

Dynamics of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in different tissues of *Crassostrea gigas* at two contrasted ecosystems: insights from growth and food sources

Emmery Antoine ^{1,2,3,4}, Alunno-Bruscia Marianne ¹, Bataillé Marie-Pierre ⁵,
 Kooijman Sebastiaan, A., L. M. ⁴, Lefebvre Sebastien ^{3,*}

¹ Ifremer, LEMAR UMR 6539, 11 Presqu'île du Vivier, 29840 Argenton-en-Landunvez, France

² Université de Caen Basse Normandie, UMR BOREA MNHN, UPMC, UCBN, CNRS-7208, IRD-207, Esplanade de la Paix, F-14032 Caen, France

³ Université de Lille, CNRS, Université Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, 62930 Wimereux, France

⁴ Vrije Universiteit, Dep. of Theoretical Biology, de Boelelaan 1085 1081 HV Amsterdam, The Netherlands

⁵ Université de Caen Basse Normandie, UMR INRA - UCBN 950 EVA, Esplanade de la Paix CS, 14032 Caen cedex 5, France

* Corresponding author : Sebastien Lefebvre, email address : sebastien.lefebvre@univ-lille1.fr

Abstract :

We studied the influence of food availability on the growth (whole body and organs) of the oyster *Crassostrea gigas* and on the dynamics of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respective values. Juvenile oysters originating from Arcachon Bay were transplanted to two contrasting ecosystems, Baie des Veys (BDV) and Rade de Brest (RDB), for a 1-yr growth survey. In BDV, chlorophyll-a concentrations ([Chl-a]) was 3 times higher than in RDB on average, which accounts for the differences in oyster growth between BDV and RDB. Differences in trophic conditions could also explain the differences in C/N ratios and $\delta^{13}\text{C}$ values between sites partly due to higher investment in gametogenesis for the oysters in BDV than in RDB; these differences widened when lipid normalized $\delta^{13}\text{C}$ values were used. Oysters clearly selected microalgae as the main food source, and especially phytoplankton. Gi (Gills), Mu (Muscle) and Re (remaining organs) clearly exhibited different isotopic enrichment levels, with $\delta\text{Mu} > \delta\text{Gi} > \delta\text{Re}$ regardless of C or N elements, culture sites and seasons. $\delta^{15}\text{N}$ discrimination between organs was rather equivalent between sites. These results would benefit from an experiment under controlled conditions along a calibration of the lipid normalization to correct $\delta^{13}\text{C}$ values in bivalves.

Keywords : food availability, isotopic discrimination, suspended particulate organic matter, oyster, bivalves, marine coastal intertidal areas, trophic ecology

45 1. Introduction

Suspension feeding bivalves are a key ecological component of marine food webs in coastal ecosystems (Gili and Coma, 1998; Jennings and Warr, 2003). Their filtration and biodeposition activities actively contribute to the transfer and recycling of particulate and enhance pelagic-benthic coupling (Doering et al., 1986). Most generalist feeders (*i.e.* those feeding on a mixture of different food sources according to their bioavailability) occupy an intermediate trophic niche between primary producers and secondary consumers (Lefebvre et al., 2009b). A major part of their diet is composed of microalgae, *i.e.* phytoplankton and/or resuspended microphytobenthos (also known as benthic microalgae) species (Kang et al., 2006; Yokoyama et al., 2005). Because suspension-feeding bivalves are sensitive to environmental fluctuations, such as anthropogenic impact (Piola et al., 2006), meteorological conditions (Grangeré et al., 2009), temperature (Laing, 2000), food quantity (Chauvaud et al., 2001), quality (Chaparro et al., 2008) and diversity (Riera, 2007), they act as ecological indicators of the trophic state of coastal ecosystems (Cloern and Jassby, 2008; Lefebvre et al., 2009a).

60 To understand the trophic role and the place of suspension feeding bivalves in ecosystems, temporal and spatial variations of the stable isotope ratios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, have been extensively used over recent decades (Boecklen et al., 2011). Utilization of stable isotope ratios in trophic studies is based on the observation that the isotope ratios of a consumer's tissues resemble those of its diet, with enrichment in ^{13}C and ^{15}N (DeNiro and Epstein, 1978, 65 1981). A common use of stable isotope ratios is to make inferences about diet from isotope values measured in tissues and food sources of a consumer (Phillips and Gregg, 2003).

Numerous studies have shown that suspended particulate organic matter (SPOM) -mainly composed of phytoplankton, microphytobenthos (MPB), macroalgal detritus, riverine particulate organic matter (rPOM), bacteria, *etc...* can contribute to the diet of benthic

70 suspension feeders (*e.g.* Dang et al., 2009; Decottignies et al., 2007; Kang et al., 1999; Marín
Leal et al., 2008; Riera and Richard, 1996). However, the main food source in most coastal
marine ecosystem is microalgae such as diatoms (*e.g.* Pernet et al., 2012). The growth of
suspension-feeding bivalves relies mainly on phytoplankton (PHY) during spring blooms and
on other sources such as MPB outside these periods (Lefebvre et al., 2009b). Relationships
75 between food availability to organisms and their physiological performances have thus been
established for bivalve species. Kang et al. (2006) showed the importance of the seasonal
development of microphytobenthos as a food source during the critical period of growth and
gonad development for intertidal suspension- and deposit-feeders *Laternula marilina* and
Moerella rutila. Sauriau and Kang (2000) showed that around 70 % of the annual production
80 of intertidal cockles (*Cerastoderma edule*) in Marennes-Oléron Bay relied on
microphytobenthos.

The dynamics of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the organs of an individual and its food sources
provide valuable information on pathways of matter in its tissues. Studies by Lorrain et al.
(2002), Deudero et al. (2009), Malet et al. (2007) and Paulet et al. (2006) used the seasonal
85 variations of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in different organs of bivalves to make a detailed
examination of energy allocation processes. Lorrain et al. (2002) showed that, for *Pecten*
maximus, the seasonal variations in the available SPOM and its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
correlated with the seasonal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the scallop adductor muscle, gonad and
digestive gland. These authors also found that the isotopic discrimination and turnover rates
90 were different between organs and used this property to investigate energy and nutrient flow
among them. Malet et al. (2007) used the same approach to explain physiological differences
between diploid and triploid oysters (*C. gigas*). By means of a diet-switching experiment
conducted in different seasons, Paulet et al. (2006) measured the isotope ratios of two
suspension feeders, *P. maximus* and *C. gigas* in the gonad, the adductor muscle and the

95 digestive gland. They showed differences in isotope incorporation and discrimination between
organs, seasons and species that reflected differences in energy allocation strategies. The
transplantation experiment of *Mytilus galloprovincialis* carried out by Deudero et al. (2009) at
a large spatial scale also showed different isotopic enrichment in the digestive gland, the
adductor muscle and the gills due to their different turnover rates and biochemical
100 composition.

The influence of food availability on marine bivalve growth as well as the contribution of
different food sources to the diet of shellfish are widely documented in the literature.
However, the seasonal dynamics of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in different tissues of the oyster *C.*
gigas in its natural environment has been rarely documented (but see Malet et al., 2007). A
105 particular attention was paid on the influence of growth and physiological state (C/N ratio) on
the isotopic discrimination between tissues (muscle, gills and remaining tissues *i.e.* mostly
digestive gland and mantle). To provide constrained growth conditions, 7-month old oysters
were transplanted in two ecosystems where food sources differ in quantity.

110 **2. Materials and methods**

2.1. Study sites

The ecosystems of Baie des Veys (BDV, Normandie) and Rade de Brest (RDB, Bretagne),
that differ in their morphodynamic and hydrobiological characteristics and in the rearing
performances of *C. gigas* (Fleury et al., 2005a,b) were studied (Fig. 1). BDV, which is located
115 in the southwestern part of the Baie de Seine, is a macrotidal estuarine system with an
intertidal area of 37 km², a maximum tidal amplitude of *ca.* 8 m and a mean depth of *ca.* 5m.
BDV is influenced by four rivers (watershed of 3500 km²) that are connected to the bay by
the Carentan and Isigny channels. In BDV, the culture site of Grandcamp (49°23'124"N,
1°05'466"W) is located in the eastern part of the bay and is characterized by muddy sand

120 bottoms. RDB is a 180 km² semi-enclosed marine ecosystem connected to the Iroise Sea by a
deep narrow strait. Half of its surface area is less than 5 m in depth (mean depth = 8m). Five
rivers flow into RDB, but two of these account for 50 % of the freshwater inputs: the Aulne in
the south (watershed of 1842 km²) and the Elorn in the north (watershed of 402 km²). In
RDB, the study site at Pointe du Château (48°20'03"N, 04°19'145"W) is located at the mouth
125 of the Daoulas river in an area with gravel and rubble bottoms that likely prevent a high
development and re-suspension of microphytobenthos species.

2.2. Environmental data: chlorophyll-*a* and temperature

Chlorophyll-*a* concentration ([Chl-*a*], µg.L⁻¹) data were provided by the Ifremer national
130 REPHY network for phytoplankton monitoring (<http://wwz.ifremer.fr/lerpc/Activites-et-Missions/Surveillance/REPHY>), at Géfosse in BDV (49°23'47"N, 1°06'36"W, *i.e.* at a
distance of 1.5 km from the culture site) and Lanvéoc in RDB (48°18'33.1"N, 04°27'30.1"W,
i.e. at a distance of 10.5 km from the culture site). [Chl-*a*] were obtained after filtering
samples of seawater and determined following Aminot & Kerouel (2004). Water samples
135 were collected at high spring tide (Fig. 1). At each site, seawater temperature was measured
continuously (high-frequency recording) using a multiparameter probe (a Hydrolab DS5-X
OTT probe in RDB and a TPS NKE probe in BDV).

2.3. Sample collection and analysis

140 2.3.1. Oysters

Juveniles of the oyster *C. gigas* (mean shell length= 2.72 cm±0.48; mean dry flesh mass=
0.020 g±0.008) originating from Arcachon Bay were split into two groups and transplanted to
the two culture sites in March 2009. Oysters were reared from March 2009 to February 2010
in plastic net culture bags attached to iron rearing tables at 60 cm above the bottom. The

145 rearing tables were placed in the intertidal area, so that the oysters were subjected to tidal
action, *i.e.* immersed *ca.* 70 % of the day (average daily immersion time over a year).
Samples were taken every two months during autumn and winter, and monthly during spring
and summer. At each sampling date, 30 oysters (with individual mass representative of the
mean population mass) were collected in the two sites. They were cleaned of epibiota and
150 maintained alive overnight in filtered seawater to evacuate their gut contents. The oysters
were individually measured (shell length), opened for tissue dissection and carefully cleaned
with distilled water to remove any shell debris. After dissection, the tissues were frozen (-
20°C), freeze-dried (48 h), weighed (total dry flesh mass, DW, g), ground to a homogeneous
powder and finally stored in safe light and humidity conditions for later isotopic analyses.
155 From March until late June 2009, five individuals out of the 30 sampled were randomly
selected for whole body stable isotope analyses. From July 2009, the gills (Gi) and the
adductor muscle (Mu) of the five oysters were dissected separately from the remaining tissues
(Re), *i.e.* mantle, gonad, digestive gland and labial palps. As for the whole body tissues, Gi,
Mu and Re were frozen at -20°C, freeze-dried (48 h) and weighed (DW_{Gi} , DW_{Mu} and DW_{Re})
160 prior to being powdered and stored until stable isotope analyses. From July 2009 the total dry
flesh mass was then estimated as: $DW = DW_{Gi} + DW_{Mu} + DW_{Re}$.

2.3.2. Organic matter food sources (OMS)

In BDV and RDB, suspended particulate organic matter (SPOM) was sampled for stable
165 isotope analyses in the surroundings of each culture site at high tide. Two replicate samples of
seawater were taken in 2 L bottles at subsurface (0-50 cm), and then filtered onto preweighed,
pre-combusted (450°C, 4 h) Whatmann GF/C ($\varnothing= 47$ mm) glass fibre filters immediately
after sampling and pre-filtering onto a 200 μm mesh to remove the largest particles. In BDV,
microphytobenthos (MPB), which can be re-suspended from the sediment by waves and tidal

170 action and contributes to the diet of *C. gigas* (Marín Leal et al., 2008) was also sampled. MPB
was collected by scraping the visible microalgal mats off of the sediment surface adjacent to
the culture site during low tide. Immediately after scraping, MPB and sediment were put into
sea water where they were kept until the extraction at the laboratory. MPB was extracted from
the sediment using Whatmann lens cleaning tissue (dimensions: 100 ×150 mm, thickness:
175 0.035 mm). The sediment was spread in a small tank and covered with two layers of tissue.
The tank was kept under natural light/dark conditions until migration of the MPB. The upper
layer was then taken and put into filtered sea water to resuspend the benthic microalgae. The
water samples were then filtered onto pre-weighed, precombusted (450°C, 4 h) Whatmann
GF/C (Ø = 47 mm) glass-fibre filters. Meiobenthic fauna (mainly nematodes) was removed
180 from the filters under a binocular microscope. No samples of MPB were collected in RDB
due to the bottom composition of the culture site (mostly gravels). The SPOM and MPB
filters were then treated with concentrated HCl fumes (4 h) in order to remove carbonates
(according to Lorrain et al., 2003), frozen (-20°C) and freeze-dried (60°C, 12 h). Both the
filters were then ground to a powder using a mortar and pestle and stored in safe light and
185 humidity conditions until isotope analyses. Phytoplankton dominated SPOM isotope values
were retrieved from Lefebvre et al. (2009b) for BDV (SPOM_{phyBDV}), and from the French
Coastal Monitoring Network SOMLIT (Service d’Observation en Milieu LITtoral,
<http://somlit.epoc.u-bordeaux1.fr/fr/>, Brest Portzic “BreP” station) for RDB (SPOM_{phyRDB}).
Finally, isotope values for epipellic MPB in BDV (MPB_{epiBDV}) were also taken from Lefebvre
190 et al. (2009b).

2.4. Elemental and stable isotope analyses

The samples of oyster tissues and OMS were analysed using a CHN elemental analyser
EA3000 (EuroVector, Milan, Italy) for particulate organic carbon (POC) and particulate

195 nitrogen (PN) in order to calculate their C/N atomic ratios (Cat/Nat). Analytical precision for the experimental procedure was estimated to be less than 2 % dry mass for POC and less than 6 % dry mass for PN. The gas resulting from the elemental analyses was introduced online into an isotopic ratio mass spectrometer (IRMS) IsoPrime (Elementar, UK) to determine the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios. Isotopic ratios are expressed as the difference between the samples
 200 and the conventional Pee Dee Belemnite (PDB) standard for carbon, and air N_2 for nitrogen, according to the following equation:

$$\delta_{ij}^0 = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000 \quad (1)$$

where δ_{ij}^0 (‰) is the isotope 0 (13 or 15) of element i (C or N) in a compound j . Subscript j stands for the whole soft tissues DW, the gills Gi, the adductor muscle Mu, the remaining
 205 tissues Re of *C. gigas* or the food sources SPOM and MPB. R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio. The standard values of R are 0.0036735 for nitrogen and 0.0112372 for carbon. When the organs were sampled, the isotopic ratio and the C/N ratio of the whole soft tissues, i.e. $\delta_{i\text{DW}}^0$ and $\text{C}/\text{N}_{\text{DW}}$, respectively, were calculated as follows:

$$210 \quad \delta_{i\text{DW}}^0 = \frac{\delta_{i\text{Gi}}^0 \text{DW}_{\text{Gi}} + \delta_{i\text{Mu}}^0 \text{DW}_{\text{Mu}} + \delta_{i\text{Re}}^0 \text{DW}_{\text{Re}}}{\text{DW}_{\text{Gi}} + \text{DW}_{\text{Mu}} + \text{DW}_{\text{Re}}} \quad (2)$$

$$\text{C}/\text{N}_{\text{DW}} = \frac{\text{C}/\text{N}_{\text{Gi}} \text{DW}_{\text{Gi}} + \text{C}/\text{N}_{\text{Mu}} \text{DW}_{\text{Mu}} + \text{C}/\text{N}_{\text{Re}} \text{DW}_{\text{Re}}}{\text{DW}_{\text{Gi}} + \text{DW}_{\text{Mu}} + \text{DW}_{\text{Re}}} \quad (3)$$

The internal standard was the USGS 40 of the International Atomic Energy Agency ($\delta^{13}\text{C} = -26.2$ ‰; $\delta^{15}\text{N} = -4.5$ ‰). The typical precision in the analyses was ± 0.05 % for C and ± 0.19 % for N. One tin caps per sample was analysed. The mean value of the isotopic ratio was
 215 considered for both animal tissues and OMS.

2.5. Statistical analyses

Firstly, comparisons of growth patterns and isotopic composition of whole body tissues between the BDV and RDB sites were based on the average individual dry flesh mass (DW), the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the C/N ratio. Differences among DW, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C/N ratio were analysed by a two-way ANOVA, with time (*i.e.* sampling date) and site as fixed factors. Secondly, repeated measures ANOVAs were used to test for differences in the individual dry mass, isotopic ratios and C/N ratio among the different organs (gills, adductor muscle and remaining tissues) of *C. gigas*, with site and sampling date as the (inter-individual) sources of variation among oysters, and organs as the (intra-individual) source of variation within oysters. In both cases, all data were square-root transformed to meet the assumptions of normality, and the homogeneity of variance and/or the sphericity assumption were checked. In cases where ANOVA results were significant, they were followed by a Tukey HSD post-hoc test (Zar, 1996) to detect any significant differences in dry mass, isotopic ratios and C/N ratio for the whole body and for each of the organs between the two sites and/or among the different organs.

2.6. Correction of isotopic values

Isotopic values were corrected for two purposes. First, the mean observed C/N ratios were found higher than 3.5 (except adductor muscle), above this value lipid normalisation is recommended (Post et al., 2007). Normalisation of $\delta^{13}\text{C}$ values was performed according to: $\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \text{ C:N}$ (Post et al., 2007). Secondly, determination of the diet was based on visual inspection of the biplot by further correcting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with the trophic enrichment factor (or trophic discrimination Δ) as measured for *C. gigas* by Dubois et al.(2007) during a diet-switch experiment: $\Delta\delta^{13}\text{C} = 1.9 \text{ ‰}$ and $\Delta\delta^{15}\text{N} = 3.8 \text{ ‰}$.

3. Results

3.1. Abiotic conditions and food availability

From March 2009 to February 2010, [Chl-*a*] was on average three times higher in BDV than
 245 in RDB, with maximum values of 9.1 $\mu\text{g.L}^{-1}$ in June 2009 in BDV and 4.4 $\mu\text{g.L}^{-1}$ in May 2009
 in RDB (Fig. 2). From March to July 2009, the average [Chl-*a*] was 2.3 $\mu\text{g.L}^{-1}$ in RDB against
 4.9 $\mu\text{g.L}^{-1}$ in BDV, *i.e.* which was relatively high compared with the average [Chl-*a*] values of
 0.5 $\mu\text{g.L}^{-1}$ in RDB and of 1.4 $\mu\text{g.L}^{-1}$ in BDV from October 2009 to February 2010 (Fig. 2).

Seawater temperature in RDB and BDV showed a typical seasonal pattern with some
 250 similarities among the 2 sites, *i.e.* increasing values between March and August 2009, up to a
 maximum value in July or August 2009, followed by a decrease during the autumn (Fig. 2).

The thermal amplitude was, however, slightly higher in BDV (15.3°C) than in RDB (13.7°C).
 In BDV, the maximum and minimum temperatures were reached in August 2009 (20.8°C)
 and March 2010 (6.6°C) respectively, while in RDB they occurred in early July 2009
 255 (19.7°C) and January 2010 (4.4°C), respectively.

3.2. Growth and elemental ratios of *C. gigas* tissues

The mass gain of oysters expressed in total dry flesh mass DW differed significantly over
 time between the two ecosystems RDB and BDV (2-way ANOVA, site \times time, $F_{7,455} = 40.79$,
 260 $P < 0.0001$; Fig. 3 A). From March to June 2009, the increase in DW was relatively slow and
 similar in BDV and RDB (from 0.02 g to 0.36 g in BDV and from 0.02 g to 0.39 g in RDB),
 with no significant differences between sites at any sampling date (Tukey HSD post-hoc test,
 $0.194 \leq P \leq 0.513$). From July 2009, DW was significantly higher in BDV than in RDB at
 each sampling date (Tukey HSD post-hoc test, $P < 0.0001$). DW increased more sharply from
 265 July until October 2009 in BDV compared with RDB where growth ceased in August and
 where a slight decrease in DW was observed until February 2010 (Fig. 3A). At the end of the
 survey (February 2010), the value for DW in BDV was 1.80 g compared with 0.55 g in RDB,

which respectively corresponds to a mass gain of *ca.* 8 times and 2.5 times from the transplantation in March 2009.

270 Similarly to DW, significant interactions between site, time and organs were noted for the dry mass of the different organs, DW_{Gi} , DW_{Mu} , and DW_{Re} , indicating that the mass gain varied over time differently among the organs between the two ecosystems (3-way ANOVA, $F_{8,307} = 8.14$, $P < 0.0001$, Fig. 3 C&E). In BDV, DW_{Re} showed an increase of *ca.* 30 % between July and October 2009, whereas it decreased by *ca.* 12 % over the same period in RDB (Fig. 3 C).

275 At both sites, DW_{Re} was stable from November 2009 until February 2010 (Fig. 3 C&E). Between August 2009 and February 2010, 70 % and 80 % of DW corresponded to DW_{Re} in RDB and BDV respectively. From July 2009 to February 2010, DW_{Mu} and DW_{Re} were significantly different between the two sites at each sampling date (Tukey HSD post-hoc test, $P \leq 0.0346$), while DW_{Gi} was not significantly different between BDV and RDB at any

280 sampling date between July and October 2009 (Tukey HSD post-hoc test, $0.0541 \leq P \leq 0.1550$). In BDV, DW_{Gi} and DW_{Mu} did not differ significantly in July 2009 (Tukey HSD post-hoc test, $P = 0.0682$; Fig. 3C); in RDB, they were also no significant differences in July, August, October 2009 or in February 2010 (Tukey HSD post-hoc test, $0.0970 \leq P \leq 0.9134$, Fig. 3E).

285 The C/N ratio of whole soft body tissues (C/N_{DW}) was significantly higher in BDV than in RDB at almost all sampling dates (2-way ANOVA, site \times time $F_{7,78} = 2.96$, $P = 0.0096$, Fig. 3B). Only in June 2009 C/N_{DW} did differ significantly between BDV and RDB (Tukey HSD post-hoc test, $P = 0.1667$). In BDV, a strong increase of *ca.* 87% was observed from April to August 2009, when C/N_{DW} reached the maximum value of 6.9. At the same time, the C/N_{DW}

290 ratio remained rather constant in RDB, with a mean value of 4.3 over the whole survey period. In BDV, C/N_{DW} in BDV fell to the value of 5.8 in February 2010 (Fig. 3B). C/N ratio of oyster organs C/N_{Re} , C/N_{Gi} and C/N_{Mu} changed over time in a different manner between

ecosystems as shown by significant interactions between site, time and organs (3-way ANOVA, $F_{8,44} = 4.21$, $P = 0.0008$, Fig. 3 D & F). C/N_{Re} showed similar variations to C/N_{DW} (Fig. 3 B, D & F). C/N_{Re} , C/N_{Gi} and C/N_{Mu} were significantly different from one another in BDV and RDB at all sampling dates (Tukey HSD post-hoc test, $P \leq 0.0372$) and ranked as follows: $C/N_{Re} > C/N_{Gi} > C/N_{Mu}$ irrespective of the study site.

3.3. Stable isotope ratios of *C. gigas* whole body

Both $\delta^{13}C_{DW}$ and $\delta^{15}N_{DW}$ of *C. gigas* (whole body) over time differed significantly between BDV and RDB ecosystems (2-way ANOVAs, site \times time, $F_{7,78} = 31.82$, $P < 0.0001$ for $\delta^{13}C_{DW}$ and $F_{7,78} = 11.23$, $P < 0.0001$ $\delta^{15}N_{DW}$, Fig. 4A&B). With the exception of February 2010 ($P=0.9568$; Fig. 4A), $\delta^{13}C_{DW}$ was significantly lower in BDV than in RDB at all other sampling dates (Tukey HSD post-hoc test, $P < 0.05$), with a mean $\delta^{13}C_{DW}$ of -20.6 ‰ in BDV and -19.5 ‰ in RDB. From March to May 2009, $\delta^{13}C_{DW}$ in RDB decreased from -19.3 ‰ to -20.6 ‰ and then increased up to -18.9 ‰ in July 2009. The highest $\delta^{13}C_{DW}$ value, -18.8 ‰, was reached in August 2009 and a slight decrease was then observed until the end of the survey in RDB. A sharp decrease in $\delta^{13}C_{DW}$ occurred in BDV from -19.3 ‰ in March 2009 to -22.1 ‰ in May 2009 (Fig. 4A). Between June and July 2009, $\delta^{13}C_{DW}$ leapt up to the value of -19.9 ‰ and remained constant until February 2010. When normalising for lipids, $\delta^{13}C_{DW}$ values followed approximately the same pattern in BDV and RDB with slightly higher values in BDV from October 2009. Except in June 2009 when no significant differences were observed (Tukey HSD post-hoc test, $P = 0.8610$), $\delta^{15}N_{DW}$ was significantly higher in BDV than in RDB ($P \leq 0.0143$; Fig. 4B). The maximum values for $\delta^{15}N_{DW}$ were 9.5 ‰ in August 2009 and 10.3 ‰ in September 2009 in RDB and in BDV, respectively.

3.4. Stable isotope ratios of *C. gigas* organs and isotopic discrimination

Temporal variations in $\delta^{13}\text{C}$ and in $\delta^{15}\text{N}$ of the different organs showed similar patterns to $\delta^{13}\text{C}_{\text{DW}}$ and $\delta^{15}\text{N}_{\text{DW}}$ at both sites (Fig. 4). The interactions between site, time and organ were significant for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, indicating that stable isotope ratios of oyster organs changed over time in a different way between ecosystems (3-way ANOVA, $F_{8,48} = 8.88$, $P < 0.0001$ and $F_{8,48} = 8.88$, $P = 0.0002$ respectively, Fig. 4 C, D, E & F).

From August 2009 to February 2010, $\delta^{13}\text{C}_{\text{Gi}}$, $\delta^{13}\text{C}_{\text{Mu}}$ and $\delta^{13}\text{C}_{\text{Re}}$ values (*i.e.* isotope ratios for the gills, the adductor muscle and the remaining tissues) values decreased for oysters in RDB, while they remained stable in oysters at BDV (Fig. 4 C & E). No significant differences were observed between BDV and RDB in October and November 2009 for the $\delta^{13}\text{C}_{\text{Gi}}$ (Tukey HSD post-hoc test, $P = 0.1037$ and $P = 0.1345$, respectively), in November 2009 and February 2010 for the $\delta^{13}\text{C}_{\text{Re}}$ (Tukey HSD post-hoc test, $P = 0.0729$ and $P = 0.6928$, respectively) and in February 2010 for the $\delta^{13}\text{C}_{\text{Mu}}$ (Tukey HSD post-hoc test, $P = 0.1279$). In October 2009, the $\delta^{13}\text{C}_{\text{Gi}}$ and the $\delta^{13}\text{C}_{\text{Mu}}$ values did not differ significantly from each other in BDV (Tukey HSD post-hoc test, $P = 0.8190$). Significant differences among $\delta^{13}\text{C}_{\text{Gi}}$, $\delta^{13}\text{C}_{\text{Mu}}$ and $\delta^{13}\text{C}_{\text{Re}}$ were found in BDV and in RDB (Tukey HSD post-hoc test, $P \leq 0.0371$) at all other sampling dates (Fig. 4 C&E).

$\delta^{15}\text{N}_{\text{Gi}}$, $\delta^{15}\text{N}_{\text{Mu}}$ and $\delta^{15}\text{N}_{\text{Re}}$ were significantly different between the two sites at most dates (Tukey HSD post-hoc test, $P \leq 0.0064$), except in July 2009 for $\delta^{15}\text{N}_{\text{Re}}$ (Tukey HSD post-hoc test, $P = 0.1041$; Fig. 4 D&F). Values were rather constant in RDB over the whole survey, while they increased from June to September 2009 in BDV and then decreased slightly and stabilised until February 2010 (Fig. 4 C&E). The $\delta^{15}\text{N}_{\text{Gi}}$, $\delta^{15}\text{N}_{\text{Mu}}$ and $\delta^{15}\text{N}_{\text{Re}}$ were significantly different from one another within BDV and RDB (Tukey HSD post-hoc test, $P \leq 0.0371$) at all sampling dates.

At the two sites, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranked in the same order: $\delta^{13}\text{C}_{\text{Mu}} > \delta^{13}\text{C}_{\text{Gi}} > \delta^{13}\text{C}_{\text{Re}}$ and $\delta^{15}\text{N}_{\text{Mu}} > \delta^{15}\text{N}_{\text{Gi}} > \delta^{15}\text{N}_{\text{Re}}$ (Fig. 4 C,D,E & F). The isotopic discrimination between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for organs (noted $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ respectively) were very close for the two sites when considering uncorrected $\delta^{13}\text{C}$ values (Fig 5A). Only for gills, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were slightly higher in BDV than in RDB. When applying lipid normalization of $\delta^{13}\text{C}$ values to the organs, this difference in $\delta^{13}\text{C}_{\text{Gi}}$ and $\delta^{15}\text{N}_{\text{Gi}}$ between RDB and BDV was widened (Fig. 5B). However, $\delta^{13}\text{C}$ values after correction for lipids were significantly higher in BDV than in RDB and differed significantly compared to the scenario without any lipid normalisation (Fig. 5A & B).

350

3.5. Stable isotope ratios of food sources and diet of *C. gigas*

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the suspended particulate organic matter SPOM food source (*i.e.* $\delta^{13}\text{C}_{\text{SPOM}}$ and $\delta^{15}\text{N}_{\text{SPOM}}$) exhibited different patterns in BDV and RDB (Fig. 6A and B). From May to September 2009, the $\delta^{13}\text{C}_{\text{SPOM}}$ decreased from -18.5 ‰ to -25.4 ‰ in RDB (Fig. 6A). From September 2009 to late October 2009, $\delta^{13}\text{C}_{\text{SPOM}}$ varied over a range of 2.4 ‰ and then stabilised. In BDV, a sharp increase in $\delta^{13}\text{C}_{\text{SPOM}}$ occurred in June and July 2009 when the maximum value was reached, *i.e.* $\delta^{13}\text{C}_{\text{SPOM}} = -18.5$ ‰ followed by a decrease of around 5.2 ‰ over the next three months (Fig. 6A). $\delta^{15}\text{N}_{\text{SPOM}}$ values in RDB varied between 6.5 ‰ and 8.1 ‰ from May to September 2009 and dropped to 5.5 ‰ in October 2009 (Fig. 6B), with an average of 7.2 ‰ over the whole sampling period. In BDV, $\delta^{15}\text{N}_{\text{SPOM}}$ was 8.4 ‰ on average, but showed high temporal variability throughout the survey. The highest $\delta^{15}\text{N}_{\text{SPOM}}$ is observed in BDV in late October 2009, reaching a value of 10.1 ‰ (Fig. 6B). It should be noticed that the $\delta^{13}\text{C}_{\text{SPOM}}$ and $\delta^{15}\text{N}_{\text{SPOM}}$ values in BDV were higher by 3.8 ‰ and 3.2 ‰ respectively than the $\text{SPOM}_{\text{phyBDV}}$ values (Fig. 7). In RDB, $\delta^{13}\text{C}_{\text{SPOM}}$ and $\delta^{15}\text{N}_{\text{SPOM}}$ were also higher than the

360

365 SPOM_{phyRDB} values by 0.9 ‰ and 1.2 ‰ respectively (Fig. 7). The opposite pattern was observed for the microphytobenthos food source in BDV: the $\delta^{13}\text{C}_{\text{MPB}}$ and $\delta^{15}\text{N}_{\text{MPB}}$ values in the present study were lower than the $\delta^{13}\text{C}_{\text{MPBepi}}$ and $\delta^{15}\text{N}_{\text{MPBepi}}$ values by 3.5 ‰ and 2.1 ‰, respectively (Fig. 7).

As for *C. gigas* tissues, the mean $\delta^{13}\text{C}_{\text{DW}}$ and $\delta^{15}\text{N}_{\text{DW}}$ values that were corrected for trophic
 370 discrimination and also lipid-normalized differed significantly from the values of SPOM in both BDV and RDB. For BDV, these values were close to those ones of SPOM_{phyBDV}. $\delta^{13}\text{C}_{\text{DW}}$ values first decreased slowly over time and then increased, while $\delta^{15}\text{N}_{\text{DW}}$ increased gradually. Except for the first two points which are below the range of SPOM_{phyBDV}, SPOM_{phyBDV} values scatter in a crescent shape plot towards the MPB values (Fig. 7). For RDB, the corrected
 375 values of $\delta^{13}\text{C}_{\text{DW}}$ equaled the SPOM_{phyRDB} values. In parallel, $\delta^{15}\text{N}_{\text{DW}}$ increased gradually over time, the first value being *ca.* 3 ‰ below $\delta^{15}\text{N}_{\text{SPOMphyBDV}}$ (Fig. 7).

4. Discussion

Oyster growth and C/N ratio vary with food availability

380 [Chl-*a*], as a quantitative proxy of microphyte food availability, influences oyster growth (in terms of dry flesh mass DW) differently at the sites BDV and RDB. The seasonal differences in [Chl-*a*] between BDV and RDB, which are particularly marked in spring and early summer, *i.e.* [Chl-*a*]_{BDV} *ca.* 4 [Chl-*a*]_{RDB}, likely account for the differences in oyster growth performances observed between the two sites (Figs. 2, 4A and 5 A). An increase in DW
 385 occurs from March to June 2009 (Fig. 4A) when Chl-*a* is likely non-limiting in both BDV and RDB sites. This suggests that the growth of *C. gigas* (as expressed in DW) in BDV and in RDB mainly relies on the microphytes food source. The growth patterns of *C. gigas* in BDV slightly differ from the results of Grangeré et al. (2009) and Marín Leal et al. (2008), who reported a sharp decrease of DW in spring, due to spawning events, and in autumn and winter,

390 probably due to low food availability. The continuous growth of *C. gigas* in DW observed from March 2009 to February 2010 in our study can be explained by the unusual [Chl-*a*] in BDV in 2009: blooms did not exceed $9.1 \mu\text{g.L}^{-1}$ and but stretched over four months (from May to August). Conversely, Grangeré et al. (2009), Jouenne et al. (2007) and Lefebvre et al. (2009b) reported larger blooms, between $12 \mu\text{g.L}^{-1}$ and $25 \mu\text{g.L}^{-1}$, earlier in the year (March
395 and April).

Similarly to DW, the dry mass and C/N ratios of the different organs, and especially the remaining tissues Re pool, were also influenced by food availability. The high contribution of DW_{Re} to the total dry mass of oysters, *i.e.* 70 % and 80 % in BDV and RDB (Fig. 3C, E), respectively, can be explained by the fact that *C. gigas* stores energy (mainly lipids and
400 glycogen) in the digestive gland, gonad and mantle during spring and summer (*e.g.* Berthelin et al., 2000; Costil et al., 2005; Ren et al., 2003; Whyte et al., 1990). The decrease in DW_{Re} for oysters in RDB from July 2009 to March 2010 may result from the low quantity of food *i.e.* [Chl-*a*] = $0.6 \mu\text{g.L}^{-1}$ observed from July 2009 to February 2010. The C/N ratios of the gills and the adductor muscle tend to remain constant over time, suggesting that these two organs
405 make very little contribution to reserve storage.

SPOM isotopic value is highly variable

With respect to the composition of available food, the temporal variations of SPOM in BDV differ from the typical patterns observed in this bay. In BDV, the SPOM source was slightly
410 higher in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, ranging from -27.2 ‰ to -21.5 ‰ and from 6.3 ‰ to 10.1 ‰, respectively, compared with previous ranges of values that have been reported, *ca.* -22 ‰ to -18 ‰ for $\delta^{13}\text{C}$ and *ca.* 3 ‰ to *ca.* 6 ‰ for $\delta^{15}\text{N}$ values in 2004 and 2005 (Lefebvre et al., 2009b; Marín Leal et al., 2008). The isotope ratios of epipellic microphytobenthos species ($\delta^{13}\text{C}_{MPBepi}$ and $\delta^{15}\text{N}_{MPBepi}$) also differed from the values usually observed in BDV

415 (Fig. 6). $\delta^{13}\text{C}_{\text{MPBepi}}$ was higher than previously reported (overall mean = -14.8 ‰), though the
 $\delta^{15}\text{N}_{\text{MPBepi}}$ were lower (overall mean = 5.5 ‰) than the MPB values found by Marín Leal et
al. (2008) in 2004 and 2005 (overall mean = -17.9 ‰ and 7.4 ‰, respectively). On the
contrary, these values are comparable to typical values of epipelagic MPB of mudflats (Malet et
al., 2008). The sampling methods of the epipelagic and epipsammic fractions of
420 microphytobenthos by Lefebvre et al. (2009b) and Marín Leal et al. (2008) may account for
the differences with our values, which only referred to diatoms that had migrated through the
sediment (Fig. 7). Moreover, the $\delta^{15}\text{N}_{\text{MPBepi}}$ was lower than the $\delta^{15}\text{N}_{\text{SPOM}}$ in our study, while
the opposite trend was previously reported (Kang et al., 2006; Riera, 2007; Yokoyama et al.,
2005). Our sampling in the immediate surroundings of the culture sites differs from Lefebvre
425 et al. (2009b) and from Marín Leal et al. (2008), *i.e.* the seaward samples of SPOM are likely
dominated by phytoplankton (PHY, Fig. 7). SPOM samples are time dynamic mixing of alive
and detritus particles in the estuarine-coastal zone (Malet et al., 2008). Additionally, isotopic
ratios of alive particles can have their own temporal dynamics depending on the availability
of nutrients: for example, $\delta^{13}\text{C}$ values of phytoplankton cells typically increase during blooms
430 when dissolved CO_2 availability decreases and so does the fractionation (Savoie et al., 2003).
Thus, it is very likely that the samples of our study contained more detritus from macroalgae
and riverine particulate organic matter (rPOM) than MPB or PHY.

Oysters select microalgae in their diet

435 However, the oyster diet in the two systems mainly relied on a marine phytoplankton food
source (PHY, *e.g.* Kang et al., 2006; Marín Leal et al., 2008), as indicated by the isotopic
enrichment of oysters relative to the SPOM_{phy} values in the two study sites (Fig. 7). The
capacity for pre-ingestive particle processing has been described in *C. gigas*, resulting in an
efficient selection of organic particles over inorganic ones (Barillé et al., 1997). The selection

440 efficiency within organic material was demonstrated, resulting in a positive selection of live cells against detritus (Ward and Shumway, 2004). This possibly explained the differences between SPOM, SPOM_{phy} and oyster tissue isotope values in our study (Fig. 7). During autumn, oysters in BDV continued to grow while their $\delta^{13}\text{C}_{\text{DW}}$ increased and their $\delta^{15}\text{N}_{\text{DW}}$ decreased (trend mostly driven by $\delta^{13}\text{C}_{\text{Re}}$ and $\delta^{15}\text{N}_{\text{Re}}$), suggesting that MPB_{epi} may have
445 contributed to the diet of *C. gigas* when PHY availability was low (Fig. 7, Marin-Leal et al., 2008). Accordingly, pelagic microphytes (mostly phytoplankton and to a less extent resuspended MPB) enumeration data have been used as a suitable trophic forcing variable to simulate the variability of growth and reproduction of *C. gigas* in RDB and BDV (Alunno-Bruscia et al., 2011, Bernard et al., 2011). The contribution of MPB to the diet of oysters that
450 was found in BDV in our study seems rather low compared to previous results on this site (Lefebvre et al., 2009b, Marin Leal et al., 2008). According to Grangeré et al. (2012), an interannual variability in the contribution of MPB to the diet of *C. gigas* was observed over 4 years (2005 to 2008): a low contribution of MPB coincided with wet years (2007 and 2008) when phytoplankton production was higher due to higher nutrient input from rivers. With
455 annual cumulated precipitations of *ca.* 870 mm (Météo France data), which is rather close to precipitations measured in 2007 (926 mm) and 2008 (918 mm), 2009 ranks as a wet year (compared to dry years i.e. 2005 and 2006 *ca.* 750 mm), possibly explaining the low contribution of MPB this year.

460 **Isotopic discrimination vary slightly between sites**

The ranking $\delta_{\text{Mu}} > \delta_{\text{Gi}} > \delta_{\text{Re}}$ is observed in the enrichment patterns of ^{13}C and ^{15}N between organs, irrespective of the study site. This pattern is consistent with results from previous studies. Malet et al. (2007) found that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were higher in the adductor muscle than in the mantle, digestive gland and gonad of diploid and triploid *C. gigas*. Paulet et al. (2006)

465 showed that the adductor muscle had higher $\delta^{13}\text{C}$ values than the gills and the gonad of oysters in a diet-shift experiment. In the same way, Yokoyama et al. (2005) pointed out that $\delta^{15}\text{N}_{\text{Mu}}$ in *C. gigas* was greater than $\delta^{15}\text{N}_{\text{Gi}}$ after reaching equilibrium with the food source. δ_{Re} vary faster over time than δ_{Mu} and δ_{Gi} (Fig. 4 C, D, E & F). These results suggest that uptake food is preferentially routed to the storage organs, *e.g.* digestive gland and mantle, 470 and/or reproductive tissues during active growth periods in spring and early summer. The narrow range of variation in δ_{Mu} (and to a lesser extent of δ_{Gi}) over the survey might be due to the lower turnover rate of this organ. The carbon incorporation index calculated by Paulet et al. (2006) in the muscle of both *C. gigas* and *P. maximus* showed the lowest value among all organs studied in all seasons. In *P. maximus*, $\delta^{13}\text{C}_{\text{Mu}}$ and $\delta^{15}\text{N}_{\text{Mu}}$ show similarly low 475 variation compared with the digestive gland and the gonad (Lorrain et al., 2002). The differences in isotopic discrimination between organs ($\Delta\delta^{13}\text{C}$) that have been reported in the literature (*e.g.* Deudero et al., 2009; Guelinckx et al., 2007; Suzuki et al., 2005; Tieszen et al., 1983) can be mainly explained by differences in the biochemical composition of the different organs. Organs containing a high proportion of lipids have a lower $\delta^{13}\text{C}$ value than organs 480 with a lower lipid content (or a higher protein content), since lipids are depleted in ^{13}C (Post et al., 2007). The low $\delta^{13}\text{C}_{\text{Re}}$ and high C/N_{Re} ratios are thus consistent with the physiological role of these tissues, *i.e.* energy storage and reproduction, since gametogenesis in spring and summer is characterised by an increase in lipids and glycogen (Berthelin et al., 2000; Soudant et al., 1999). The relatively high values of $\delta^{13}\text{C}_{\text{Mu}}$ and $\delta^{15}\text{N}_{\text{Mu}}$, combined with a high protein 485 concentration in the adductor muscle (low C/N ratio), supports the idea that the adductor muscle is not a storage compartment supplying the energetic needs of reproduction (Berthelin et al., 2000). The simultaneous increases of DW_{Re} and C/N_{Re} and the low values of the $\delta^{13}\text{C}_{\text{Re}}$ from February to October 2009 in BDV confirm that most of the energy assimilated during spring and summer is directed to the reserve tissues (Figs. 3D and 4C). The same pattern was

490 previously shown by Malet et al. (2007) for *C. gigas* and by Smaal and Vonk (1997) for
Mytilus edulis. The $\delta^{13}\text{C}$ lipid normalisation after Post et al. (2007) confirms the impact of the
C/N ratio on $\delta^{13}\text{C}$ values (Fig 5B), and more particularly for the remaining tissues Re in
which a significant part of glycogen accounts for high C/N. However, such a relationship has
never been reported for bivalves. When applying the lipid normalisation, the enrichment in
495 ^{13}C for Re compared to Gi (Fig. 5B) may have two non exclusive origins, either higher $\delta^{13}\text{C}$
values in the food that oyster ingested before sampling, or an overestimation of the lipid
correction. Finally, except for the gills (Gi), the $\delta^{15}\text{N}$ isotopic discrimination in organs
($\Delta\delta^{15}\text{N}$) was very close between the two sites, thus leading to a constant $\Delta\delta^{15}\text{N}$ whatever the
growth. Though surprising, this is an interesting result since, at the whole body level,
500 trophic fractionation (or trophic enrichment) is supposed to decrease with growth (Lefebvre &
Dubois, this issue) or with food level (Emmery et al., 2011). An experiment under controlled
food and temperature conditions would certainly help deciphering the causes for $\Delta\delta^{13}\text{C}$ and
 $\Delta\delta^{15}\text{N}$ differences between organs, e.g. different turnover rates, food level and/or dynamic
isotopic values of food sources.

505

Concluding remarks

As a conclusion, the results of our transplantation experiment showed that temporal variations
in food availability (quantity, [Chl-*a*] and stable isotope ratios) and also in growth (in dry
flesh mass, DW) and physiological state (C/N ratio) of *C. gigas* both influenced the dynamics
510 of stable isotopes of the whole body tissues and of the organs (gills, adductor muscle and
remaining tissues) of oysters. The oyster diet was mainly composed of microalgae and
especially phytoplankton confirming that *C. gigas* is an opportunistic suspension feeder that
can modify its diet as a function of available food on temporal and spatial scales (Lefebvre et
al., 2009b). A calibration of the lipid correction for $\delta^{13}\text{C}$ values should be done on bivalves,

515 taking into account the high content of glycogen in digestive gland at certain periods of the
year. Finally, these results and the constant isotopic discrimination between organs would
benefit to be confirmed under controlled conditions.

Acknowledgments

520 A. Emmery was supported by funding from *Région Basse-Normandie* and Ifremer. This PhD
project was part of the Observatoire Conchylicole network
(http://wwz.ifremer.fr/observatoire_conchylicole_eng/Presentation). We would like to thank
F. Maheux, S. Parrad, M. Ropert and A. Gangnery from the Ifremer Environment &
Resources laboratory in Port-en-Bessin, as well as P. Le Souchu and S. Pouvreau from the
525 Ifremer laboratory at Argenton for their technical help. We also thank J. Le Poittevin for her
technical assistance with the isotopic samples. We are also very grateful to A. Sedon for her
help with the sample preparation. We finally thank N. Savoye, S. L'Helguen, P. Rimelin-
Mauryfor, E. Grossteffan and K. Charlier for providing us with a useful dataset from the
French Coastal Monitoring Network SOMLIT (Service d'Observation en Milieu LITora,
530 <http://somlit.epoc.u-bordeaux1.fr/fr/>). We finally thank F. Jean from the LEMAR for their
advice and encouragement.

References

- Alunno-Bruscia M, Bourlès Y, Maurer D, Robert S, Mazurié J, Gangnery A, Gouilletquer P,
535 Pouvreau S 2011. A single bio-energetics growth and reproduction model for the oyster
Crassostrea gigas in six atlantic ecosystems. *J Sea Res* 66: 340-348
- Aminot A, Kérouel R 2004. *Hydrologie des écosystèmes marins: paramètres et analyses*
Ifremer, 336 pp

- Barillé L, Prou J, Héral M, Razet D 1997. Effects of high natural seston concentrations on the
540 feeding, selection, and absorption of the oyster *Crassostrea gigas* (Thunberg). J Exp
Mar Biol Ecol 212: 149-172
- Bernard I, de Kermoisan G, Pouvreau S 2011. Effect of phytoplankton and temperature on
the reproduction of the pacific oyster *Crassostrea gigas*: Investigation through DEB
theory. J Sea Res 66: 349-360
- 545 Berthelin C, Kellner K, Mathieu M, 2000. Storage metabolism in the Pacific oyster
(*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West
Coast of France). Comp Biochem Physiol Part B: Biochem Mol Biol 125: 359-369
- Boecklen WJ, Yarnes CT, Cook BA, James AC 2011. On the use of stable isotopes in trophic
ecology. Annu Rev Ecol Evol Syst 42: 411-440
- 550 Chaparro O, Segura C, Montiel Y, Thompson R, Navarro J 2008. Variations in the quantity
and composition of seston from an estuary in southern chile on different temporal
scales. Estuar Coast Shelf Sci 76: 845-860
- Chauvaud L, Donval A, Thouzeau G, Paulet YM, Nézan E 2001. Variations in food intake of
Pecten maximus (L.) from the Bay of Brest (France): influence of environmental factors
555 and phytoplankton species composition. Life Sci 324: 743-755
- Cloern JE, Jassby AD 2008. Complex seasonal patterns of primary producers at the land-sea
interface. Ecol Lett 11: 1294-1303
- Costil K, Royer J, Ropert M, Soletchnik P, Mathieu M, 2005. Spatiotemporal variations in
biological performances and summer mortality of the Pacific oyster *Crassostrea gigas*
560 in Normandy (France). Helgol Mar Res 59: 286-300
- Dang C, Sauriau P, Savoye N, Caill-Milly N, Martinez P, Millaret C, Haure J, de
Montaudouin X 2009. Determination of diet in Manila clams by spatial analysis of
stable isotopes. Mar Ecol Prog Ser 387: 167-177

- Decottignies P, Beninger PG, Rincé Y, Robins RJ, Riera, P 2007. Exploitation of natural food
565 sources by two sympatric, invasive suspension feeders: *Crassostrea gigas* and
Crepidula fornicata. Mar Ecol Prog Ser 334: 179-192
- DeNiro M, Epstein S 1978. Influence of diet on the distribution of carbon isotopes in animals.
Geochim Cosmochim Acta 42: 495-506
- DeNiro M, Epstein S 1981. Influence of diet on the distribution of nitrogen isotopes in
570 animals. Geochim Cosmochim Acta 45: 341-351
- Deudero S, Cabanellas M, Blanco A, Tejada S 2009. Stable isotope fractionation in the
digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis*.
J Exp Mar Biol Ecol 368: 181-188
- Doering PH, Oviatt CA, Kelly JR 1986. The effects of the filter-feeding clam *Mercenaria*
575 *mercenaria* on carbon cycling in experimental marine mesocosms. J Mar Res 44: 839-
861
- Dubois S, Blin JL, Bouchaud B, Lefebvre S 2007. Isotope trophic step fractionation of
suspension-feeding species: Implications for food partitioning in coastal ecosystems. J
Exp Mar Biol Ecol 351: 121-128
- 580 Emmery A, Lefebvre S, Alunno-Bruscia M, Kooijman S 2011. Understanding the dynamics
of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in soft tissues of the bivalve *Crassostrea gigas* facing environmental
fluctuations in the context of dynamic energy budgets (DEB). J Sea Res 66: 361-371
- Fleury PG, Simonne C, Claude S, Palvadeau H, Guilpain P, D'Amico F, Le Gall P, Vercelli
C., Pien C 2005a. REseau Mollusques des Rendements Aquacoles (REMORA - huître
585 creuse); résultats des stations NATIONALES; Année 2003. Technical Report. Ifremer.
URL: <http://archimer.ifremer.fr/doc/00000/2412/>
- Fleury PG, Simonne C, Claude S, Palvadeau H, Guilpain P, D'Amico F, Le Gall P, Vercelli
C, Pien C 2005b. REseau Mollusques des Rendements Aquacoles (REMORA - huître

- creuse); résultats des stations NATIONALES; Année 2004. Technical Report. Ifremer.
590 URL: <http://archimer.ifremer.fr/doc/00000/2413/>
- Gili JM, Coma R 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends Ecol Evol* 13: 316-321
- Grangeré K, Ménesguen A, Lefebvre S, Bacher C, Pouvreau S 2009. Modelling the influence of environmental factors on the physiological status of the Pacific oyster *Crassostrea*
595 *gigas* in an estuarine embayment; The Baie des Veys (France). *J Sea Res* 62: 147-158
- Grangeré K, Lefebvre S, Blin JL 2012. Spatial and temporal dynamics of biotic and abiotic features of temperate coastal ecosystems as revealed by a combination of ecological indicators. *Estuar Coast Shelf S* 108: 109-118
- Guelinckx J, Maes J, Van Den Driessche P, Geysen B, Dehairs F, Ollevier F 2007. Changes
600 in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in different tissues of juvenile sand goby *Pomatoschistus minutus*: a laboratory diet-switch experiment. *Mar Ecol Prog Ser* 341: 205-215
- Jennings S, Warr K 2003. Environmental correlates of large-scale spatial variation in the $\delta^{15}\text{N}$ of marine animals. *Mar Biol* 142: 1131-1140
- Jouenne F, Lefebvre S, Veron B, Lagadeuc Y 2007. Phytoplankton community structure and
605 primary production in small intertidal estuarine bay ecosystem (eastern English channel, France). *Mar Biol* 151: 805-825
- Kang C, Lee Y, Choy E, Shin J, Seo I, Hong J 2006. Microphytobenthos seasonality determines growth and reproduction in intertidal bivalves. *Mar Ecol Prog Ser* 315: 113-127
- 610 Kang C, Sauriau P, Richard P, Blanchard G 1999. Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. *Mar Ecol Prog Ser* 187: 147-158

- Laing I 2000. Effect of temperature and ration on growth and condition of king scallop
615 (*Pecten maximus*) spat. *Aquaculture* 183: 325-334
- Lefebvre S, Harma C, Blin J 2009a. Trophic typology of coastal ecosystems based on $\delta^{13}\text{C}$
and $\delta^{15}\text{N}$ ratios in an opportunistic suspension feeder. *Mar Ecol Prog Ser* 390: 24-37
- Lefebvre S, Marín Leal J, Dubois S, Orvain F, Blin J, Bataillé M, Ourry A, Galois R 2009b.
620 Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in
two shellfish culture dominated ecosystems. *Estuar Coast Shelf Sci* 82: 415-425
- Lefebvre S, Dubois S in press. The stony road to understand isotopic enrichment and turnover
rates: insight into the metabolic part. *Vie milieu* (This volume)
- Lorrain A, Paulet YM, Chauvaud L, Savoye N, Donval A, Saout C 2002. Differential $\delta^{13}\text{C}$
and $\delta^{15}\text{N}$ signatures among scallop tissues: implications for ecology and physiology. *J*
625 *Exp Mar Biol Ecol* 275: 47-61
- Lorrain A, Savoye N, Chauvaud L, Paulet YM, Naulet N 2003. Decarbonation and
preservation method for the analysis of organic C and N contents and stable isotope
ratios of low-carbonated suspended particulate material. *Anal Chim Acta* 491: 125-133
- Malet N, Sauriau PG, Faury N, Soletchnik P, Guillou G 2007. Effect of seasonal variation in
630 trophic conditions and the gametogenic cycle on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels of diploid and
triploid Pacific oysters *Crassostrea gigas*. *Mar Ecol Prog Ser* 346: 203-217
- Malet N, Sauriau PG, Ryckaert M, Malestroit P, Guillou G 2008. Dynamics and sources of
suspended particulate organic matter in the Marennes-Oléron oyster farming bay:
Insights from stable isotopes and microalgae ecology. *Estuar Coast Shelf S* 78: 576-586
- 635 Marín Leal JC, Dubois S, Orvain F, Galois R, Blin JL, Ropert M, Bataille MP, Ourry A,
Lefebvre S 2008. Stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and modelling as tools to estimate the
trophic ecology of cultivated oysters in two contrasting environments. *Mar Biol* 153:
673-688

- Paulet YM, Lorrain A, Richard J, Pouvreau S 2006. Experimental shift in diet $\delta^{13}\text{C}$: A
640 potential tool for ecophysiological studies in marine bivalves. *Org Geochem* 37: 1359-
1370
- Pernet F, Malet N, Pastoureaud A, Vaquer A, Quéré C, Dubroca L 2012. Marine diatoms
sustain growth of bivalves in a mediterranean lagoon. *J Sea Res* 68, 20-32
- Phillips D, Gregg J 2003. Source partitioning using stable isotopes: coping with too many
645 sources. *Oecologia* 136: 261-269
- Piola RF, Moore SK, Suthers IM 2006. Carbon and nitrogen stable isotope analysis of three
types of oyster tissue in an impacted estuary. *Estuar Coast Shelf Sci* 66: 255-266
- Post D, Layman C, Arrington D, Takimoto G, Quattrochi J, Montaña C 2007. Getting to the
fat of the matter: models, methods and assumptions for dealing with lipids in stable
650 isotope analyses. *Oecologia* 152: 179-189
- Riera P 2007. Trophic subsidies of *Crassostrea gigas*, *Mytilus edulis* and *Crepidula fornicata*
in the Bay of Mont Saint Michel (France): A $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ investigation. *Estuar Coast
Shelf Sci* 72: 33-41
- Sauriau PG, Kang C 2000. Stable isotope evidence of benthic microalgae based growth and
655 secondary production in the suspension feeder *Cerastoderma edule* (Mollusca,
Bivalvia) in the Marennes-Oléron Bay. *Hydrobiologia* 440: 317-329
- Smaal A, Vonk A 1997. Seasonal variation in C, N and P budgets and tissue composition of
the mussel *Mytilus edulis*. *Mar Ecol Prog Ser* 153: 167-179
- Soudant P, Van Ryckeghem K, Marty Y, Moal J, Samain J, Sorgeloos P 1999. Comparison of
660 the lipid class and fatty acid composition between a reproductive cycle in nature and a
standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*. *Comp Biochem
Physiol Part B: Biochem Mol Biol* 123: 209-222

- Suzuki K, Kasai A, Nakayama K, Tanaka M 2005. Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. *Can J Fish Aquat Sci* 62: 671-678
- 665
- Tieszen L, Boutton T, Tesdahl K, Slade N 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57: 32-37
- Ward J, Shumway S 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. *J Exp Mar Biol Ecol* 300: 83-130
- 670
- Whyte J, Englar J, Carswell B 1990. Biochemical composition and energy reserves in *crassostrea gigas* exposed to different levels of nutrition. *Aquaculture* 90: 157-172
- Yokoyama H, Tamaki A, Koyama K, Ishihi Y, Shimoda K, Harada K 2005. Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. *Mar Ecol Prog Ser* 304: 101-116
- 675
- Zar J 1996. Multiple regression and correlation. *Biostatistical analysis*. 3rd ed. upper saddle river, NJ: Prentice hall ed, 662 p.

680 Figure captions

Figure 1: Geographic location of the two study ecosystems, Baie des Veys (BDV) and Brest Harbour (RDB), along the Channel and Atlantic coasts of France, respectively. White circles indicate the oyster culture sites and black circles indicate the locations where chlorophyll-*a* concentration [Chl-*a*] and temperature were monitored.

685

Figure 2: Temporal variations in chlorophyll-*a* concentration ([Chl-*a*], $\mu\text{g.L}^{-1}$, solid lines) and water temperature (Temp., $^{\circ}\text{C}$, dashed lines) from March 2009 to March 2010 at two sites: Baie des Veys in Normandy (BDV, grey lines) and Rade de Brest in North Brittany (RDB, black lines). The arrow indicates the oyster transplantation date (start of the survey).

690

Figure 3: Temporal variations in mean individual dry flesh mass (DW, g, left panels) and C/N ratio (right panels) of *Crassostrea gigas* tissues from March 2009 to February 2010 at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols). Panels A and B shows values of the whole body tissues. Panels C, D, E, F shows the values of the organs: the remaining tissues Re (including the mantle, gonad, digestive gland and labial palps), the adductor muscle Mu and the gills Gi. The vertical bars indicate $\pm\text{SD}$ of the mean for $n = 30$ oysters. Note that in E, Gi and Mu are surimposed.

695

Figure 4: Temporal variations in mean individual $\delta^{13}\text{C}$ (‰, left panels) and $\delta^{15}\text{N}$ (‰, right panels) values of *Crassostrea gigas* tissues from March 2009 to February 2010 at two sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black symbols). Panels A and B shows values of the whole body tissues. Panels C, D,

700

E, F shows the values of the organs: the remaining tissues Re (including the mantle, gonad,
 705 digestive gland and labial palps), the adductor muscle Mu and the gills Gi. In panels, C, E
 grey symbols shows the variation of $\delta^{13}\text{C}$ values for gills and remaining tissues that were for
 lipid normalised according to Post et al.(2007). The vertical bars indicate $\pm\text{SD}$ of the mean for
 $n = 5$ oysters.

710 Figure 5: Mean $\pm\text{SD}$ of isotopic discrimination for $\delta^{13}\text{C}$ ($\Delta\delta^{13}\text{C}$) and for $\delta^{15}\text{N}$ values ($\Delta\delta^{15}\text{N}$)
 between organs (the remaining tissues Re, the adductor muscle Mu and the gills Gi) at two
 sites: Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany
 (RDB, black symbols). For both sites, the reference values(=0) are uncorrected $\delta^{13}\text{C}$ values
 of Re. A : uncorrected $\delta^{13}\text{C}$ values (Re values for BDV were slightly repositionned for visual
 715 purpose). B : lipid normalised $\delta^{13}\text{C}$ values according to Post et al. (2007). Note that $\delta^{15}\text{N}$ for
 the adductor muscle was not corrected as C/N ratio was below 5.

Figure 6: Temporal variations of $\delta^{13}\text{C}$ (‰, graph A) and $\delta^{15}\text{N}$ (‰, graph B) values of the
 suspended particulate matter SPOM from March 2009 to February 2010 at two sites: Baie des
 720 Veys in Normandy (BDV, white symbols) and Rade de Brest in North Brittany (RDB, black
 symbols). The vertical bars indicate $\pm\text{SD}$ of the mean for two replicate samples.

Figure 7 : Variations in $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) values of *Crassostrea gigas* and the potential
 food sources at Baie des Veys in Normandy (BDV, white symbols) and Rade de Brest in
 725 North Brittany (RDB, black symbols) from March to October 2009. $\delta^{13}\text{C}$ (‰) values were
 lipid normalized (Post et al., 2007). $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) were corrected for trophic
 discrimination (Dubois et al., 2007). SPOM (Suspended Particulate Organic Matter). MPB

(microphytobenthos). MPB_{epi} (epipellic microphytobenthos). $SPOM_{\text{phy}}$ (phytoplankton dominated SPOM). The vertical bars indicate $\pm SD$ of the mean for $n = 12$ (SPOM), $n = 7$
730 (MPB). Arrows indicate chronological samples of *C. gigas* tissues.

Fig. 1

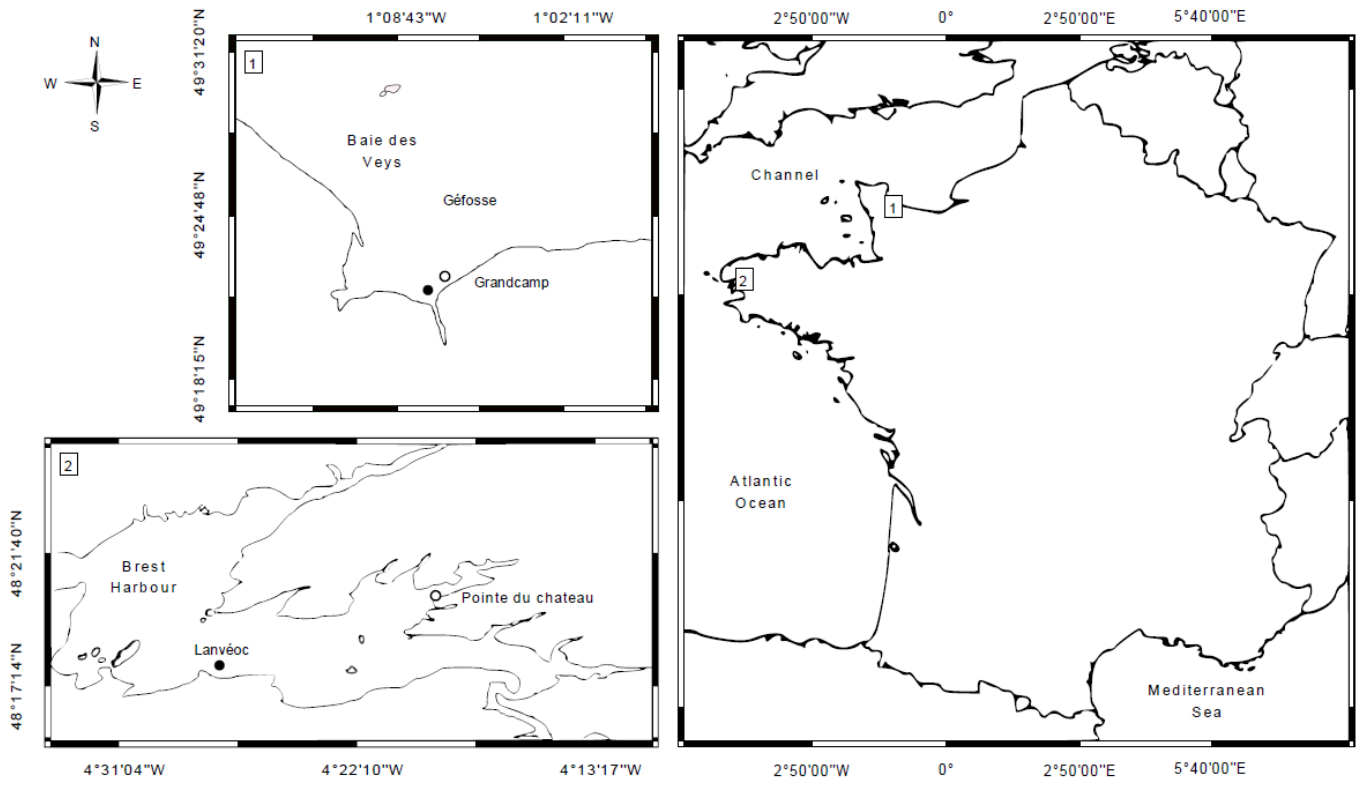


Fig. 2

740

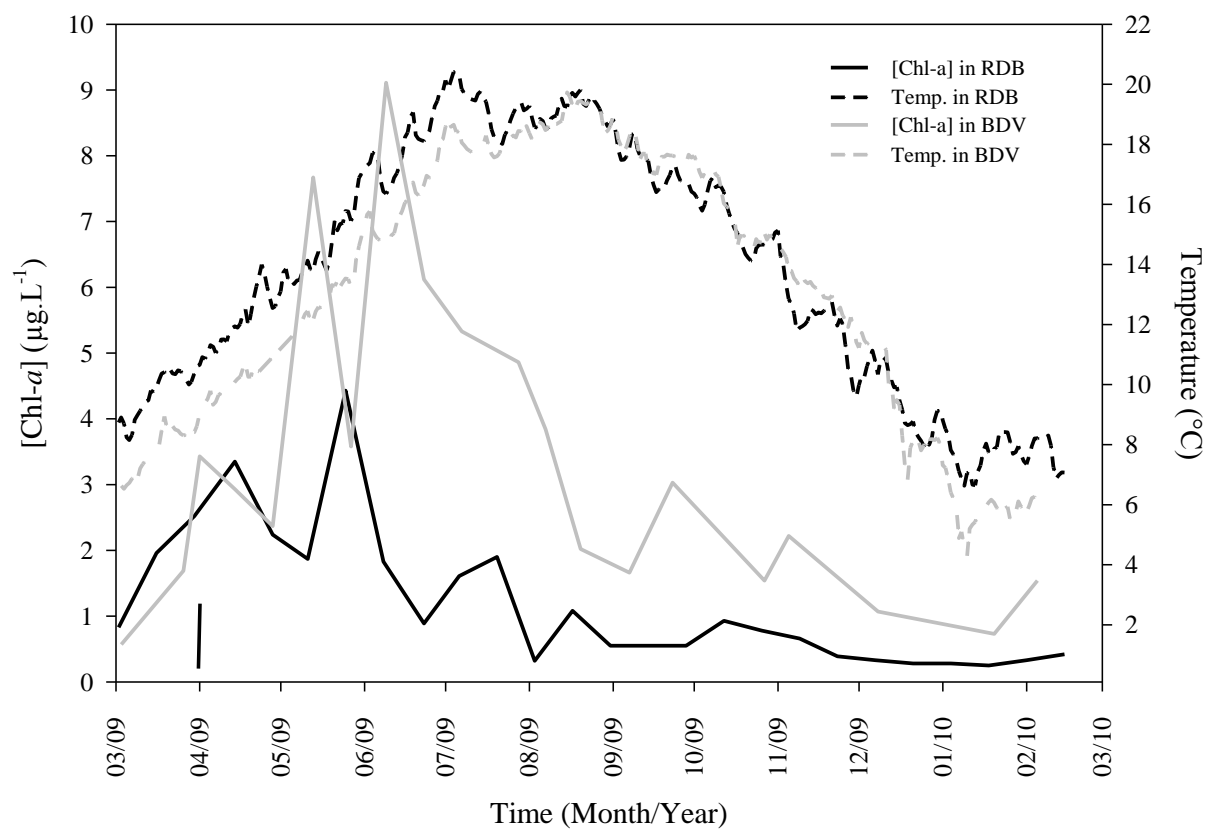


Fig. 3

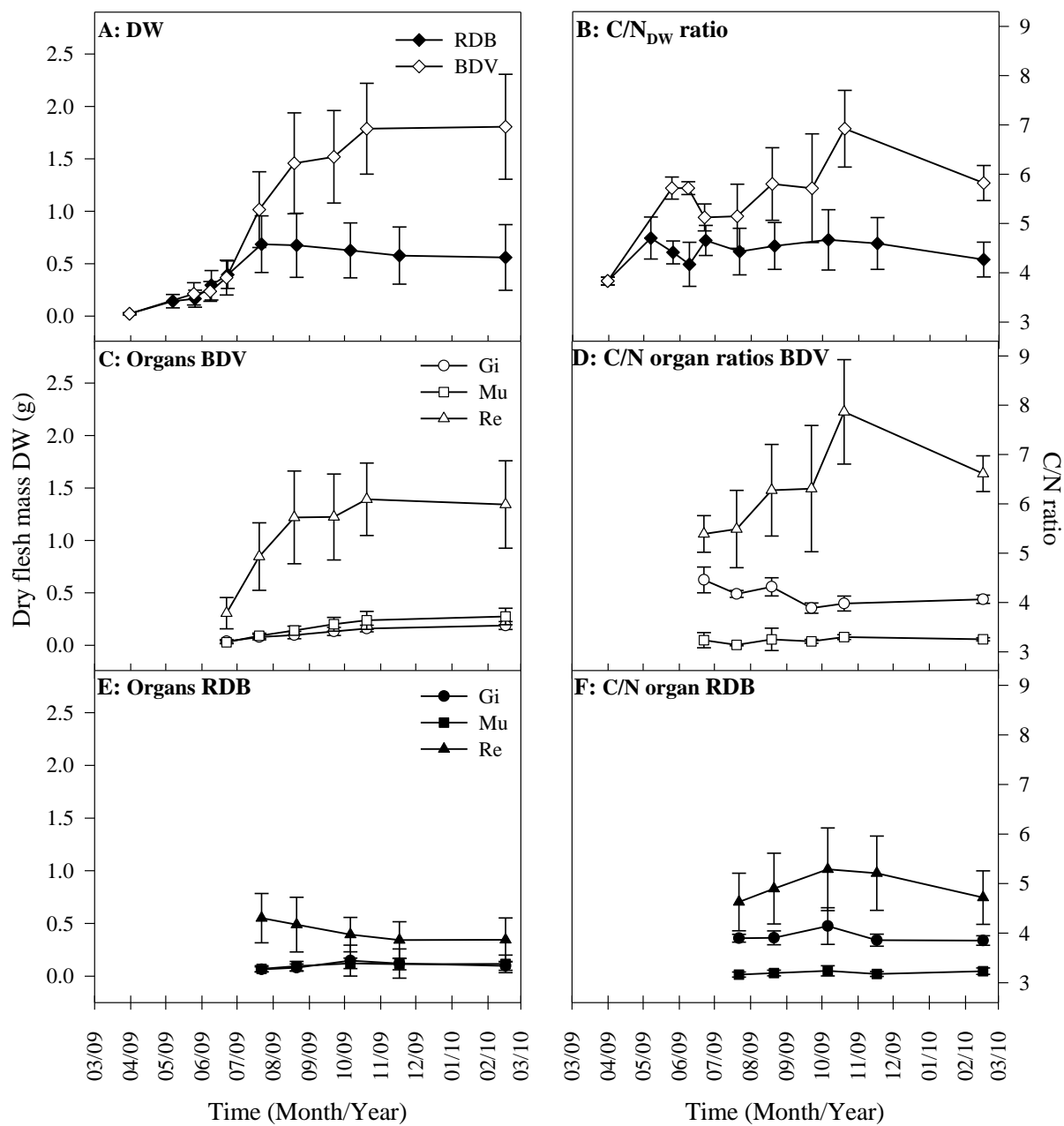


Fig. 4

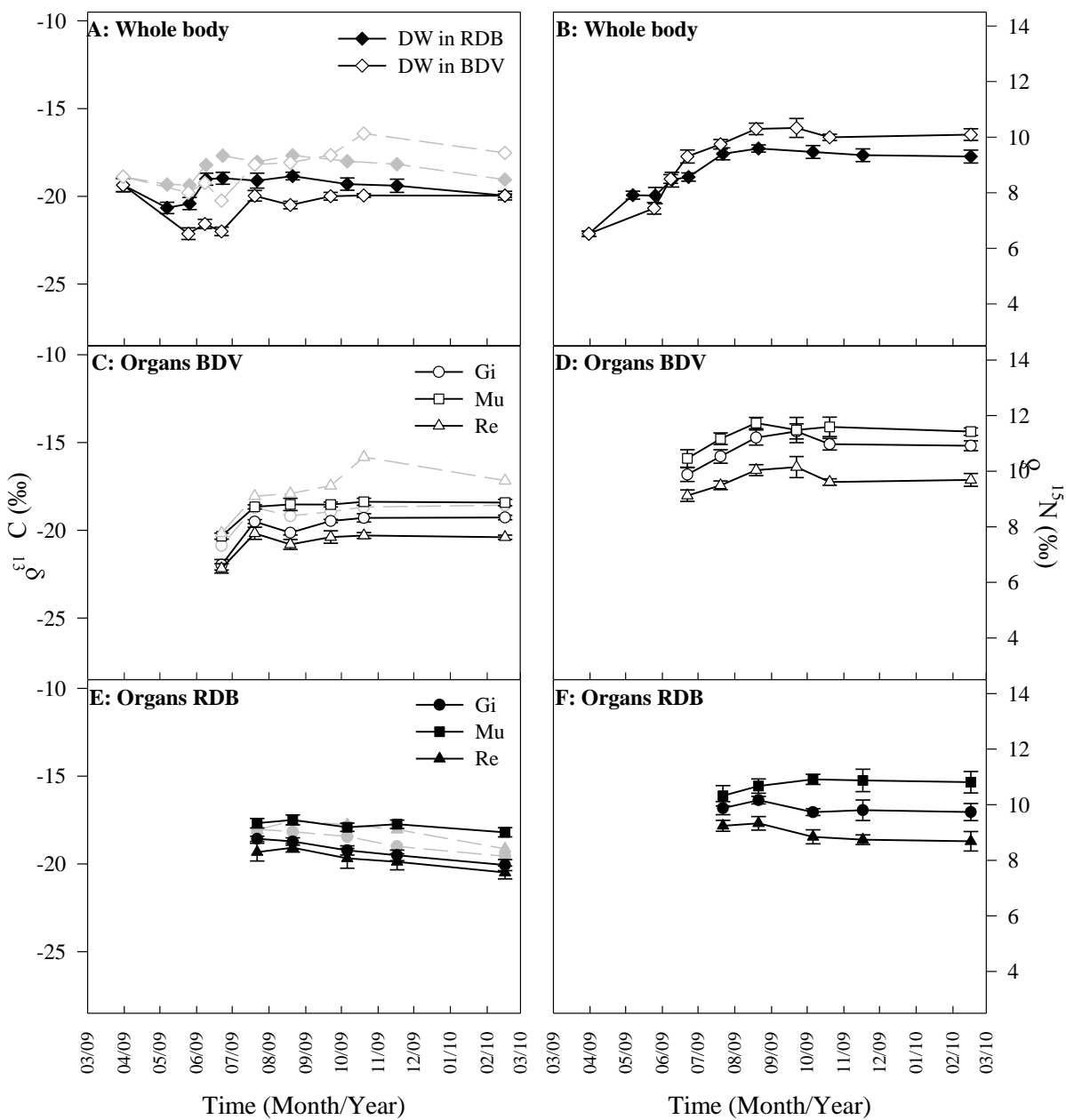


Fig. 5

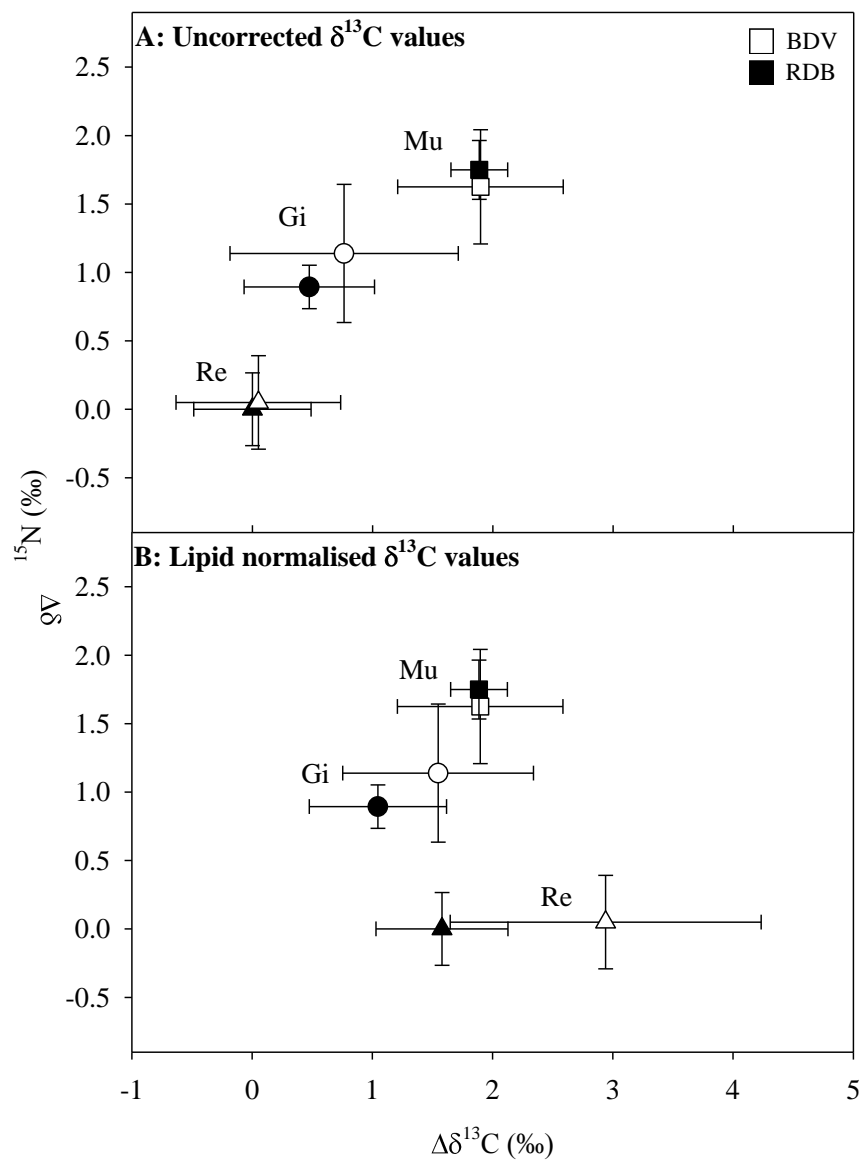
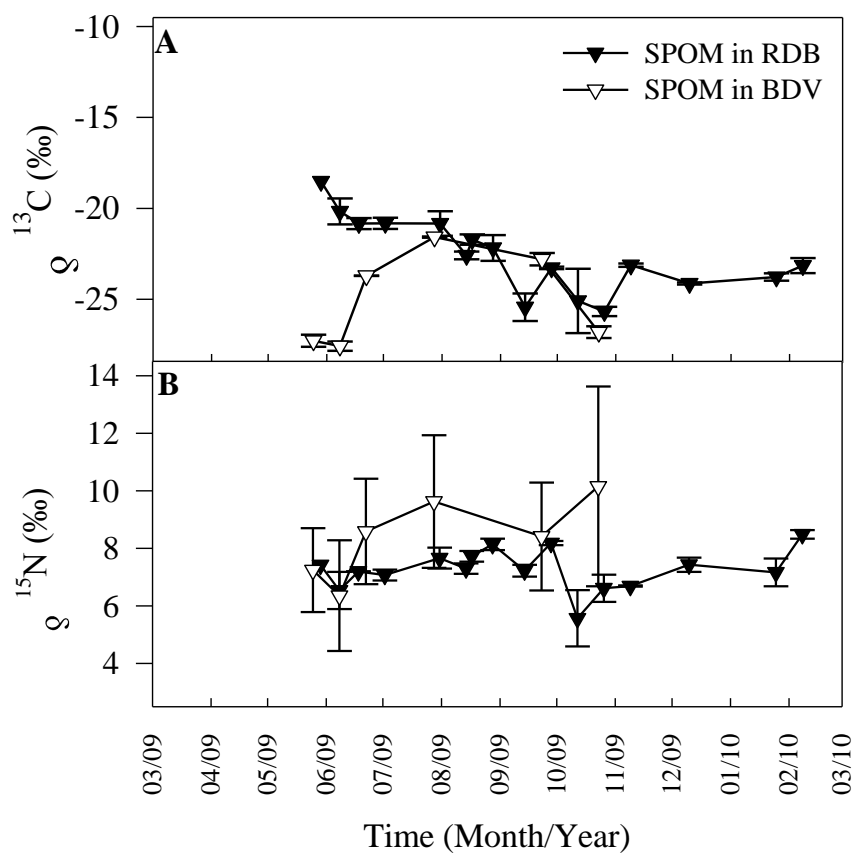


Fig. 6



760

Fig 7

