne, while larvae were observed at Roscoff only from mid July. The ingestion rate calculated by applying the equation of conservation of matter was then lower at Boulogne than at Roscoff. The assimilation efficiency was also slightly lower at Boulogne than at Roscoff with (I-Eg):I ratios respectively of 0.40 and 0.44 in terms of carbon and 0.47 and 0.49 in terms of nitrogen. The higher C:N ratios of the ingested matter compared to the available matter, particularly at Boulogne, suggested a selective feeding, the carbon uptake being enhanced relative to the nitrogen uptake. However, the C:N ratios were lower for the assimilated than the ingested matter suggesting the ability of *O. fragilis* to selectively digest organic material with a greater absorption efficiency for nitrogen than carbon. Comparing stable isotope signatures and fatty acid profiles of *O. fragilis* to the ones of other brittle-stars lacking subcuticular bacteria, McKenzie *et al*. (2000) suggested that the bacteria might contribute to the

nitrogen budget of the host. Even if the brittle-stars derive the bulk of their nutrition from conventional feeding, an additional contribution of symbionts could also explain the discrepancy in the fate of available particulate matter and assimilated matter.

Based on metabolic budget and stable-isotope analysis approaches, which both showed that the assimilated matter composition differed from the POM composition, a selective feeding was hypothesized for *Ophiothrix fragilis*. Due to high levels of silt found in stomach contents, selection of particles was not thought to take prior the ingestion in *O. fragilis* (Warner & Woodley 1975) even though selective ingestion (by rejection of some whole boluses in which food had been collected) had been reported for other ophiuroid species (Pentreath 1970). During experiments with polystyrene microspheres, behavior assumed to be active rejection was nevertheless observed in *O. fragilis*, but was not confirmed with nutritive particles (Allen 1998). Further experimental study using naturally occurring nutritive and non-nutritive particles is then required to examine this possible active selection. A second hypothesis was the contribution of the subcuticular bacterial symbionts to the nutrition of their host. Stable isotope tracer experiments are needed for testing the possible transfer of SCB-derived matter to their host. Finally, the matter really available at the bottom for the suspension-feeder should have not been analyzed and complementary sediment trap samples would be useful.

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