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Trophic niche of two co-occurring ophiuroid species in impacted coastal systems, derived from fatty acid and stable isotope analyses

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ABSTRACT: The trophic niches of 2 common co-occurring ophiuroids, *Ophiocomina nigra* and *Ophiothrix fragilis* (Echinodermata, Ophiuroidea), in 2 contrasting coastal systems of Brittany (France) were investigated. We used a combination of fatty acid biomarkers derived from neutral lipids and stable isotopic compositions to explore the contributions of oceanic versus continental inputs to the ophiuroids' diet. We investigated 2 different systems with an inshore versus offshore comparison. We sampled potential food sources and surveyed organisms every 2 mo for 1 yr. Spatio-temporal variations in stable isotopes and fatty acid profiles of the ophiuroids were generally low compared to interspecific differences. Fatty acid markers showed that both ophiuroids relied on diatom inputs. However, a more δ^{15}N-enriched isotopic composition as well as a more balanced plant- versus animal-derived fatty acid composition in *O. nigra* suggest that a broader range of food sources are being used by this species irrespective of location or sampling time. The positive correlation between the 18:1n-9/18:1n-7 fatty acid ratio and δ^{15}N values indicates a higher trophic position for *O. nigra* (suggesting an omnivorous feeding mode), whereas *O. fragilis* appears to be more herbivorous. Moreover, the low polyunsaturated/saturated fatty acid ratio associated with elevated bacterial fatty acid markers indicates that *O. nigra* preferentially consumes detritus, while *O. fragilis* relies more on fresh phytoplankton-derived material. Both stable isotope and fatty acid analyses suggest that terrestrial inputs do not contribute significantly to the diet of these ophiuroids. However, phytodetritus derived from decomposing green macroalgae contributed to the diet of *O. nigra* in the Bay of Douarnenez.

KEY WORDS: Echinoderm · Feeding ecology · *Ophiocomina nigra* · *Ophiothrix fragilis* · Trophic markers · Green algae

INTRODUCTION

Echinoderms often exhibit high population density fluctuations — also called 'outbreak' or 'die-off' events — in coastal and deep water ecosystems, where they play key structural and functional roles. Such events are often facilitated by anthropogenic factors (e.g. nutrient inputs, over-fishing) and the life history traits of the echinoderms (adult feeding mode, high fecundity, high longevity, larval type), and could trigger dramatic changes in the marine ecosystem structure (Uthicke et al. 2009).

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In European coastal waters, benthic assemblages are often dominated by dense populations of benthic suspension-feeders as well as opportunistic deposit-feeders which can take advantage of the high primary production (Hily 1991, Carvalho et al. 2011). In widespread habitats, ophiuroids account for a majority of the benthic biomass, where they play a key role in the coupling of benthic–pelagic systems (Allen 1998). The Land's End of Brittany (France) hosts dense populations of both *Ophiocomina nigra* and *Ophiothrix fragilis* (Echinodermata, Ophiuroid) (Abildgaard), which occur either in mixed or monospecific beds across a wide range of sedimentary features (Hughes 1998). Although this species assemblage is reported to be stable over time and is usually dominated by *O. fragilis* (Hily 1991), recent investigations in Brittany revealed long-term density changes and spatial shifts in *O. nigra* and *O. fragilis* beds between 1987 and 2011 (Blanchet-Aurigny et al. 2012a, Guillou et al. 2013). It was hypothesized that a combination of changes in food supply and the biological traits of *O. nigra* were a key factor of its proliferation in this area (Blanchet-Aurigny et al. 2012a). As demonstrated with other echinoderms, it was suggested that flexibility in feeding habits (Thrush & Cummings 2011) as well as an increase in fecundity and fertilization (Sewell & Levitan 1992) contributed to the species' success. While both adult ophiuroid species are reported to be suspension-feeders (Aronson 1989), *O. nigra* exhibits a much wider trophic plasticity and displays a versatile feeding behavior (Fontaine 1965). Indeed, *O. nigra* is more mobile at the sediment–water interface and commonly consumes a variety of deposited material (Vevers 1956), or behaves as a scavengerous species, foraging on detritus or carrion (Nagabhushanam & Colman 1959).

In coastal marine and estuarine systems, the energy supply for benthic consumers is derived from a high diversity of food sources, with strong variation in space and time (Peterson 1999). Such ecosystems often exhibit a great diversity of primary producers which are conveyed into higher trophic levels (Mann 1988). During the last several decades, abundant production of green macrophytes *Ulva* sp. have been an increasing issue in Brittany's coastal waters (Dion & Le Bozec 1996, Merceron et al. 2007), providing a large supply of macroalgae detritus for many species that potentially benefit. Benthic invertebrate assemblages are often dominated by dense populations of benthic suspension-feeders as well as opportunistic deposit-feeders taking advantage of this abundant primary production (Hily 1991, Carvalho et al. 2011).

Stable isotope analysis has been successfully used to study the trophic links from primary producers to higher trophic levels, indicating different utilization of food sources by benthic and pelagic invertebrates (Dubois & Grall 2013). Indeed, stable isotope signatures in consumers provide time-integrated information on food sources. The carbon and nitrogen isotope ratios in an animal's tissues closely reflect those in its diet, with a slight enrichment of heavier isotopes (^{13}C, ^{15}N) due to preferential respiration of lighter ^{12}C and excretion of lighter ^{14}N (DeNiro & Epstein 1978). Lipid markers also provide complementary information on the type and quality of resources assimilated by animals (Dalsgaard et al. 2003). Marine bacteria, diatoms, dinoflagellates, terrestrial inputs, macroalgae and vascular plants show different combinations of specific fatty acids. As recently discussed, the use of fatty acids for dietary studies can be constrained by the degree to which the fatty acid composition of an organism is obscured by factors that have the capacity to alter metabolic processes (Kelly et al. 2008, 2009, Guest et al. 2010, Dethier et al. 2013). In such cases, the use of fatty acid composition from neutral lipid contents (storage lipids) alone may reduce confusion on dietary interpretation (Pernet et al. 2012, 2014). When used together, fatty acid and stable isotope techniques show promise for resolving the trophic ecology of complex aquatic ecosystems (Kharlamenko et al. 2001, Kelly & Scheibling 2012).

In the present study, we aimed to investigate the trophic niche of the 2 ophiuroid species *O. nigra* and *O. fragilis*, using stable isotope and fatty acid biomarkers. We compared temporal variations in diet composition of the ophiuroids at sites with different hydrodynamic conditions and levels of anthropogenic influence (e.g. nutrient inputs, green macrophytes). In the inshore zone of the Bay of Douarnenez, where the green algae *Ulva* sp. proliferates, we evaluated whether the expanding population of *O. nigra* benefits from this resource. We also examined patterns of resource overlap to determine the possibility of resources competition between species, as well as to better understand the success of *O. nigra* over *O. fragilis* in this region.

MATERIALS AND METHODS

Study areas

The Bay of Brest (BB) is a shallow marine coastal system of 180 km^2 that is connected to the Atlantic Ocean by a narrow opening 1.8 km wide (Fig. 1).

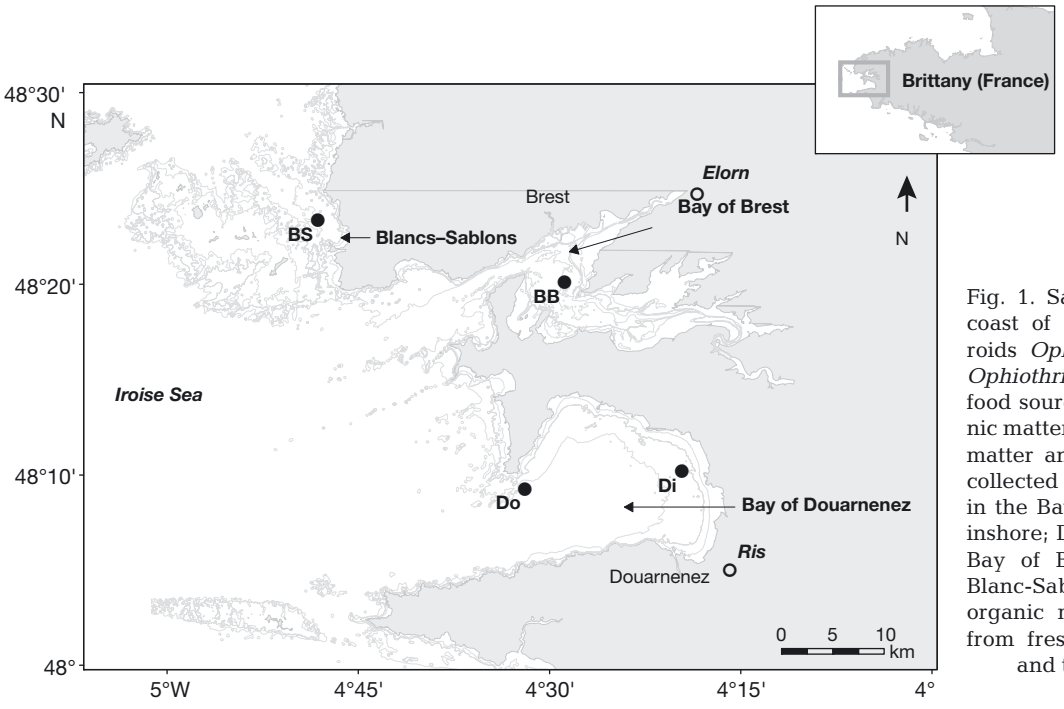

Fig. 1. Sampling sites off the coast of Brittany. The ophiuroids *Ophiocomina nigra* and *Ophiothrix fragilis* and their food sources (suspended organic matter, sedimented organic matter and macroalgae) were collected at 4 sites (●): 2 sites in the Bay of Douarnenez (Di: inshore; Do: offshore), 1 in the Bay of Brest (BB) and 1 at Blanc-Sablons (BS). Terrestrial organic matter was collected from freshwater at the Elorn and the Ris rivers (○)

This Bay is a macrotidal system that receives anthropogenic nutrient inputs from 2 rivers (Aulne and Elorn), which are facilitated by high hydrodynamic mixing conditions (Le Pape et al. 1996). The Bay is characterized by large amounts of phytoplankton biomass, and benthic communities are dominated by dense populations of suspension-feeders (Hily 1989, 1991). To the north of the BB, the site of Blanc-Sablons (BS) experiences strong hydrodynamic conditions and is directly exposed to oceanic inputs. South of these 2 sites, the Bay of Douarnenez is a shallow marine coastal system of about 350 km^2 connected to the Atlantic Ocean by an opening 9 km wide. The inshore zone of the Bay of Douarnenez (Di) is highly impacted by green macroalgae blooms, also called 'green tide' (Merceron et al. 2007). The offshore zone of the Bay (Do) is influenced by strong hydrodynamic oceanic conditions.

Sample collections

Food sources and ophiuroid specimens were sampled every 2 mo between June 2010 and April 2011 in BB, BS, and in Do and Di. Four potential food sources were analyzed: suspended particulate organic matter (SPOM), terrestrial organic matter (TOM), sediment organic matter (SOM) and the green macroalgae *Ulva* sp. in the Bay of Douarnenez. Water samples for SPOM analyses were collected at 50 cm depth at high tide, close to the ophiuroid bed. Freshwater samples were collected from 2 rivers (Elorn and Ris) for TOM analyses. Water was pre-filtered through a 200 µm mesh screen to remove the largest particles, and then filtered through pre-combusted Whatmann GF/C filters. The surface sediment of 1 cm was scraped from a core for SOM analyses. Sediment samples were sifted through 500 and 200 µm mesh screens to remove the largest particles and frozen at −80°C. Sediment samples of the <200 µm fraction were then freeze-dried and grounded to a fine powder. Finally, samples of *Ulva* sp. (stranded and floating blades) were collected in the Bay of Douarnenez, cleared of epibionts and frozen at −80°C. Samples were freeze-dried and ground to powder before stable isotope analyses.

On each sampling date, ophiuroid specimens (30 individuals of each species) were kept in filtered seawater using a flow-through system for 24 h to clear their stomachs. Animals from each location were maintained in separate tanks. A pool of 10 individuals (ca. 10 mm disk diameter) per replicate and 3 replicates per species were sampled, rinsed with filtered seawater, and frozen at −80°C. Each replicate was ground to powder with a Dangoumau homogenizer, and sub-sampled for stable isotope and fatty acid analyses. Sub-samples were stored at −80°C before being analyzed.

Stable isotope analyses

SPOM and TOM samples collected on GF filters were treated with HCl fumes (10N) for 24 h to remove carbonates, and then dried at 40°C before $\delta^{13}C$ analysis. SOM samples were decarbonated with 1 N HCl; sub-samples of ground animal tissues were freeze-dried and also decarbonated with 1 N HCl. Lipids were removed using a cyclohexane solution (Kojadinovic et al. 2008). Lipid-free tissues were dried at 48°C for 24 h prior to acid treatment. Untreated samples were used for $\delta^{15}N$ analyses.

Isotopic analyses were performed with a Finnigan MAT Delta Plus isotope ratio mass spectrometer (IRMS) coupled with a Carlo Erba NC2500 elemental analyser in the Isotope Stable Laboratory at Cornell University, New York. The analytical error was 0.2‰ for both N and C (as measured with internal laboratory standards). Stable isotopic data are expressed as the relative per ml differences between the samples and the conventional standard Pee Dee Belemnite (PDB) for carbon and air N_2 for nitrogen, according to the following equation:

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

where X (in ‰) is the ^{13}C or ^{15}N abundance and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

To interpret changes in stable isotopic compositions and to assess food source contributions in consumers' tissues, estimates of trophic discrimination factors (TDFs, hereafter denoted as Δ) are of critical importance (DeNiro & Epstein 1978, Minagawa & Wada 1984). A previous feeding experiment showed that the TDF in *Ophiothrix fragilis* is lower than in *Ophiocomina nigra*, irrespective of food sources (Blanchet-Aurigny et al. 2012b). Therefore, isotopic compositions of *O. fragilis* were normalized with respect to those of *O. nigra* by adding +0.8 and +0.5‰ respectively to the original $\delta^{13}C$ and $\delta^{15}N$ values (Blanchet-Aurigny et al. 2012b). The food sources were also corrected using specific TDFs: TDFs for SPOM and SOM ($\Delta\delta^{13}C$ = +2‰; $\Delta\delta^{15}N$ = +3.6‰) were estimated via a feeding experiment with filter-feeding species using phytoplankton, which is the main component of these 2 food sources (Dubois et al. 2007a); TDFs for *Ulva* sp. ($\Delta\delta^{13}C$ = −2.35‰; $\Delta\delta^{15}N$ = +3.4‰) were calculated using a feeding experiment involving *O. nigra* and *O. fragilis* fed with fresh *Ulva* sp. The unexpected negative value of $\Delta\delta^{13}C$ was due to the small amount of digestible material in this macroalgae (Blanchet-Aurigny et al. 2012b). After adjustment by trophic discrimination factors (fractionation factors), the diet of a given consumer was determined based on the close similarity of $\delta^{13}C$ and $\delta^{15}N$ values between potential food sources and that given consumer.

Lipid analyses

Approximately 200 mg of powdered animal samples per replicate (3 replicates per analysis) for each species was placed in glass vials filled with 3 ml of dichloromethane-methanol ($CHCl_2$-MeOH mixture) (2:1 v/v) prior to total lipid extraction following Folch et al. (1957). An aliquot of the lipid extract was evaporated to dryness and lipids were recovered with three 0.5 ml washings with chloroform-methanol ($CHCl_3$-MeOH mixture) (98:2 v/v). The lipids were placed at the top of silica gel microcolumn (30 × 5 mm internal diameter; Kiesegel Merk; 70 to 230 µm mesh; previously heated to 450°C and deactivated with 6% water). The neutral lipids were eluted with 10 ml $CHCl_3$-MeOH mixture (98:2 v/v). The polar lipids were eluted with 15 ml methanol. A known amount of 23:0 fatty acid was added as an internal standard to both neutral and polar fractions of the animal samples. Lipids were transesterified with 10 wt. % boron trifluoride-methanol (Metcalfe & Schmitz 1961) and analysed according to the method described by Marty et al. (1992); the fatty acid methyl esters were analyzed in a gas chromatograph with an on-column injector, a DB-Wax (30 m × 0.25 mm; 0.25 µm film thickness) capillary column and a flame ionization detector.

Fatty acids were identified by comparing their retention times with those of known standards: a 37 component fatty acid methyl ester (FAME) mix, bacterial acid methyl ester (BAME) mix and polyunsaturated fatty acid—PUFA No. 3 (from Menhaden oil) (Supelco). Because polar lipids are less sensitive to dietary changes (Lee et al. 1971), only fatty acid profiles of neutral lipids of both *O. nigra* and *O. fragilis* were included in this study. All fatty acids contributing to at least 0.5% of the total were taken into account. Table 1 shows a summary of the dietary fatty acid markers investigated in the present study.

Statistical analyses

A time series of fatty acids (mean of 3 replicates) for each species and sampling site (BB, BS, Di, Do, see Fig. 1) were associated using a hierarchical clustering method based on the Euclidian distance and Ward's linkage methods (Ward 1963). The clustering

and related time series were represented using heat map plots of the raw dataset (Eisen et al. 1998). A heat map is a graphical representation of data where the values of a variable on a 2-dimensional map are represented by squares with colour gradients, and variables (here fatty acid compositions) are ordered according to the dendrogram defined by the hierarchical clustering. Each of the fatty acids contributing to at least 0.5 % of the neutral lipids is represented on the heat map. The multivariate analysis and heat map were performed with R (R Development Core Team 2012).

In addition to multivariate analyses, factorial 3-way split-plot analyses of variance (ANOVAs) were conducted to determine differences in stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and selected fatty acid combinations according to species (fixed between-subjects factor with 2 levels: *O. fragilis* and *O. nigra*), locations (latitude, fixed between-subjects factor with 2 levels: north [BB + BS] and south [Di + Do]; and exposure and terrestrial inputs, fixed between-subjects factor with 2 levels: Inshore [BB + Di] and offshore [BS + Do], and their interactions), sampling dates (random factor with 5 levels of repeated measurements: June 2010, August 2010, October 2010, January 2011, April 2011) and their mutual interactions (see Table 2). Significant differences between all possible combinations of sample means were assessed using least-square means multiple comparisons tests ($p < 0.05$). Homogeneity of variance-covariance matrices were graphically assessed. All ANOVAs were performed with SAS 9.0 (SAS Institute).

The package Stable Isotope Bayesian Ellipses in R (SIBER) (R Development Core Team 2012) was used to investigate the overall variability (including all sites and dates) in isotopic values for each ophiuroid population. In this study, 2 metrics were applied: (1) the total area (TA), calculated from a convex hull drawn around the most extreme data points in a $\delta^{13}C$ versus $\delta^{15}N$ biplot, which emphasized the overall dispersion of individuals' diets within the trophic niche proxied by the isotopic space; and (2) the standard ellipses area (SEAc), calculated as a measure of the mean core population isotopic niche. SEAc is designed to cope with differences in sampling efforts (none here) and to provide a statistical background to facilitate comparisons since it is based on the variability in isotopic compositions (Jackson et al. 2011, Brind'Amour & Dubois 2013). These 2 metrics were used as a quantitative proxy of the overall annual diversity in trophic niches of the 2 co-occurring species (Layman et al. 2012): high metric values would be linked to a larger diversity in trophic sources and/or a larger plasticity. The TA or SEAc in a δ-space could then be seen as a proxy for the trophic niche of the ophiuroids, and overlap in TA or SEAc would indicate potential direct inter-specific competition.

Finally, relationships between (1) $\delta^{15}N$ and the ratio of the fatty acids 18:1n-9 to 18:1n-7 (omnivory index), and (2) the fatty acids trans-16:1n-13 and bacterial markers (iso and anteiso 15:0 + 17:0) were estimated to provide a more accurate depiction of the spatio-temporal variability among ophiuroids, specifically re-

Table 1. Dietary fatty acid markers discussed in this study

Marker	Diet	Source
20:5n-3/22:6n-3	Diatoms to flagellates	Budge & Parrish (1998)
16:4n-1	Diatoms	Dalsgaard et al. (2003)
16:1n-7/16:0	Diatoms	
15:0 + 17:0[a]	Bacteria	Volkman et al. (1980)
PUFA/SFA[b]	Phytoplankton vs. detritus	Biandolino et al. (2008), Pommier et al. (2010), Maazouzi et al. (2007), Prato et al. (2012)
Trans-16:1n-13	Higher plants	Nichols et al. (1982)
	Green macroalgae	Nelson et al. (2002)
	Phytoplankton	Dunstan et al. (1992), Terasaki et al. (2002), Veloza et al. (2006)
18:2n-6 + 18:3n-3	Terrestrial vascular plants	Dalsgaard et al. (2003)
	Green macroalgae	Kelly & Scheibling (2012), Khotimchenko et al. (2002)
18:1n-9/18:1n-7	Omnivory	Auel et al. (2002), Stevens et al. (2004), Graeve et al. (1997), El-Sabaawi et al. (2009, 2010)
20:1 and 22:1 isomers[c]	Copepods	Ackman et al. (1980), Budge et al. (2002), Sargent & Whittle (1981), Kattner et al. (2012)

[a]Includes iso and anteiso branched chains containing 15 to 17 carbon atoms
[b]Sum of polyunsaturated over the sum of saturated fatty acids
[c]Includes monounsaturated fatty acids containing 20 or 22 carbon atoms (20:1n-11, 20:1n-9, 22:1n-9)

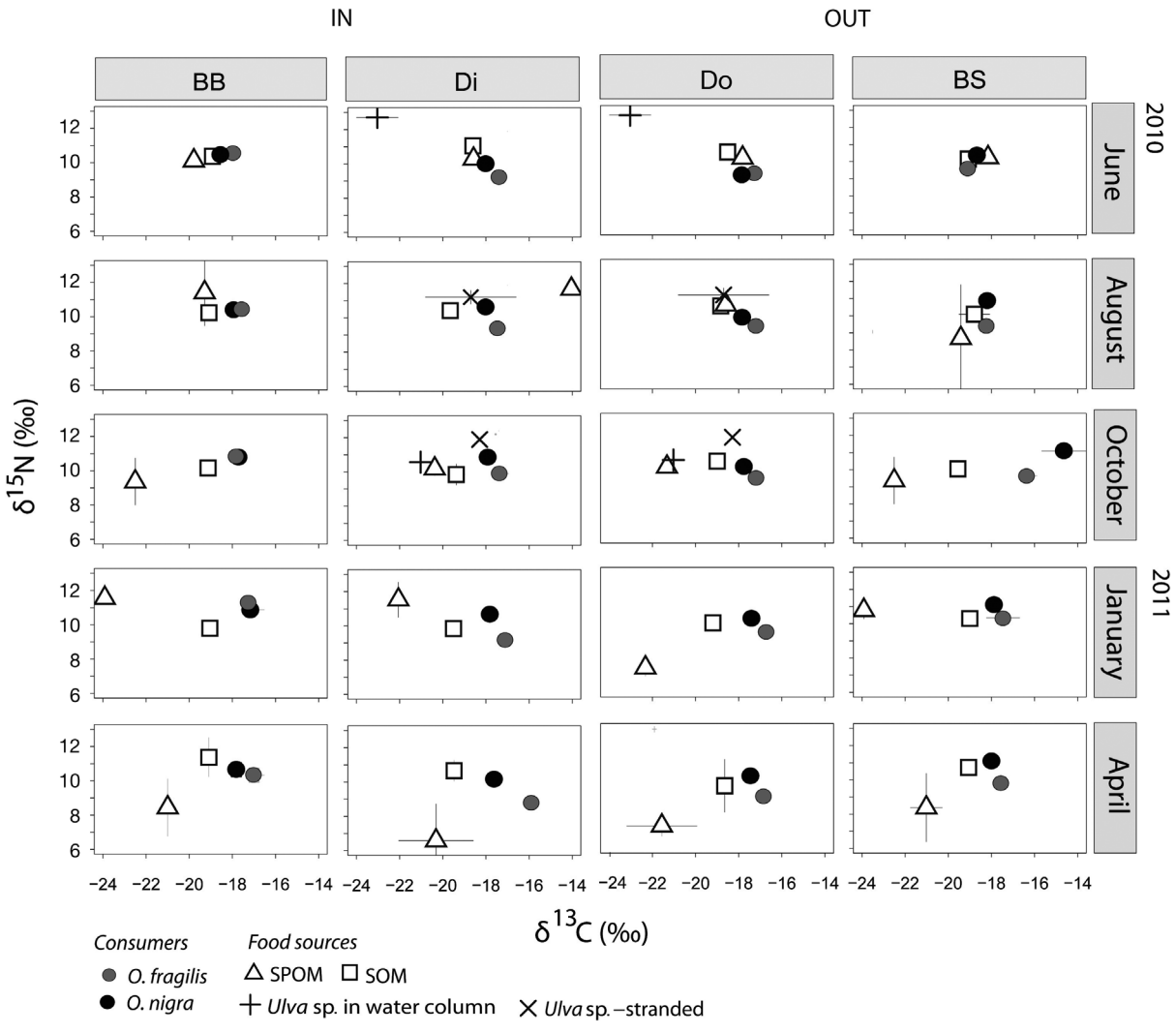

Fig. 2. $\delta^{15}N$ and $\delta^{13}C$ in *Ophiocomina nigra* and *Ophiothrix fragilis* tissues (whole animal) and in their food sources — suspended organic matter (SPOM), sedimented organic matter (SOM), and *Ulva* sp. — by sites (Bay of Douarnenez inshore [Di] and offshore [Do], Bay of Brest [BB] and Blanc-Sablons [BS]), sampling dates and exposure (IN and OUT). Error bars are SD

garding their relative trophic positions and their relative consumption of plant detritus. The relationships were investigated by calculating the Pearson's correlation coefficients (r) (R Development Core Team 2012).

RESULTS

Stables isotopes of food sources and ophiuroids

Stable isotope values of potential food sources and both ophiuroid species are presented in Table S1 in the Supplement at www.int-res.com/articles/suppl/m525p127_supp.pdf. The biplots ($\delta^{13}C$ and $\delta^{15}N$) of isotopic compositions of organic food sources (corrected for trophic discrimination) and both ophiuroids are presented in Fig. 2.

Isotopic signatures of the food sources (SPOM, SOM, TOM and *Ulva* sp.) varied according to location and time. Temporal variations in $\delta^{13}C$ in SPOM were similar between locations. In contrast, location and time seemed to interact in their effects on $\delta^{15}N$ in SPOM. In contrast to SPOM, $\delta^{13}C$ and $\delta^{15}N$ values in SOM were fairly constant irrespective of location and time. Similarly, $\delta^{13}C$ values of TOM remained relatively constant over time, although were severely depleted compared to SPOM. However, $\delta^{15}N$ values of TOM varied markedly with time (Table S1).Values of $\delta^{13}C$ and $\delta^{15}N$ in stranded samples of *Ulva* sp. (Di and Do) were similar to those of floating specimen

collected on 10 August. However, some changes were observed in the 10 October sample, where values of $\delta^{13}C$ and $\delta^{15}N$ in stranded algae were more enriched than those of floating *Ulva* sp. (Table S1, Fig. 2).

Among the 4 potential food sources (SPOM, SOM TOM and *Ulva* sp.), only SPOM, SOM, and *Ulva* sp. likely contributed to the ophiuroid diets. Moreover, the contributions of these food sources varied slightly as a function of species, location and sampling time. On 10 June and 10 August, both ophiuroid species relied on SPOM and SOM overall, regardless of location. From 10 October to 11 April, SOM likely contributed more than SPOM to the diet of the ophiuroids. However in BS on 10 October, and in Di only for *O. fragilis* from January to 11 April, both species were far away from sampled food sources. From August to 10 October in the southern areas (Do and Di), *Ulva* sp. contributed more to the diet of *O. nigra* than *O. fragilis* (Fig. 2).

The 2 ophiuroid species showed distinct stable isotope signature: values of $\delta^{15}N$ in *O. fragilis* were generally lower than those observed in *O. nigra*, whereas values of $\delta^{13}C$ overlapped between the 2 species (Fig. 3). Values of $\delta^{13}C$ generally ranged between −17.6 and −18.6‰ for *O. nigra*, and between −16.6 and −19.6‰ for *O. fragilis*. Isotopic metrics revealed little overlap between the 2 ophiuroids (only the convex hull areas overlapped). *O. nigra* occupied a smaller isotopic niche space (SEAc = 0.49, TA = 1.50) than *O. fragilis* (SEAc = 1.32, TA = 3.70). The $\delta^{13}C$ and $\delta^{15}N$ ranges were both larger for *O. fragilis* (Fig. 3).

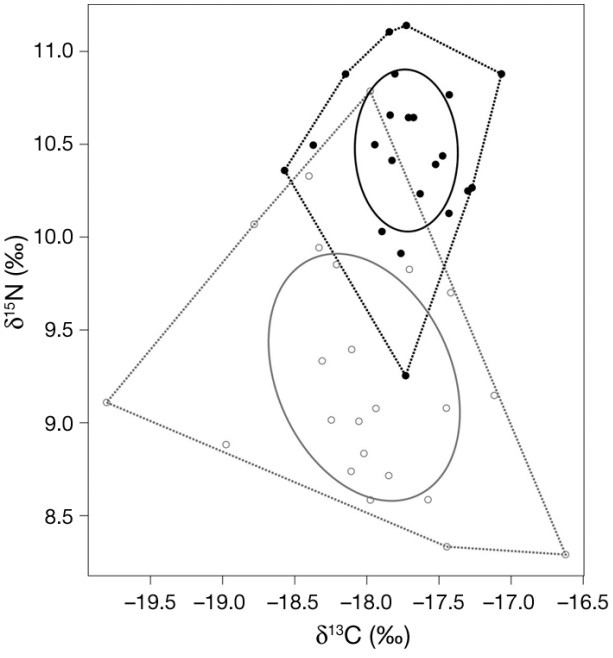

Fig. 3. Population isotope niche widths of both ophiuroids (all sites and all dates). Standard ellipses areas (SEAc, solid lines) and total area from convex hull (TA, dotted lines) estimations for *Ophiocomina nigra* (black spots and lines) and *Ophiothrix fragilis* (grey spots and lines) populations using Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al. 2011)

Fatty acid compositions of ophiuroids

Fatty acid compositions of neutral lipids in soft tissues of *O. fragilis* and *O. nigra* are presented in Table S2 in the Supplement, and summarized using a heat map (Fig. 4). Two clusters (I and III) of fatty acids appeared, distinctly and clearly separating the 2 ophiuroids (Fig. 4).The first cluster consisted of fatty acids characteristic of *O. nigra*, namely 14:0, 16:0, 20:1n-11, 16:1n-7, 24:6n-3, 16:4n-3, 22:1n-9, 18:1n-9, 22:6n-3, non-methylene interrupted (NMI) fatty acids (20:2 and 22:2), bacterial fatty acids, and trans-16:1n-13. The second cluster consisted of fatty acids that occurred in similar proportions in both ophiuroids, namely 18:3n-3, 18:2n-6, 16:3n-6, 18:0, 20:4n-6, 16:3n-3. Finally, the third cluster consisted of fatty acids which occurred in higher proportions in *O. fragilis*, and included 20:5n-3, 16:4n-1, 18:4n-3, 18:1n-7 and 18:1n-11 (Fig. 4).

Although ophiuroid species, location and sampling date effects on 16:1n-7/16:0 interacted (species × latitude × exposure × time; $p < 0.0001$, Table 2), this diatom marker varied only slightly (from 0.63 to 0.93, except in *O. nigra* collected at BS, where values higher than 1.0 were recorded) and no clear spatio-temporal pattern emerged (Fig. 5A). As observed for 16:1n-7/16:0, species, location and sampling date effects on 20:5n-3/22:6n-3 interacted ($p < 0.0001$; Table 2, Fig. 5B). However, this diatom marker was 3 to 6 times higher in *O. fragilis* than observed in *O. nigra*, irrespective of location or sampling date ($p < 0.001$). It is also noteworthy that values of 20:5n-3/22:6n-3 were particularly high in both ophiurid species, ranging from 15.5 to 58.4 in *O. fragilis* and from 3.6 to 21.7 in *O. nigra*, reflecting low levels of 22:6n-3 (see Table S2). In *O. nigra*, values of 20:5n-3/22:6n-3 increased by 2 and 6 times from 10 October onward at Do and Di, respectively, whereas they remained fairly constant at BB and BS. In *O. fragilis*, values of 20:5n-3/22:6n-3 increased from 10 October onward at Di and Do as observed in *O. nigra*; however, at BB and BS, ratio values in *O. fragilis* peaked on 11 January, reaching 58.4 and 46.9, respectively.

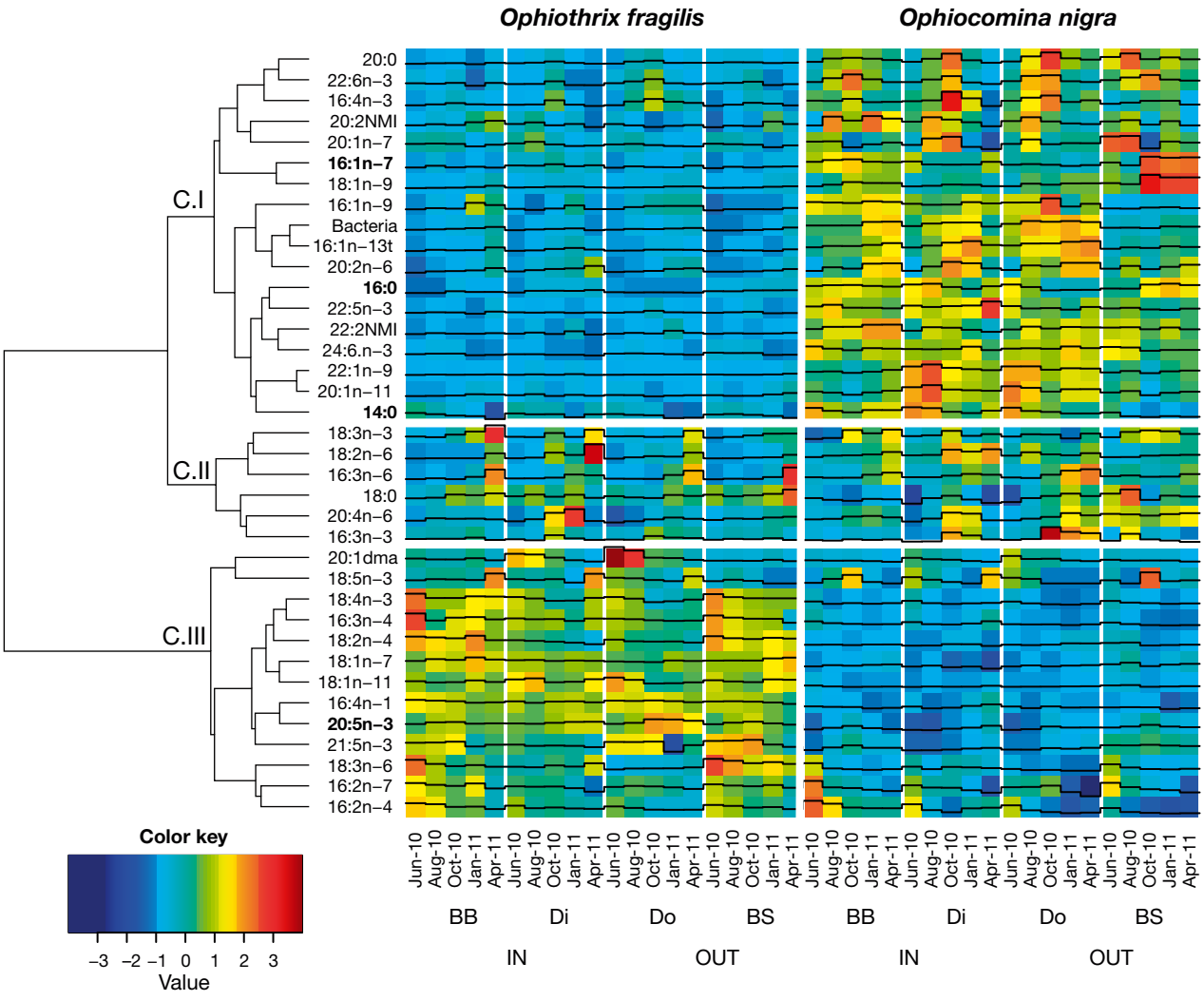

Fig. 4. Heat map plots showing time-series of fatty acids contributing >0.5% mass of total fatty acids in *Ophiothrix fragilis* and *Ophiocomina nigra*, averaged by sampling dates, sites (BB: Bay of Brest; Di and Do: inshore and offshore zones of Bay of Douarnenez; BS: Blanc-Sablons) and exposure (IN versus OUT). Fatty acids were reordered according to the hierarchical clustering result given by the dendrogram. The horizontal white lines separate the 3 clusters (C.I, C.II, C.III) of fatty acids. The vertical white lines separate the sampling sites and species. Fatty acids in bold are dominant. Bacteria include the sum of iso and anteiso branched chains containing 15–17 carbon atoms; NMI: Non-Methylene_Interrupted; t: Trans; dma: total of dimethyl acetals

The polyunsaturated/saturated (PUFA/SFA) ratio was about 2 times higher for *O. fragilis* than *O. nigra* ($p < 0.0001$; Table 2, Fig. 5C) regardless of location and sampling date, and remained at that level over time for both species.

Values of 18:2n-6 + 18:3n-3 ranged from 0.7 to 1.9%. There was a significant interaction of species, location and sampling date effects on this indicator ($p < 0.0001$; Table 2, Fig. 5D). These values were generally higher in *O. nigra* compared to those observed in *O. fragilis*, and remained fairly constant at Do, whereas they increased regularly at BB and BS, and chaotically at Di. Values of 18:2n-6 + 18:3n-3 recorded at the end of the study period showed a 32% increase inside the bays (BB and Di, mean = 1.55%) compared to those observed outside (BS and Do, mean = 1.18%), irrespective of ophiuroid species.

The sum of iso- and anteiso-branched chain fatty acids and unbranched 15:0 and 17:0 varied as a function of the interaction among species, location and sampling date ($p < 0.0001$; Table 2, Fig. 5E). Overall, values for this bacterial marker were lower in *O. fragilis* (between 0.8 and 1.9%) than in *O. nigra*, in which they ranged from 1.6 to 3.2%. For *O. nigra*, these values increased gradually between 10 June and 11 January at BB and Di to reach 3.2%. At Do, bacterial fatty acids were initially higher than that observed at BB and Di ($p < 0.001$, Fig. 5E) and

Table 2. Factorial 3-way repeated measure ANOVA results for the effect of latitude (north: BB + BS versus south: Di + Do), region (inshore: BB + Di versus offshore: BS +Do), species (*Ophiocomina nigra* versus *Ophiothrix fragilis*), and date of sampling (5 sampling dates) on stable isotopes and selected fatty acid dietary markers (ratio and/or sum of fatty acids). See Fig. 1 for location abbreviations. Bacteria: sum of iso and anteiso branched chain fatty acids. *p < 0.05 (n = 3); rep: replicate

Source of variation	$\delta^{13}C$	$\delta^{15}N$	16:1n-7/ 16:0	20:5n-3/ 22:6n-3	∑PUFA/ ∑SFA	18:2n-6 +18:3n-3	Bacteria	20:1n-11	18:1n-9/ 18:1n-7
Between-subjects									
Species	<0.0001*	<0.0001*	0.0013*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Region	0.3712	<0.0001*	0.0729	<0.0001*	0.0012*	<0.0001*	0.0057*	0.3422	0.0013*
Latitude	0.0053*	<0.0001*	0.0007*	0.0481*	0.2299	<0.0001*	<0.0001*	<0.0001*	0.0026*
Region × Latitude	0.6205	0.0236*	0.2436	0.0002*	0.0005*	0.0007*	<0.0001*	0.0641	0.0142*
Region × Species	0.0010*	0.0032*	0.0083*	<0.0001*	0.1517	0.1935	0.0835	0.2804	0.0015*
Latitude × Species	<0.0001*	0.0039*	<0.0001*	0.4436	0.2542	0.2875	<0.0001*	<0.0001*	0.0004*
Region × Latitude × Species	0.0687	<0.0001*	0.0663	0.3350	0.0001*	0.4154	<0.0001*	0.2081	0.0075*
Error a = rep(Region × Latitude)									
Within-subjects									
Date	<0.0001*	<0.0001*	0.0140*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Region × Date	<0.0001*	<0.0001*	0.0003*	<0.0001*	0.0005*	<0.0001*	<0.0001*	0.0102*	0.2037
Latitude × Date	<0.0001*	<0.0001*	0.3133	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0005*
Species × Date	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0812	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Region × Species × Date	0.0208*	0.0795	0.1090	<0.0001*	0.0299*	<0.0001*	0.0654	0.2844	0.1305
Latitude × Species × Date	0.0092*	0.0007*	0.0081*	<0.0001*	0.1098	<0.0001*	<0.0001*	0.1717	0.0002*
Region × Latitude × Species × Date	<0.0001*	0.1134	<0.0001*	<0.0001*	0.0023*	<0.0001*	<0.0001*	<0.0001*	0.0002*
Error b = Species × rep(Region × Latitude)									

remained fairly stable. In both species, values of this bacterial marker remained fairly constant in BS. The fatty acid trans-16:1n-13 was positively correlated with bacterial fatty acids ($p < 0.001$, Pearson's $r = 0.93$). Again, specimens belonging to *O. nigra* were on the top right and corner of the biplot, while *O. fragilis* specimens were on the bottom left hand corner (Fig. 6). The highest values of bacterial and trans-16:1n-13 fatty acids were observed in Di and Do for *O. nigra*.

Values of 20:1n-11 varied as a function of the interaction among species, location and sampling date ($p < 0.0001$, Table 2). The levels of this fatty acid varied from 3.8 to 13% for *O. nigra* as a function of location and time, whereas those of *O. fragilis* remained relatively constant over time (~4%) irrespective of location. At Di and Do, the level of 20:1n-11 was 2 times higher in *O. nigra* than in *O. fragilis* and approximately 3 times higher in June and August 2010 (Fig. 5F). Although values of 18:1n-9/18:1n-7 varied as a function of the interaction among species, location and sampling date ($p = 0.0002$, Table 2), values observed in *O. nigra* were 3 times higher than those in *O. fragilis*, regardless of location or sampling date (Fig. 5G). Temporal variations in 18:1n-9/18:1n-7 were rather low, except for *O. nigra* collected at BS, where the values increased by 3 times between 10 August and 10 October. Interestingly, 18:1n-9/18:1n-7 increased with increasing $\delta^{15}N$ in the ophiuroids ($p < 0.0001$, Pearson's $r = 0.70$). Moreover, all specimens of *O. nigra* were distributed on the top right and corner of the biplot while *O. fragilis* specimens were on the bottom left hand corner (Fig. 7). Note that no relationship between 18:1n-9/18:1n-7 and $\delta^{15}N$ occurred for *O. fragilis* in BB.

DISCUSSION

The present study investigated the trophic niches of 2 co-occurring ophiuroid species, *Ophiocomina nigra* and *Ophiothrix fragilis*, at 4 locations in Brittany (France) over a 1 yr period, by means of stable isotope and fatty acid analyses. Our study provides evidence that although these species could potentially compete for similar food resources, they are segregated as a result of using different feeding strategies, leading to differences in their trophic niche.

Trophic relationships between ophiuroid species

The relationship between $\delta^{15}N$ and 18:1n-9/18:1n-7 revealed differences in relative trophic positions between ophiuroid species which can be used to dis-

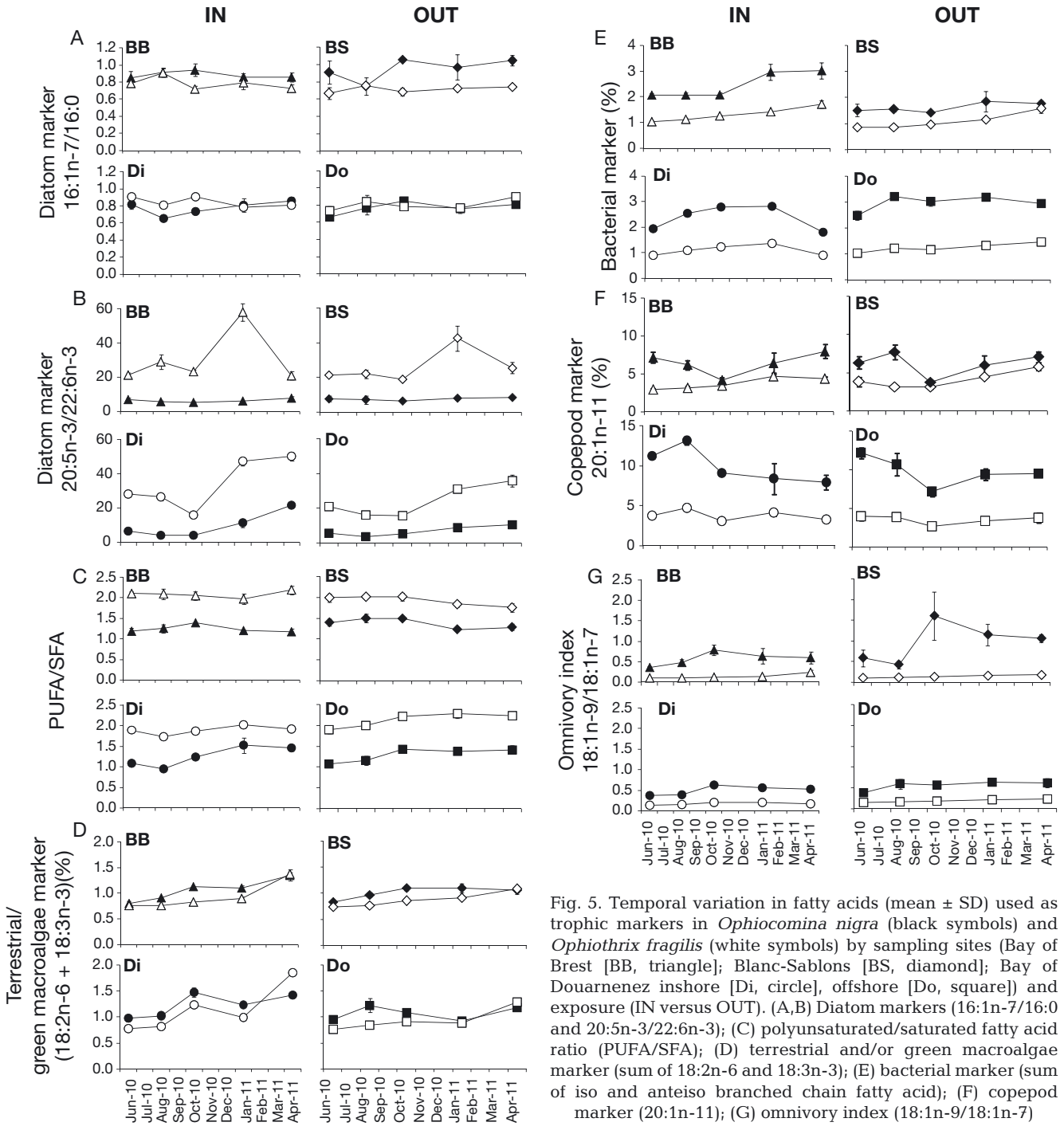

Fig. 5. Temporal variation in fatty acids (mean ± SD) used as trophic markers in *Ophiocomina nigra* (black symbols) and *Ophiothrix fragilis* (white symbols) by sampling sites (Bay of Brest [BB, triangle]; Blanc-Sablons [BS, diamond]; Bay of Douarnenez inshore [Di, circle], offshore [Do, square]) and exposure (IN versus OUT). (A,B) Diatom markers (16:1n-7/16:0 and 20:5n-3/22:6n-3); (C) polyunsaturated/saturated fatty acid ratio (PUFA/SFA); (D) terrestrial and/or green macroalgae marker (sum of 18:2n-6 and 18:3n-3); (E) bacterial marker (sum of iso and anteiso branched chain fatty acid); (F) copepod marker (20:1n-11); (G) omnivory index (18:1n-9/18:1n-7)

tinguish patterns of trophic differentiation. Higher values of $\delta^{15}N$ in *O. nigra* compared to *O. fragilis* suggests that *O. nigra* occupies a higher trophic position. This was corroborated by the fact that values of 18:1n-9/18:1n-7, which reflect an omnivorous feeding mode, were markedly higher in *O. nigra*, and correlate positively with $\delta^{15}N$. *O. nigra* is reported to be a suspension-feeding species (Aronson 1989), but stable isotope and fatty acid analyses both corroborated the idea that this ophiuroid is more generally an opportunistic feeder. This feeding behaviour has been reported for many other ophiuroids (Warner 1982). Based on observations of stomach contents as well as tooth morphology (Warner & Woodley 1975), *O. fragilis* is also capable of scavenging and predation (Boos 2012). However, our results suggested that

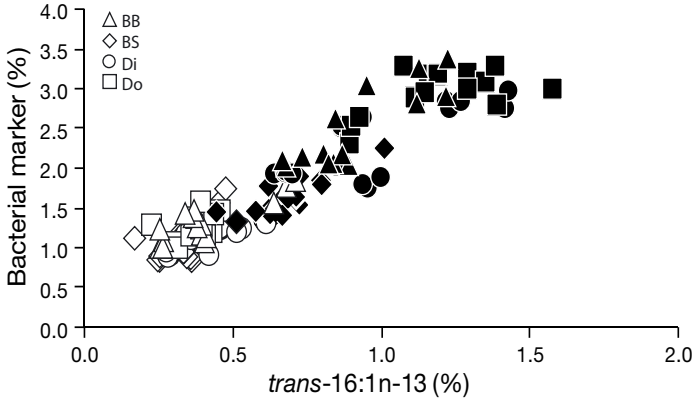

Fig. 6. Relationship between *trans*-16:1n-13 and bacterial markers in both *Ophiocomina nigra* (black) and *Ophiothrix fragilis* (white) by site: Bay of Douarnenez inshore (Di) and offshore (Do), Bay of Brest (BB) and Blanc-Sablons (BS). (Pearson's r = 0.93, p < 0.0001)

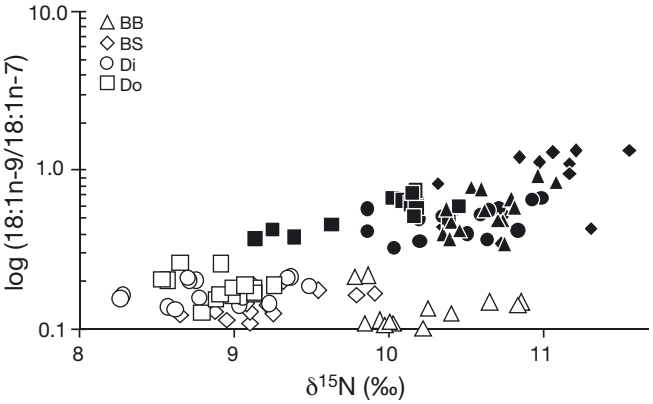

Fig. 7. Ratio of 18:1n-9/18:1n-7 (log transformed) versus $\delta^{15}N$ in both *Ophiocomina nigra* (black) and *Ophiothrix fragilis* (white) by site: Bay of Douarnenez inshore (Di) and offshore (Do), Bay of Brest (BB) and Blanc-Sablons (BS). (Pearson's r = 0.70, p < 0.0001)

assimilation of animal prey by *O. fragilis* is of minor importance. Values of 18:1n-9/18:1n-7 were consistent with those reported for the ophiuroids *Ophiura sarsi* and *Ophiacantha borealis*, which are considered to be carnivorous (ratio = 1.4) and suspension feeders (ratio = 0.1), respectively (Graeve et al. 1997).

In the present study, *O. nigra* exhibited high levels of 20:1n-11, likely reflecting the contribution of copepods to the diet, which could be ingested either along with detritus from the sediment or by active hunting (Ratnayake & Ackman 1979, Drazen et al. 2008, Würzberg et al. 2011). However, levels of 20:1n-11 did not show a consistent rise with increasing levels of 18:1n-9/18:1n-9 and $\delta^{15}N$. It is therefore possible that ophiuroids synthesize these monounsaturated isomers de novo (Drazen et al. 2008).

The ratios of 20:5n-3/22:6n-3, together with the fatty acids 16:4n-1 and 16:3n-4, were consistently higher in *O. fragilis* than in *O. nigra*. Therefore, it appears that *O. nigra* relies less on diatoms than *O. fragilis*, irrespective of location or sampling time. However, ratios of 20:5n-3/22:6n-3 were unusually high (>20) compared to values reported in the literature (Dalsgaard et al. 2003). It was previously reported that levels of 22:6n-3 are low in several ophiuroid species (including *O. nigra* and *O. fragilis*), because this fatty acid would have been elongated to 24:6n-3 (Takagi et al. 1986, McKenzie et al. 2000, Drazen et al. 2008). Thus, the species-specific differences in the ratios of 20:5n-3/22:6n-3 in our study may not reflect differences in food sources, but rather mirror the selective incorporation and elongation processes of fatty acids according to species-specific physiological requirements.

Values of PUFA/SFA, an indication of the relative importance of phytoplankton versus detritus (Maazouzi et al. 2007, Biandolino et al. 2008, Pommier et al. 2010, Prato et al. 2012), were lower in *O. nigra* than in *O. fragilis*. However, bacterial fatty acids were higher in *O. nigra* than in *O. fragilis*. These results suggest that *O. nigra* relies less on diatoms and more on detritic organic matter and bacteria than *O. fragilis*. Additionally, *O. nigra* exhibited non-negligible levels of trans-16:1n-13, which correlated positively with bacterial fatty acids. Considering that trans-16:1n-13 is a marker for phytodetritus, which could be derived from green algae, these results would imply that *O. nigra* grazed on microbial material (see references in Table 1). This result is also supported by higher levels of 18:2n-6 and 18:3n-3 in *O. nigra*, which can be used as a macroalgal signal when terrestrial inputs are absent or limited (Kelly & Scheibling 2012).

Stable isotopes alone revealed that dietary overlap (TA and SEAc) between the 2 ophiuroid species was very limited and trophic separation was mainly detectable according to their $\delta^{15}N$ values. Thus, as previously suggested for other coexisting ophiuroid species (Iken et al. 2001), *O. nigra* and *O. fragilis* maintained some degree of trophic niche separation by differences in selective ingestion and feeding strategies. When organic matter consists of mixed sources, inter-specific differences in particle capture strategies may reduce competition among ophiuroids, as previously investigated for suspension-feeder trophic niches (Dubois et al. 2007b,c, Lefebvre et al. 2009, Pernet et al. 2012, Dubois & Colombo 2014). While both adult ophiuroid species are re-

ported to be suspension-feeders, collecting particles from the water-column with their arms (Aronson 1989), *O. nigra* exhibits a much wider trophic plasticity and more versatile feeding behavior (Fontaine 1965).

Unexpectedly, *O. nigra* exhibited a somewhat lower overall annual variability in isotopic signatures than *O. fragilis*. We hypothesized that since the population of *O. nigra* assimilated a broad range of food sources with no marked dietary changes regardless of season or location, its isotope signature variability could be buffered.

Spatio-temporal variations in stable isotope and fatty acid profiles of ophiuroids

In our study, spatial and temporal variations in stable isotopes and fatty acid profiles of ophiuroids were generally low compared to inter-specific differences. This result was somewhat unexpected since these systems (the Bay of Brest and Bay of Douarnenez) differ in terms of their hydrodynamic patterns (e.g. depth, residency time and water currents), and both open sea off-shore stations are very different from their respective coastal and highly productive inshore stations. Sites located inside the bays exhibit frequent green macroalgae blooms (Ménesguen et al. 2006), predominantly in the inshore zone of the Bay of Douarnenez (e.g. up to 14 000 t of fresh spin-dried *Ulva* spp. recorded in 2009; CEVA 2011). The Bay of Brest is much more influenced by freshwater inputs rich in nitrates than the inshore zone of the Bay of Douarnenez, but is less susceptible to eutrophication due to favourable hydrodynamic conditions.

Stable isotope and fatty acid results were both consistent with a diet that is phytoplankton-oriented in summer, indicating that both ophiuroids feed on SPOM and/or SOM regardless of location. Nevertheless, suspension or deposit feeding modes are difficult to separate for these ophiuroids, since the SPOM and SOM were not isotopically discriminated. In other seasons, although stable isotope analysis highlighted that organic matter in the ophiuroid diets is apparently derived from sediment, fatty acids provide evidence that the use of food sources varies between species and locations to some degree.

As previously reported for several small invertebrates (Fujiwara & Highsmith 1997, Kamermans et al. 2002, Catenazzi & Donnelly 2007, Dubois et al. 2007b,c, Prato et al. 2012), our results support the relative importance of *Ulva* spp. as a direct or indirect food source to the ophiuroid diet. During the summer and fall in the Bay of Douarnenez, the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of the ophiuroids revealed a contribution of *Ulva* sp. to their diet. During the fall in the inshore zone of Douarnenez as well as in spring inside the bays, despite low levels of 18:2n-6 and 18:3n-3, a slight increase of these fatty acids was observed for the 2 ophiuroid species compared to those observed outside. This is corroborated by non-negligible levels of the fatty acids 16:3n-3 and 16:4n-3 observed mostly in *O. nigra*, supporting a green macrophyte dietary origin (Fleurence et al. 1994, Khotimchenko et al. 2002).

It is therefore likely that decomposing *Ulva* sp. locally contributed to the diet of *O. nigra*, especially in the Bay of Douarnenez. This seems consistent since elevated levels of bacterial inputs with the fatty acid trans-16:1n-13 were observed in *O. nigra*. In shallow coastal areas, microbial processes become dominant during deposition and decomposition of macroalgal blooms at the sediment interface (Lomstein et al. 2006). Decomposing *Ulva* sp. could have resulted in the oxidation of 18:2n-6 and 18:3n-3 as reported for seagrass (Tenore et al. 1984, Kharlamenko et al. 2001, Alfaro et al. 2006). Such a phenomenon would potentially lead to an underestimation of the contribution of *Ulva* sp. to the ophiuroid diet. Benthic invertebrates have the capacity to significantly modify their dietary fatty acids. This is supported by feeding experiments on sea urchins, which revealed that 18:2n-6 and 18:3n-3 decreased in abundance from algae (source) to urchins (consumers), indicating that these fatty acids were affected by consumer metabolism (Castell et al. 2004, Kelly et al. 2009). As recommended by Kelly & Scheibling (2012), further controlled feeding experiments are necessary to distinguish dietary tracer fatty acids from those that are modified by the consumer.

The relationship between 18:1n-9/18:1n-7 and $\delta^{15}N$ (up to 11.4‰) clearly showed that *O. nigra* occupies a higher trophic position in the BS site compared to those recorded in other locations. This suggests a higher contribution of animal material to its diet, other than that of copepod origin, since levels of the fatty acid 20:1n-11 were low at this location.

This study demonstrates that the cross-validation of isotope and fatty acid biomarkers is a powerful tool to investigate some aspects of the feeding ecology of benthic organisms, here applied to *O. nigra* and *O. fragilis*. Although our findings are in accordance with the results of previous studies reported for these species elsewhere (Mc Kenzie et al. 2000), our results clearly suggest that the trophic plasticity of *O. nigra*

allows adaptation to fluctuating food sources and expansion of its trophic niche in order to gain a competitive advantage over *O. fragilis*.

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