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Effect of seasonal variation in trophic conditions and the gametogenic cycle on $\delta^{13}C$ and $\delta^{15}N$ levels of diploid and triploid Pacific oysters *Crassostrea gigas*

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Abstract:

Carbon and nitrogen stable isotopes were investigated in separate organs of diploid and sterile triploid Pacific oysters *Crassostrea gigas* for 13 mo, together with changes in chemical and isotope composition of suspended matter sampled from an intertidal mudflat within Marennes-Oléron Bay, France. Particulate organic matter (POM) was a mixture of pelagic and benthic material with a predominance of neritic phytoplankton in spring, and resuspended microphytobenthos in summer and autumn. A remarkable shift of +3‰ in $\delta^{13}C$ was reflected in both diploids and triploids from spring to summer, and further temporal differences were observed amongst their tissues. Seasonal changes in POM $\delta^{15}N$ were also reflected in oyster tissues, with digestive gland and muscle tissues showing the largest and the least variability, respectively. Use of $\delta^{13}C$ and C:N ratio relationships in separate tissues allowed for an assessment of the influences of trophic condition, seasonal changes, and gametogenic cycle on tissue $\delta^{13}C$. Diploid digestive gland $\delta^{13}C$ matched those of gonads, and differences between diploids and triploids in digestive gland and mantle $\delta^{13}C$ were less than –1‰ during gametogenesis. The reproductive and rest periods were easily distinguished in these tissues and were characterised by enriched $\delta^{13}C$ values in summer–autumn compared with spring, which is consistent with POM $\delta^{13}C$ seasonal changes. A similar trend was observed in muscle, with a preferential incorporation of ^{13}C-enriched carbon during the summer–autumn growing season. However, despite the similar roles of mantle and digestive gland in lipid synthesis in both diploids and triploids, the correlation of $\delta^{13}C$ with the C:N ratio highlighted the transfer of lipids to gonads in diploids and their differential allocation to growing tissues in sterile triploids.

Keywords: *Crassostrea gigas* · Stable isotopes · Reproduction · Ploidy · Lipids

INTRODUCTION

For decades, stable isotope ratios have been recognised as efficient tools for the identification of dietary sources incorporated by consumers (Fry & Sherr 1984), and have been consequently used as natural tracers of organic matter flow in aquatic food webs (Michener & Schell 1994). Routine reconstruction of diets from stable isotope ratios in whole animal body is commonly summarised by the maxim “you are what you eat” or more properly "you are what you assimilate plus a few per mil" (DeNiro & Epstein 1978, 1981, Fry & Sherr 1984). Indeed, stable isotopes in consumers provide time-integrated information, averaging the natural environment variability in dietary components. They also give us a clue on how animal tissues turn over in relation to growth and/or metabolic replacement (Tieszen et al. 1983). Comparative analysis of fast vs. slow turn over tissues (e.g. digestive gland vs. muscle) may reveal short- and long-term changes in food source composition, respectively (Tieszen et al. 1983, Fry & Sherr 1984). The $\delta^{13}C$ values of consumers reflect the $\delta^{13}C$ values of their diet with small changes (< 1 ‰), whereas $\delta^{15}N$ values show a larger discrimination of 3-4 ‰ per trophic level (DeNiro & Epstein 1978, 1981, Vander Zanden & Rasmussen 2001). However, animal-diet differences (δ_{tissue} - δ_{diet}) may vary among species (DeNiro & Epstein 1978, 1981, Peterson & Fry 1987), and within species among ontogenic stages and sizes (Gearing et al. 1984), physiological states (Hobson et al. 1993), body tissues (Tieszen et al. 1983, Piola et al. 2006) and biochemical compounds. For instance, in a given animal body, lipids are generally more depleted in ^{13}C than carbohydrates and proteins (DeNiro & Epstein 1978), the last being incorporated without significant isotopic changes from dietary protein. These variations reflecting biological processes and physiology-driven kinetics has not yet been widely determined (see e.g. Hobson et al. 1993) and, as argued by Gannes et al. (1997), still requires experimental investigation.

In estuarine and coastal ecosystems, benthic bivalve molluscs have been successfully used to trace mixing processes between terrestrial, marine and autochthonous organic materials for both spatial (Incze et al. 1982, Stephenson & Lyon 1982, Peterson et al. 1985, Riera & Richard 1996, Machás & Santos 1999) and temporal scales (Riera & Richard 1997, Piola et al. 2006). These organisms may reflect the degree of benthic-pelagic coupling in shallow waters through the utilisation of food sources of different origin (Incze et al. 1982, Gearing et al. 1984, Peterson et al. 1985, Sauriau & Kang 2000). So far, metabolic processes that modify the diet derived isotopic composition of bivalves have not been investigated in detail. Different bivalve tissues trophic enrichments have already been reported (Stephenson & Lyon (1982), Machás & Santos (1999) and Piola et al. (2006). Similarly, in view of the respective roles of different tissues in energy allocation between maintenance, growth and the reproductive cycle in bivalves (e.g. Gabbott 1983), Lorrain et al. (2002) suggested that seasonal changes in the magnitude of metabolic transfers between germinal and somatic tissues in the scallop *Pecten maximus* have significant consequences for their $\delta^{13}C$ and $\delta^{15}N$ compositions, irrespective of diet resources. The relative contributions of growth and metabolic replacement to isotopic turnover was also investigated by Dattagupta et al. (2004) using transplanted methanotrophic mussels *Bathymodiolus childressi* between different hydrocarbon seep sites.

To investigate these issues in a bivalve species representative of estuarine areas, temporal changes in the $\delta^{13}C$ and $\delta^{15}N$ compositions of separate tissues of the Pacific oyster *Crassostrea gigas* (Thunberg) were compared to changes in the isotopic composition of its food originating from a tidal mudflat of the Marennes-Oléron Bay. Previous contributions to the comparative analysis of stable isotope ratios in food resources (Galois et al. 1996, Richard et al. 1997) and *C. gigas* in this bay (Riera & Richard 1996, 1997) have revealed significant seasonal trends and spatial heterogeneity in trophic conditions. Riera & Richard (1996) suggested that *C. gigas* collected on bare mudflats at the mouth of the Charente estuary (north of Marennes-Oléron Bay) are mainly fuelled by benthic diatoms. However, depending on their geographic location within its salinity gradient, oysters may reflect short-term incorporation of continental materials, as revealed by the more negative $\delta^{13}C$ values of their whole body following periods of high river discharge (Riera & Richard 1997). The aim of this study was to investigate the isotopic composition of Pacific oysters *C. gigas* experimentally reared on sandy mudflats located in the southern part of the Marennes-Oléron Bay, far from direct estuarine influence, and then to test the hypothesis that reared oysters from this culture site reflect the isotopic signature of the mudflats based food web irrespective of estuarine influence.

The annual reproductive cycle in bivalve molluscs is closely linked to other metabolic functions involved in the energy storage-utilisation cycle, with an important biochemical pathway between carbohydrates and lipids (see the review of Gabbott 1983). This cycle differs between species and depends on the type of tissues and cells involved in the storage and mobilization of energy (Mathieu & Lubet 1993). It is recognised that gonad development and accumulation of energy reserves in *Crassostrea gigas* may overlap temporally during spring and summer periods (Deslous-Paoli & Héral 1988, Kang et al. 2000, Matus de la Parra et al. 2005). The second hypothesis for our study was that biochemical modifications linked to gamete build-up would modify the isotopic signals in oyster tissues involved in energy transfers to the gonads. Thus, we analysed all oyster tissues separately i.e. mantle, gills, gonads and particular attention was paid to muscle with long-turnover times and digestive gland with short-turnover times to track temporal changes in diet assimilation (Tieszen et al. 1983, Fry & Sherr 1984). Moreover, to the best of our knowledge, this is the first time that *in situ* experiments simultaneously involve diploid and triploid oysters in stable isotope studies. In the latter, reproductive potential is reduced due to disruption of meiosis, whereas growth of somatic organs is enhanced (Beaumont & Fairbrother 1991, Garnier-Géré et al. 2002). Consequently, triploids as sterile animals are expected to provide a useful tool to unmask metabolic processes linked to reproduction.

MATERIALS AND METHODS

Study area. Our study was deployed at Ronce-les-Bains, an intertidal oyster culture area (175 ha) located in the southern part of the Marennes-Oléron Bay (French Atlantic coast north of the Gironde estuary) (Fig. 1). Details of hydrobiology features, shellfish activities, sedimentary conditions and benthic ecology of the study site have been previously given by Soletchnik et al. (1998), Goulletquer & Héral (1997), Kang et al. (1999) and Sauriau & Kang (2000), respectively. *Crassostrea gigas* were cultured off-bottom using iron frames on which oyster bags were fastened (Fig. 1, see picture).

Figure 1

Sample collection. Adult diploid and triploid oysters originated from a commercial oyster farm at La Tremblade. Triploids were produced at Ifremer La Tremblade by mating tetraploids and diploids (Guo et al. (1996). Both diploid and triploid oysters were over-wintered in salt marsh-based earth ponds for conditioning, ca. 7 months for diploids and 1 month for triploids such as traditional rearing practice within the Marennes-Oléron Bay.

At the start of the experiment triploid oysters (58.8 ± 7.5 mm, n = 15) were significantly longer than diploids (40.7 ± 5.9 mm, n = 15) (one-tailed Student's t-test, $p < 0.001$); however, total dry weights were similar at 0.15 ± 0.05 g and 0.13 ± 0.04 g (n=15, two-tailed Student's t-test, $p = 0.17$), respectively. Every month from March 2002 to April 2003, samples of 35 diploid and triploid oysters were collected. During the summer reproductive period from May to August 2002 sampling intervals were shortened (see Table 1). Oysters were kept overnight in filtered seawater to remove gut contents. From each sample, 5 oysters were frozen and stored at -20°C for later dissection into mantle, gills, digestive gland, muscle and gonads, and subsequent isotope analyses. Labial palps were not separated from mantle tissues. To minimise seepage of tissue fluids, particularly the gonads, dissection was performed on frozen oysters. The remaining 30 oysters were split into three groups, which were frozen, freeze dried, and analysed separately for lipid content according to the procedure by Deslous-Paoli & Héral (1988).

Hydrobiological parameters. Water samples were collected twice a month from March 2002 to May 2003 at Ronce-les-Bains within the first two hours of the flood tide. About 5 L of water was collected and pre-filtered with a 63 µm screen to remove any zooplankton or algae debris. Total particulate matter (TPM) was determined after filtration through precombusted and preweighed Whatman GF/C filters and dried for 24 h at 60° and then particulate inorganic matter (PIM) after filters had been combusted for 4 h at 450°. Chlorophyll *a* was extracted from GF/F Whatman filter according to the method of Holm-Hansen & Riemann (1978 cited in Richard et al. 1997) and its concentration was determined using a Turner fluorometer at 665 nm. All hydrobiological parameters were determined in triplicate. A last sample of water was filtered on a single precombusted Whatman GF/C filter and frozen for subsequent C and N stable isotope analyses.

Stable isotope analysis. Five frozen oysters were carefully dissected to separate the adductor muscle, digestive gland and gonads from gills and mantle. To prevent contamination of tissues by shell fragments, oyster tissues, except the gonads were quickly rinsed in 10 % v:v HCl, and briefly rinsed twice in de-ionised water. To confirm that the acidification did not modify the isotopic values, supplement analysis were performed on subdivided organ of 4 oysters either acidified with HCl and then rinsed twice in de-ionised water, or just rinsed twice in de-ionised water. The paired t-test did not show any difference between the mean of both treatment ($n = 16$, for carbon $p = 0.54$, for nitrogen $p = 0.71$). Digestive gland, mantle, gills and gonads were freeze-dried, ground thoroughly to a fine powder whereas muscle tissues were cut into fine pieces with a scalpel. Water sample filters were acidified with 2N HCl acid vapour in a glass desiccator 4h at room temperature to remove carbonates and kept frozen at -20°C until analysed. Particulate organic matter was scraped from the fibreglass filters.

Carbon and nitrogen isotope ratios of the oyster tissues (n=3), and particulate organic matter samples were measured by CF-IRMS analysis using an IsoPrime stable isotope mass spectrometer (Micromass, Manchester, UK) interfaced to an elemental analyser EuroEA3024-IRMS (Eurovector, Milan, Italy). The analytical precision for 10 consecutive measurements was < 0.15 ‰ for both N and C isotope ratios. Data were expressed in the standard δ notation as parts per thousand (‰) relative to the Peedee Belemnite Limestone (PDB) and atmospheric N_2 for carbon and nitrogen, respectively. The stable isotopic ratio is reported as $\delta^{A}X = ((R_{sample} / R_{standard}) - 1) * 10^3$ (‰), where A is the atomic mass of the heavy stable isotope of the element X, and $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ for nitrogen, respectively.

Statistical analyses. Basic statistics and analyses of variance were performed using the Minitab Release 10.2 package. Homoscedasticity of data was tested prior to analysis of variance and non-parametric tests used in case of rejection (Sokal & Rohlf 1981). Stable isotope ratio time-series in oyster tissues were tested using a two-way ANOVA with replication, and with ploidy type (diploid vs. triploid) and sampling date as fixed factors. The non-parametric test for association using Kendall's coefficient of rank correlation (τ) was also used in case of non-linear relationship between two variables and/or data known not to be normally distributed (Sokal & Rohlf 1981, p. 601), as is the case for tidal range. Regressions Model II were performed and a non-linear algorithm (SigmaPlot 1.02 curve fitter based on the Marquardt-Levenberg algorithm) in order to estimate the standard deviations of all regression parameters. Biometrics (shell length, tissue dry weight) were presented as mean ± SD. Significant differences in biometrics, stable isotope ratios between oysters and/or tissues were tested using one-tailed or two-tailed tests at a significance level of 0.05.

RESULTS

Figures 2 and 3

Environmental conditions and quality of available food sources

Temperature and salinity presented similar seasonal cycles at Ronce-les-Bains with maximum values from spring to early autumn and minimum values in winter (Fig. 2a). The temperature of the flooding tide reached 25.9°C in summer and 5.0°C in winter with a slow decrease from August 2002 to January 2003. Higher salinity values higher of 32 were observed from April to October 2002 and lower salinity values of 22 in March of both years (Fig. 2a).

Total particulate matter (TPM) concentrations were always higher than 100 mg l^{-1} (Fig. 2b) and reached extremely high values of 560 and 721 mg l^{-1} in May 2002 and January 2003, respectively. TPM concentrations between 200 and 400 mg l^{-1} were more frequently recorded in winter and spring than in other seasons. A significant Kendal's rank correlation was found between tidal range and both TPM and particulate inorganic matter (PIM) concentrations (τ = 0.161, p = 0.030, n = 84 and τ = 0.183, p = 0.014, n = 84 for TPM and PIM, respectively). This suggests a significant proportion of high and low TPM and PIM values were recorded during spring and neap tide periods, respectively. The particulate organic matter fraction (POM to TPM ratio) averaged 16 ± 4 %, with the lowest values in spring 2002 (Fig. 2b).

Chlorophyll *a* concentrations presented no clear seasonal trend but showed maximal values (> 15 µg l^{-1}) in spring, summer and winter, and minimal values (< 5 µg l^{-1}) in autumn and early winter (Fig. 2c). Pheopigment concentrations did not followed the same pattern of variation although several maximal values of Chl *a* and pheopigments matched (Fig. 2c). PIM and pheopigments were highly significantly correlated (r = 0.67, p < 0.001, n = 42) and a significant Kendal's rank correlation was found between tidal range and pheopigment concentrations (τ = 0.176, p = 0.020, n = 81). POM and Chl *a* did not show significant correlation for the whole set of dates sampled in 2002 and 2003 but high significant correlation during spring and early summer 2002 (r = 0.87, p < 0.001, n = 10).

Combining $\delta^{13}C$, the C:N ratio of POM and(POC:Chl *a* ratio, revealed three distinct periods between which oyster food quality differed (Fig. 3):

1) Early spring i.e. March to April 2002 was characterised by low POM concentrations closely associated with low Chl *a* concentrations, high pheopigments and PIM concentrations and with POC:Chl *a* values < 100 (Fig. 3b). Such values indicated that much of the particulate organic carbon derived from living algal sources. Accordingly, high C:N ratios (10.4 ± 0.4) associated with isotopic carbon depleted values of -23.2 ± 0.5 ‰ (Fig. 3a) reflect the contribution of early spring phytoplankton blooms to bulk POM.

2) Spring, summer and autumn i.e. May to November 2002 was defined by large pigment variability with several peaks in May, July and September. High chloropigment and PIM concentrations together with enriched $\delta^{13}C$ values of -20.9 ± 0.4 ‰ (Fig. 3a) indicated that benthic organic matter episodically contributed to the bay organic pool. Values of POC:Chl *a* fluctuated between 26 to 254 in relation to the relative abundance of phytoplankton vs. by resuspended benthic algae.

3) Early winter to spring i.e. December 2002 to April 2003 was characterised by high TPM concentration but with Chl *a* unrelated to pheopigment concentrations (Fig. 2c). Mean $\delta^{13}C$

values (-21.8 ± 0.4 ‰) were associated with C:N ratios ranging from 6.5 to 8.1 (Figs. 3a, b). These low values reflected estuarine organic matter with lower contributions by benthic sources than in summer and early autumn. Moreover, very high POC:Chl *a* ratios suggest that detrital materials were always a major constituent of POM (Fig. 3b) particularly in December 2002 and January 2003.

Table 1 and Figure 4

Comparative changes in oyster biometrics and proximate lipid contents

Oyster total tissue dry weight increased by approximately 10 fold between the start and the end of the experiment i.e. 0.13 ± 0.04 g to 1.33 ± 0.22 g for diploids, and 0.15 ± 0.05 g to 1.75 ± 0.24 g for triploids (Table 1). Oyster growth occurred in spring and early summer for diploids (Fig. 4a), and until early autumn for triploid (Fig. 4b). The initial difference in shell size between diploids and triploids became insignificant after the two months of cultivation because diploids exhibited compensatory shell growth (Table 1). For both diploid and triploid oysters, the digestive gland and the gills accounted for 30 to 40 % of total tissue dry weight and muscle and mantle only for 10 to 20 %. Major month-to-month changes in tissue dry weight occurred in the digestive gland and the gonads for diploids (Fig. 4a) and the digestive gland and the gills for triploids (Fig. 4b).

Abrupt % gonad changes occurred in June and one mass-spawning event occurred at the end of July (Fig. 4a, Table 1). During these respective periods, gonads represented between 27 to 54 % of the total tissue dry weight in diploid oysters before gamete release (Table 1). As a consequence of biochemical replacement during reproduction, the lipid content of diploid oysters varied from 9.7 and 15.8 % of total tissue dry weight during the spawning season (Table 1). In contrast, variation in the lipid content of triploid oysters was less abrupt than in the diploids, ranging from 9.4 to 12.2 %. From June to mid-August 2002 and outside periods of gamete release (mid-June and August), all lipid contents for diploid oysters were significantly higher than those of triploid oysters (t test, $p < 0.001$ $n = 6$, for 7 out of 9 sampling dates between 5th June and 21st August, Table 1).

Figure 5

Comparative changes in $\delta^{13}C$ in diploid and triploid oyster tissues

Before the start of the experiment at Ronce-les-Bains in March 2002, oysters were grown in intertidal areas for one year and then stored in oyster ponds during the autumn and winter. Their stable-isotope signatures were modified depending on the time spent in oyster ponds. Diploid oysters stored 7 months in oyster ponds reached a $\delta^{13}C$ value of -23.7 ± 0.3 ‰ for digestive gland, -23.0 ± 0.4 ‰ for mantle and -22.6 ± 0.3 ‰ for muscle (Fig. 5a). However, $\delta^{13}C$ values of triploid oysters, which spent only 1 month in oyster ponds, remained more enriched i.e. -21.7 ± 0.3 ‰, -19.8 ± 0.2 ‰ and -18.1 ± 0.4 ‰ for digestive gland, mantle and muscle, respectively (Fig. 5b). $\delta^{13}C$ values of gills, for clarity not shown, were intermediate between those of muscle and mantle tissues whatever the ploidy group. At the end of the experiment in April 2003, there were no significant differences in $\delta^{13}C$ values for either digestive gland or mantle between diploid and triploid oysters. However, the muscle tissues of triploids remained significantly more enriched in ^{13}C by ca > 1 ‰ than those of diploids most of the time (Figs. 5a, b).

Over one year of presence in the intertidal area, both diploid and triploid oysters showed significant seasonal changes in digestive gland, mantle and muscle isotopic composition (two-way ANOVA, $p < 0.001$). Although a rather similar pattern was observed in $\delta^{13}C$ month-to-month changes in their digestive glands (Figs. 5a, b), highly significant differences occurred within the first 3 months and in autumn and early winter ($p < 0.001$ for the interaction between ploidy type and date). There was also a clear discrepancy between the two time series in muscle $\delta^{13}C$ values of diploid and triploid oysters for the first 5 months and autumn to early winter period (two-way ANOVA, $p < 0.001$ for date, ploidy type and interaction).

Gill and mantle $\delta^{13}C$ values were nearly always intermediate between digestive gland and muscle $\delta^{13}C$ for both diploids and triploids, and significant differences between diploids and triploids were showed within the first 4 months and in early winter 2003 (Figs. 5a, b).

Figure 6

Comparative changes in $\delta^{15}N$ in diploid and triploid oyster tissues

$\delta^{15}N$ values between diploid and triploid whatever the tissues at the start of the experiment did not show significant difference (Fig. 6). Mean values of $\delta^{15}N$ ranged from 7.6 to 8.7 ‰ for diploid and triploid oysters with similar digestive glands values in March 2002 (Fig. 6).

Diploid *versus* triploid oyster $\delta^{15}N$ mean values in digestive gland did not show significant difference (two-way ANOVA, $p = 0.375$ for ploidy type). However, month-to-month changes in digestive gland $\delta^{15}N$ values were highly significant ($p < 0.001$) and strong first order interaction was indicative of varying overlap between the two time series ($p < 0.001$, Fig. 6). Similar results were obtained when $\delta^{15}N$ time series for mantle and gills (not shown for clarity) were compared, time series for the gills being intermediate between those of muscle and mantle tissues. Seasonal changes in muscle $\delta^{15}N$ were also highly significant (two-way ANOVA, $p < 0.001$) but since the first order interaction was not significant ($p = 0.246$), the highly significant effect of ploidy type ($p < 0.001$) suggested diploid and triploid oysters have different muscle $\delta^{15}N$ values. One-tailed Student's t test thus showed that muscle $\delta^{15}N$ mean values in triploid oysters were significantly higher than those of diploid oysters ($p < 0.004$) with a difference of +0.2 ‰ from April to July 2002 and +0.4 ‰ from August to April 2003.

Diploid vs. triploid oyster $\delta^{13}C$ and $\delta^{15}N$ relationships **Figure 7**

The relationships between digestive gland $\delta^{13}C$ in diploid and triploid oysters for the period May 2002 to April 2003 (excluding the acclimation period of March to April 2002) showed two groups of data in relation to the reproductive season (Fig. 7a). From the end of

May to August 2002 i.e. during the reproductive period, diploid oyster digestive glands were significantly more depleted in ^{13}C than triploid oysters (one-tailed Student's t test, $p < 0.001$). In the opposite, during the resting period, from mid-August 2002 to April 2003, diploid oyster digestive glands were significantly more enriched in ^{13}C than triploid oysters (one-tailed Student's t test, $p < 0.017$). Moreover, coupled changes in ^{13}C values of diploids and triploids occurred during the rest period because of the significant correlation between $\delta^{13}C_{triploids}$ and $\delta^{13}C_{diploids}$ ($r^2 = 0.49$, $n = 27$, $P < 0.001$) with the Y-intercept and the slope not significantly different from 0 and 1 (1.01 ± 0.01), respectively.

Due to the large differences in initial values of $\delta^{13}C$ of muscle between diploid and triploid oysters (Figs. 5a, b) and since muscle is a slow-turnover tissue, changes in $\delta^{13}C$ in muscle in diploid and triploid oysters were not comparable. However, even after 7 months on the same intertidal mudflats, diploid oysters muscle was always more depleted in ^{13}C than triploid oysters. Mantle tissues exhibited an intermediate situation between digestive gland and muscle tissues (scatter plot not shown) with significant different $\delta^{13}C$ for the first 4 months and then overlapped (Fig. 5).

The scatter plot of the relationship between digestive gland $\delta^{15}N$ in diploid and triploid oysters did not show any significant differences related to their reproductive activity (Fig. 7b). The linear regression for the whole data set was highly significant ($\delta^{15}N_{triploids} = 1.01 \pm 0.01\ \delta^{15}N_{diploids}$, $r^2 = 0.69$, $n = 57$, $p < 0.001$) and not significantly different from a 1:1 linear relationship.

$\delta^{13}C$ vs. C:N ratio in diploid and triploid oyster tissues **Figure 8**

During the reproduction period, from April to August 2002, digestive gland $\delta^{13}C$ values and C:N ratios were significantly and negatively correlated for both diploid and triploid oysters ($\delta^{13}C_{diploids} = -0.29\ (\pm 0.14)\ C:N - 20.7\ (\pm 0.7)$, $r^2 = 0.14$, $n = 30$, $p < 0.05$; $\delta^{13}C_{triploids}$

= - 0.37 (± 0.07) C:N – 19.6 (± 0.4), r^2= 0.46, n = 30, p < 0.001) (Fig. 8a). Gonads in diploid oysters also exhibited a similar negative correlation ($\delta^{13}C_{diploids}$ = - 0.46 (± 0.08) C:N – 19.9 (± 0.4), r^2 = 0.61, n = 23, p < 0.001). Slopes of the 3 linear regressions did not significantly differ. During the rest period, from August 2002 to April 2003, no significant correlations were observed in either diploid or triploid oysters, but $\delta^{13}C$ values for digestive gland were significantly greater than during the reproductive period (Fig. 8a).

During the reproductive period, $\delta^{13}C$ values and C:N ratios were significantly and negatively correlated for both diploid and triploid mantle but only for triploids during the rest period: $\delta^{13}C_{diploids}$ = - 0.63 (± 0.20) C:N - 18.5 (± 0.9) (r^2 = 0.27, n = 30, p < 0.01), $\delta^{13}C_{triploids}$ = - 0.48 (± 0.10) C:N - 18.2 (± 0.6) (r^2 = 0.46, n = 30, p < 0.001) and $\delta^{13}C_{triploids}$ = - 0.60 (± 0.08) C:N - 16.4 (± 0.5) (r^2 = 0.72, n = 27, p < 0.001), respectively (Fig. 8b). Due to large confidence intervals, relationships in diploids and triploids were not significantly different during the reproductive period, but the relationship found in triploids differed from the two others during the rest period. The range of variation in mantle tissue C:N ratios was larger in triploids (4 to 8.5) than in diploids (4 to 6) (Fig. 8b).

Muscle $\delta^{13}C$ values in both diploid and triploid oysters exhibited little scatter in C:N ratios without significant correlation (Fig. 8b). Muscle C:N ratios averaged 3.30 ± 0.16 (n = 104). $\delta^{13}C$ values in both diploids and triploids significantly differed between the two periods with $\delta^{13}C_{rest\ period} > \delta^{13}C_{reproductive\ period}$. A significant positive correlation was found between C:N ratios and muscle $\delta^{13}C$ values in diploids during the summertime rest period ($\delta^{13}C_{diploids}$ = 3.61 (± 1.29) C:N – 31.3 (± 4.2), r = 0.48, n = 27, p < 0.01).

DISCUSSION

Seasonal changes in sources of particulate organic matter (POM)

POM samples were collected from a bare sandy mudflat, where both *Zostera noltii* meadows and green macroalgae are rare (Kang et al. 1999, Sauriau & Kang 2000), the latter being confined to the vicinity of oyster culture structures (Fig. 1). For the same site, Kang et al. (1999) reported similar $\delta^{13}C$ POM values (1995: -24 to -20 ‰). Previous hydrological analysis performed within the Marennes-Oléron Bay (Galois et al. 1996, Riera & Richard 1996, Richard et al. 1997) suggest that a mix of various particulate organic matter sources was available to suspension-feeders. At that tidal site, water-column mixing is likely to occur as follows: 1) current and wind-driven resuspension acting on sedimentary materials (Kang et al. 1999); 2) tidal exchanges through the nearest marine inlet i.e. Pertuis de Maumusson (Soletchnik et al. 1998) and 3) north-to-south residual advection of water bodies that are characterised by high inorganic loads (Zurburg et al. 1994) and influenced by Charente river and Gironde estuary discharges (Soletchnik et al. 1998).

A significant contribution by benthic microalgae to the water column was expected because of large and persistent over season microphytobenthos mudflat biomass in that part of the bay (Kang et al. 1999). $\delta^{13}C$ values of microphytobenthos have been reported to range from -15 to -17 ‰ in the Marennes-Oléron Bay (Riera & Richard 1996). It could be deduced from the temporal changes in $\delta^{13}C$ POM values (Fig. 3a) that resuspended microphytobenthos material contributed to suspended POM mainly from mid spring to early winter. It is consistent with the occurrence of significant relationships between tidal ranges, particulate inorganic materiel (PIM) and pheopigments (Fig. 2c), most of POC:Chl *a* ratios lower than 200 indicative of fresh algal material (Cifuentes et al. 1988), and C:N ratios ranging from 6 to 9 (Fig. 3b). Such values higher than 5.6 (the Redfield ratio for phytoplankton) are indicative of carbon rich organic fresh detritus, representative of large amount of chloropigments in the water column. Zurburg et al. (1994) also reported similar values of C:N ratios (4 to 15 by weight) for resuspended material coming from an adjacent tidal site within the bay of

Marennes-Oléron. However, the difference between the $\delta^{13}C$ of benthic microalgae (-15 to -17 ‰) and POM (within the range -22 to -20 ‰ from May to November, Fig. 3a) implies that microphytobenthos carbon is not the major component of the bulk particulate organic carbon (POC) but only one end-member.

Oceanic and/or neritic phytoplankton would be another end-member because phytoplankton blooms are dominant in the water column in spring and early summer (Soletchnik et al. 1998). A large body of literature, including discrete measurements made off Marennes-Oléron Bay (Fontugne & Jouanneau 1987, Richard et al. 1997) indicates that $\delta^{13}C$ values of marine phytoplankton vary between -22 and -18 ‰ in temperate seas (e.g. Goericke et al. 1994). Furthermore, true oceanic plankton species are very scarce in taxonomic records made within the Marennes-Oléron Bay (Ifremer-REPHY network, Ryckaert M., com. pers.). Similarly, the hydrodynamic and hydrological features of this bay create habitats favouring neritic and estuarine phytoplankton species (Soletchnik et al. 1998), as previously concluded from $\delta^{13}C$ and lipid biomarker analyses (Galois et al. 1996, Richard et al. 1997). These authors indicated that, within the Pertuis d'Antioche (Fig. 1), POM was characterised by aged and refractory terrestrial material (1990: $\delta^{13}C$ from -27 to -26 ‰ and C:N ratios > 20) during winter months with low Charente river discharge, and fresh estuarine phytoplankton (1991: $\delta^{13}C$ from -24 to -23 ‰, C:N ratios < 10 and POC:Chl *a* < 100) during blooms in spring (diatoms), summer and fall (flagellates). That estuarine phytoplankton is an end-member in early spring is consistently supported by ranges in $\delta^{13}C$ (-22.8 to -23.8 ‰), $\delta^{15}N$ (+5 to +8), C:N ratios (10-11) and POC:Chl *a* ratios < 100 (Fig. 3) recorded from March to April 2002 at Ronce-les-Bains. However, a contribution by sedimentary materials cannot be excluded. The water column was thus characterised at that time by estuarine salinity (22 to 32) and very high turbidity (> 100 up to 600 mg l^{-1}, Figs. 2a, b), which originated from local resuspension and/or advection of estuarine waters through the Pertuis de Maumusson (Fig. 1). In fact, sedimentary particulate organic matter from lower reaches of many estuaries matches these δ^{13}C values, e.g. Gironde (Fontugne & Jouanneau 1987), Tay (Thornton & McManus 1994) and Schelde estuaries (Middelburg & Nieuwenhuize 1998), as the result of a progressive dilution of riverine (^{13}C-depleted POC source) with marine organic matter (^{13}C-enriched POC source). Within the Bay of Biscay and off the Marennes-Oléron Bay, marine organic matter comprised neritic species with ^{13}C values similar to marine plankton (Fontugne & Jouanneau 1987, Riera & Richard 1996, 1997), and are likely to fuel the studied mudflats every flood tide from mid spring to early winter.

In winter 2003, freshwater discharges from both the Charente River and Gironde estuary were much higher than in 2002, with flood conditions from mid-November 2002 to mid-March 2003. Consequently, salinity < 25-30, δ^{13}C values around -22 ‰, C:N ratios < 9 and POC:Chl *a* ratios with transient values higher than 300 were indicative of degraded phytoplankton and resuspended sedimentary material contributions to the bulk of the estuarine organic matter.

Stable-isotope composition in *Crassostrea gigas* tissues

Initial vs. final stable-isotope ratios in oyster tissues

Before the start of the experiment, adult oysters originating from intertidal areas were transferred into oyster ponds or "claires" that are traditionally used for oyster refining in late autumn and winter prior to marketing (Goulletquer & Héral 1997). Oysters placed in such a shallow and rich environment during autumn and winter continue to grow, improve their body condition and biochemical compositions (Deslous-Paoli et al. 1982). They consequently show significant increases in carbohydrate content compared to oysters reared on tidal flats (Deslous-Paoli & Héral 1988). Transplanted oysters in this study presumably acquired new stable isotope signatures reflecting the incorporation of new dietary items due to both growth and metabolic tissue replacement (DeNiro & Epstein 1978, Dattagupta et al. 2004). $\delta^{13}C$ values of diploid oyster tissues that had spent 7 months in oyster ponds before being transplanted back to tidal areas in March 2002 was depleted (-22.2 to -24.1 ‰, Fig. 5). This suggests that either a significant quantity of C3-terrestrial organic matter or locally produced $\delta^{13}C$-depleted plankton was lowering the initial stable isotope composition of those oyster tissues, even though salt marsh-based oyster ponds are fuelled with Marennes-Oléron bay waters every spring tide. Seasonal changes in stable-isotope signatures of water-column POM in the oyster ponds clearly validated this hypothesis because $\delta^{13}C$ and $\delta^{15}N$ values ranged respectively from -29 to -22.5 ‰ and +4.5 to +12.5 ‰ over a one-year cycle (Malet, unpub. data). Similar values have been reported worldwide in shallow, semi-enclosed salt marches or nearshore systems free of C4-plants, where mixing of organic materials from different origins may occur (Fry & Sherr 1984, Peterson & Fry 1987, Michener & Schell 1994).

In contrast, due to practical supply difficulties, adult triploid oysters spent only 1 month in oyster ponds. Triploid tissues retained the isotopic composition they previously gained from their intertidal rearing location. Digestive gland in triploid oysters exhibited moderate changes in $\delta^{13}C$ compared to the more depleted values recorded for diploid oysters. Mantle tissues presumably have a lower turnover rate than digestive gland and their $\delta^{13}C$ values were intermediate between those of digestive gland and muscle. Moreover, muscle in triploid oysters kept an isotope carbon ratio close to -18 ‰. Recorded differences before the start of our experiment in $\delta^{13}C$ ratios between diploid and triploid muscle, mantle and digestive glands are consistent with this time-integrated approach. Muscle tissue is recognised as a long-term integrated of dietary sources due to its slower turnover relative to more metabolically active tissues such as digestive gland, liver and mantle (Tieszen et al. 1983).

Seasonal changes in the stable-isotope signature of oyster tissues

A large mid-summer seasonal shift of ca +3.0 ‰ occurred in $\delta^{13}C$ of digestive gland and mantle of triploid oysters and in all diploid oyster tissues, except gonads. This seasonal shift differs between ploidy groups and within groups, among their tissues. This may be due to 1) a major seasonal change in the availability and/or incorporation by oyster tissues of pelagic vs. benthic food resources, 2) a differential ingestion and/or assimilation of specific compounds relative to the bulk POM, or 3) indirect consequences of the spring to summer reproductive activity in diploid *Crassostrea gigas*.

The first hypothesis is based on the recognition that in marine environments, the signature in ^{13}C of benthic microalgae is more enriched that of phytoplankton (Fry & Sherr 1984, Peterson & Fry 1987). Since the $\delta^{13}C$ values of a consumer are closely related to that of its food (DeNiro & Epstein 1978), the assimilation of ^{13}C enriched food sources (i.e. resuspended benthic microalgae from intertidal mudflats) through summer and autumn could explain the enriched ^{13}C values recorded in oyster tissues for the two seasons (Fig. 5). The progressive incorporation of ^{13}C enriched food lead to more ^{13}C depleted oyster tissues over high growth rates periods of diploid and particularly triploid tissues in summer and autumn 2002 (Figs. 4 & 5). However, temporal changes in $\delta^{13}C$ values of oyster tissues did not closely match those of the POM pool in spring (Figs. 3 & 5), POM being more enriched in early May. This relatively short time lag (< 2 months) is nevertheless consistently linked to growth exhibited by somatic tissues i.e. muscle and mantle in both diploids and triploids, and digestive gland in triploids (Fig. 5). Specific-tissue differences in $\delta^{13}C$ time-series suggested the influence of metabolic functions other than growth during that period of intense reproductive activities. Longer time lags are also expected during non-growing seasons and values > 3 months can be deduced from the comparative analysis of POM and oyster-tissue $\delta^{13}C$ time-series in autumn and early winter, with respective decreases recorded in October 2002 and January 2003 (Figs. 3 & 5). Similar lags between POM and oyster-body $\delta^{13}C$ time-series were also reported by

Riera & Richard (1997) for oysters from the upper reaches of the Charente estuary following a high flood period in winter 1992.

Similarly, summer-time changes in $\delta^{13}C$ of muscle in both diploid and triploid oysters (Figs. 5) substantiated the diet change hypothesis. As in most bivalve species, muscle tissues in *Crassostrea gigas* are mainly constituted by protein, ca 60 to 85 % of dry weight (Berthelin et al. 2000), and contained much lower proportions of lipids and glycogen than other tissues (Whyte et al. 1990, Berthelin et al. 2000). As reported in this study, muscle tissues logically exhibited small C:N ratios in both ploidy groups. Consequently, most of their seasonal changes in $\delta^{13}C$ values should have paralleled those of dietary protein components. Since $\delta^{13}C$ in the muscle of diploids matched those of triploids (-18 to -19 ‰) at the onset of winter (December 2002-January 2003, Fig. 8), it could be concluded that they had been fuelled by similar ^{13}C-enriched dietary sources throughout the summer-autumn growing season. This conclusion is also in agreement with our results in tissue $\delta^{15}N$ time-series, suggesting similar trophic levels in both ploidy groups (Fig. 6). A systematic positive difference in muscle $\delta^{15}N$ was however found between triploids and diploids. This difference is approximately 10 times lower than the accepted average value of 3-4 ‰ in $\delta^{15}N$ representing the trophic discrimination at each trophic level (DeNiro & Epstein 1981, Vander Zanden & Rasmussen 2001). It is too small to discern any differences in the trophic regime between triploid and diploid oysters. Moreover, it is recognised that higher growth performances in triploid *C. gigas* (Beaumont & Fairbrother 1991) are consistently linked to genetic and physiological differences, such as heterozygosity and higher metabolic process efficiencies (Garnier-Géré et al. 2002 and references therein). This may lead to subtle changes in morphology, anabolism and/or biochemical features of adductor muscle in triploid *C. gigas*. This hypothesis would require further experimental investigations in *C. gigas* because differences found between ploidy groups in other bivalve families *Pectinidae* and *Veneridae* may not be applicable to *Ostreidae* (Beaumont & Fairbrother 1991).

The second interpretation is linked to the ingestion and/or assimilation of specific fractions and components among the ambient POM pool because of the ability of *Crassostrea gigas* to select ingest algae. (Cognie et al. 2003). Similarly, Bougrier et al. (1997) observed that *C. gigas* may preferentially filter and reject (as pseudofaeces) diatoms relative to flagellates depending on their shape and flexibility. As in most coastal systems within the Bay of Biscay (Gailhard et al. 2002), in Marennes-Oléron Bay too, flagellates dominate phytoplankton blooms only in the fall (Galois et al. 1996 and references therein), partly due to lower turbulence conditions (Gailhard et al. 2002). Flagellates are also known to be more depleted in ^{13}C than pelagic diatoms (Cifuentes et al. 1988) and Gearing et al. (1984) showed that microflagellates may be 2 ‰ more negative than the diatom *Skeletonema costatum*, which is also a common neritic phytoplankton species in spring and autumn in coastal areas of the Bay of Biscay (Gailhard et al. 2002). However, time series in $\delta^{13}C$ oyster tissues (Fig. 5) did not exhibit any potential influences of ^{13}C-depleted phytoplankton species in the food regime of oysters in fall. The relative proportions of phytoplankton vs. microphytobenthos species and ratios of inorganic vs. organic materials should considerably influence the food regime of opportunistic suspension-feeders such as *C. gigas* in the Marennes-Oléron Bay Bougrier et al (1997) and Riera & Richard (1997) (Change under endnot).

The third interpretation is connected with the time course of gametogenesis in *Crassostrea gigas* and associated biochemical changes. Gonad development is an energy-demanding process that mobilises nutrients from assimilated food and utilises reserves previously stored in somatic tissues. Although the relative balance and time course of processes between these two pools of energy to sustain gametogenic demands are family or species-specific, glycogen is regarded as the major source of energy in marine bivalves and used for lipid synthesis (see Gabbott 1983 for a review). Increasing lipid contents with gonad build-up and ripe gamete production in *C. gigas* could explain the ^{13}C depletion in oyster tissues involved in reproduction because lipids are much more ^{13}C-depleted than proteins and carbohydrates. This is ascribed to lipid synthesis discriminating against ^{13}C in favour of the lighter isotope ^{12}C (DeNiro & Epstein 1977). In Marennes-Oléron Bay, reproductive activities in *C. gigas* usually occur from May to mid-August with maximum lipid contents recorded just before the mid-summer mass spawning event (Deslous-Paoli & Héral 1988, Matus de la Parra et al. 2005). Our data agree with this pattern, which could not be generalised because timing and duration of gametogenesis are different in other *C. gigas* populations (Ruiz et al. 1992, Pazos et al. 1996, Kang et al. 2000, Li et al. 2000). As is usually found in animals, lipid classes differ in their metabolic roles, neutral lipids (triacylglycerols) being used as energy reserves and polar lipids (phospholipids) being structural components of cells and membranes. Pazos et al. (1996), Li et al. (2000), and Matus de la Parra (2005) have all indicated that fluctuations in lipid content in whole oyster bodies and/or separate organs are largely due to changes in triacylglycerols over the reproductive period. In *C. gigas* females, the triglyceride content of the ovaries faithfully reflects the course of sexual maturation showing the highest levels when oocytes have grown sufficiently and are ready to be spawned (Li et al. 2000). Our stable isotope and proximate lipid analysis results are in accordance with these biochemical findings. Time series in digestive gland $\delta^{13}C$ in diploids differed from those of triploids until early August (Figs. 5) when mass spawning occurred in diploid oysters (Fig. 4). After that event, mid-summer to early winter changes in $\delta^{13}C$ of digestive gland and mantle tissues in diploids paralleled those of triploids, suggesting that reproduction blurs $\delta^{13}C$ signals in spring and early summer. During that period, $\delta^{13}C$ values and $\delta^{13}C$ to C:N ratios of gonads were remarkably close to those of digestive gland in diploids, highlighting the major role of the digestive gland in controlling nutrient fluxes to gonads. Small variations in $\delta^{13}C$ time-series of gonads and digestive glands also occurred but as reported in other *C. gigas* populations, they may be linked to differences between females and males or partial gamete releases prior to the occurrence of a mass spawning event. Significantly higher lipid content has been reported in females over the reproductive periods (Deslous-Paoli & Héral 1988). This higher content is mainly linked to higher proportions of neutral lipids (triacylglycerols) in gonads (Li et al. 2000, Matus de la Parra et al. 2005) and digestive glands (Matus de la Parra et al. 2005) of female *C. gigas*. Partial gamete releases before mass spawning have not been clearly established in *C. gigas*. In contrast, a second autumnal peak in the reproductive cycle may occur, as reported in Spain (Pazos et al. 1996), or gametes may degenerate and be resorbed without release, as reported in Ireland (Steele & Mulcahy 1999). Similarly, the pattern of reproductive events may be greatly affected by unfavourable thermic and food conditions, as reported in the Marennes-Oléron Bay in 1981 (Deslous-Paoli & Héral 1988). This reinforces the view of a highly flexible potential in the reproductive cycle in *C. gigas* (Kang et al. 2000) and the importance of food availability to the production of ripe gametes (Ruiz et al. 1992). In this opportunistic species, the autumn-winter period constitutes a stage of sexual resting, and glycogen reserves stored over phytoplankton blooms from spring onwards are simultaneously used for both growth and lipid accumulation for gamete build-up (Deslous-Paoli & Héral 1988, Pazos et al. 1996, Kang et al. 2000, Matus de la Parra et al. 2005). Furthermore, differences in $\delta^{13}C$ values amongst gonads and digestive gland, and mantle and muscle, reflect the phenomenon of isotopic routing (Gannes et al. 1997), with differential allocation of dietary components to different tissues. $\delta^{13}C$ values of digestive gland in diploids closely followed those of triploids after the mass spawning had occurred (Fig. 5). This may reflect the abrupt breakdown of lipid transfer from digestive gland to gonads for further summer-autumn gametogenic development.

Some gonad tissues may develop in triploids both in females and males (Beaumont & Fairbrother 1991), because in triploid oysters meiosis is disrupted at first prophase. Thus during periods favourable for gonad ripening in diploid oysters, gonadal tubules in triploids may contain pre-meiotic cells that, however, subsequently abort. This explanation is consistent with our observations that all triploids used in our analyses were sterile but may explain some unexpected parallel fluctuations in $\delta^{13}C$ values of digestive gland and mantle tissues in both diploids and triploids over the beginning of the reproductive period prior to mass spawning (Fig. 5).

Implications of somatic tissue reserves in the gametogenic cycle

Our results are consistent with the view that the digestive gland in *Crassostrea gigas* might be an essential organ controlling nutrient fluxes not only for gametogenic development but also for other maintenance and growth functions (Berthelin et al. 2000, Matus de la Parra et al. 2005). Our results suggest that in triploids the digestive gland contributes to energy storage and transfer to other organs, lipids being used for gonad development in diploid oysters but lost in gamete releases by diploids when unconverted glycogen was used for enhanced somatic growth in triploids (Beaumont & Fairbrother 1991). Gametogenesis in molluscs has been reported to be sustained by mobilisation of reserves from tissues other than the digestive gland (Gabbott 1983, Mathieu & Lubet 1993). For example, in *Pecten maximus* the most important storage tissue is the adductor muscle with the digestive gland involved secondarily depending on the season (Lorrain et al. 2002). However, Berthelin et al. (2000) concluded that muscle in *C. gigas* did not represent a storage compartment supplying the energy cost of reproduction. Its dry weight represents less than 15-20 % of total oyster dry weight and its biochemical composition is largely dominated by proteins with glycogen and lipids remaining always at low level (Berthelin et al. 2000). Most recently, Matus de la Parra et al. (2005) performed proximate biochemical and lipid class analyses on labial palps, gonads and digestive gland in *C. gigas* of Marennes-Oléron Bay. They concluded that labial palps are an organ of glycogen and triacyglycerol reserves, which are transferred to the gonads during the last stage of ripening. However, the weight-to-weight proportion of labial palps vs. soft parts revealed that labial palps represent less than 6% of the whole oyster soft body (Matus de la Parra et al. 2005). In mytilids, such as *Mytilus edulis*, the mantle tissue is the principal organ of glycogen reserve and the site of gonad development (Gabbott 1983 and references therein, Mathieu & Lubet 1993). However, from both the analyses of Berthelin et al. (2000) and our data set, it appears that mantle tissues comprise reserves to fuel the reproductive cycle in *C. gigas*. Similar correlations found in this study between $\delta^{13}C$ and C:N ratios in gonads, digestive gland and mantle in diploid oysters during their reproductive period might confirm this hypothesis but large scatter appeared in $\delta^{13}C$ mantle values (Fig. 8). A more definitive answer to this hypothesis must await further biochemical comparisons between diploid and triploid oyster tissues.

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Figure caption

Figure 1: Map of the Marennes-Oléron Bay with location of the oyster culture site at Ronce-les-Bains: oyster leasing ground (shaded areas), experimental oyster-culture site (1) and hydrobiological sampling station (2). Inserted picture shows off-bottom cultures based on iron tables on which oyster bags were fastened.

Figure 2: Variation over time in sea-surface temperature (SST: *) and salinity (○) (a), total particulate matter (TPM: ■), particulate organic and inorganic matter (POM: *, PIM: □) (b) and chloropigments (chlorophyll-*a*: ■ and pheopigments: □) (c) at Ronce-les-Bains from March 2002 to May 2003. Mean ± SD (n = 3). Sampling periods during spring tides are indicated by vertical lines.

Figure 3: Variation over time of stable carbon (*) and nitrogen (○) isotope ratios (a) and POC:Chl *a* (*) and C:N ratios (○) (b) of particulate organic matter in the water column at Ronce-les-Bains from March 2002 to May 2003. The shaded area between 100 and 200 separates POC:Chl *a* into new (< 100) and detritical (> 200) organic matter.

Figure 4: Variation over time of dry weight tissues of digestive gland (■, □), gills (♦, ◊), mantle (●, ○), muscle (▲, Δ) and gonads (*) in diploid (filled symbols) (a) and triploid (open symbols) (b) *Crassostrea gigas* at Ronce-les-Bains from March 2002 to May 2003. Mean ± SD (n = 5).

Figure 5: Variation over time in $\delta^{13}C$ values of digestive gland (■, □), mantle (●, ○), muscle (▲, Δ) and gonads (*) of diploid (filled symbols) (a) and triploid (open symbols) (b) *Crassostrea gigas* from March 2002 to April 2003. Gills not shown for clarity (see text).

Areas in doted lines represent spawning periods recorded in diploid oysters in 2002. Mean ± SD (n = 3).

Figure 6: Variation over time in $\delta^{15}N$ values of digestive gland (■, □), mantle (●, ○), muscle (▲, Δ) and gonads (*) of diploid (filled symbols) and triploid (open symbols) *Crassostrea gigas* from March 2002 to April 2003. Gills not shown for clarity (see text). Areas in doted lines represent spawning periods recorded in diploid oysters in 2002. Mean ± SD (n = 3).

Figure 7: Scatter plot of diploid vs. triploid *Crassostrea gigas* digestive gland values for $\delta^{13}C$ (a) and $\delta^{15}N$ (b) during the reproductive (large squares) and resting (small squares) periods. The solid line depicts a 1:1 correspondence. Dashed lines indicate significant correlations (see text for details). Data from March to April 2002 are omitted (see text and Figs. 5 and 6 for initial values).

Figure 8: Scatter plot of $\delta^{13}C$ values vs. C:N ratios for digestive gland (■, □) and gonads (*) (a), muscle (▲, Δ) and mantle (●, ○) (b) of diploid (filled symbols) and triploid (open symbols) *Crassostrea gigas* according to the reproductive (large symbols) and resting periods (small symbols). Solid and dashed lines indicate significant correlations for diploid and triploid oysters, respectively (see text for details). Data from March to April 2002 are omitted.

Table 1: Total shell length (mm), total tissue dry weight (g), proportion of gonad and total lipid content relative to dry weight (%) (mean ± S.D, n = 5, 5, 5 and 3 pools of 10, respectively) for diploid and triploid oysters at Ronce-les-Bains. nd = no data.

	Diploid oysters				Triploid oysters		
Sampling date	Shell length (mm)	Tissue dry weight (g)	Gonads (% dry weight)	Total lipid content (% dry weight)	Shell length (mm)	Tissue dry weight (g)	Total lipid content (% dry weight)
03/13/2002	40.7 ± 5.9	0.13 ± 0.04	0	8.0 ± 0.5	58.9 ± 7.5	0.15 ± 0.05	7.5 ± 0.3
04/25/2002	56.2 ± 7.4	0.56 ± 0.18	0	9.0 ± 0.7	59.1 ± 9.7	0.41 ± 0.17	8.9 ± 0.1
05/14/2002	57.2 ± 7.5	0.72 ± 0.20	14.7 ± 7.8	9.7 ± 0.7	64.3 ± 3.2	0.56 ± 0.08	9.6 ± 0.4
05/27/2002	58.1 ± 3.1	0.77 ± 0.12	17.7 ± 7.6	11.6 ± 1.5	62.2 ± 3.5	0.59 ± 0.15	12.0 ± 0.9
06/05/2002	57.5 ± 8.1	0.81 ± 0.11	37.2 ± 4.9	12.1 ± 0.2	65.3 ± 3.9	0.69 ± 0.14	9.4 ± 0.1
06/12/2002	54.3 ± 4.2	0.56± 0.15	17.3 ± 19.4	12.5 ± 0.9	71.7 ± 6.2	0.95 ± 0.15	10.2 ± 0.5
06/20/2002	65.1 ± 7.0	0.68 ± 0.33	26.5 ± 17.4	12.9 ± 0.7	71.4 ± 6.8	0.76 ± 0.12	11.5 ± 0.3
06/26/2002	71.7 ± 9.5	0.58 ± 0.14	5.2 ± 6.9	10.1 ± 0.4	71.7 ± 7.2	0.85 ± 0.24	11.7 ± 0.3
07/03/2002	61.3 ± 6.1	0.59 ± 0.09	17.4 ± 21.5	13.5 ± 0.7	68.4 ± 4.2	0.85 ± 0.24	11.8 ± 0.3
07/10/2002	66.3 ± 8.3	1.14 ± 0.26	54.4 ± 6.7	15.0 ± 0.4	69.6 ± 1.7	0.96 ± 0.15	10.0 ± 0.4
07/25/2002	71.4 ± 19.4	0.63 ± 0.09	0	15.1 ± 0.5	62.2 ± 7.7	0.59 ± 0.19	10.2 ± 0.5
08/08/2002	59.9 ± 5.2	0.55 ± 0.11	0	15.8 ± 1.8	79.2 ± 2.3	1.04 ± 0.13	12.2 ± 0.5
08/21/2002	70.2 ± 2.5	0.69 ± 0.17	0	9.0 ± 0.2	73.7 ± 4.4	0.85 ± 0.22	10.6 ± 0.3
09/11/2002	66.5 ± 8.4	0.65 ± 0.16	0	9.1 ± 0.2	84.5 ± 3.6	1.24 ± 0.26	11.5 ± 0.6
10/08/2002	64.5 ± 6.6	0.73 ± 0.14	0	nd	83.3 ± 5.6	1.52 ± 0.31	nd
11/05/2002	72.5 ± 7.9	1.01 ± 0.20	0	nd	92.1 ± 6.0	1.44 ± 0.26	nd
12/02/2002	55.4 ± 5.1	0.48 ± 0.17	0	nd	89.3 ± 6.5	1.58 ± 0.25	nd
01/06/2003	66.9 ± 4.5	0.84 ± 0.23	0	nd	89.9 ± 11.1	1.01 ± 0.28	nd
02/04/2003	65.1 ± 1.9	0.77 ± 0.19	0	nd	80.5 ± 12.1	0.83 ± 0.32	nd
04/12/2003	72.6 ± 4.3	1.33 ± 0.22	0	nd	95.6 ± 5.7	1.75 ± 0.24	nd

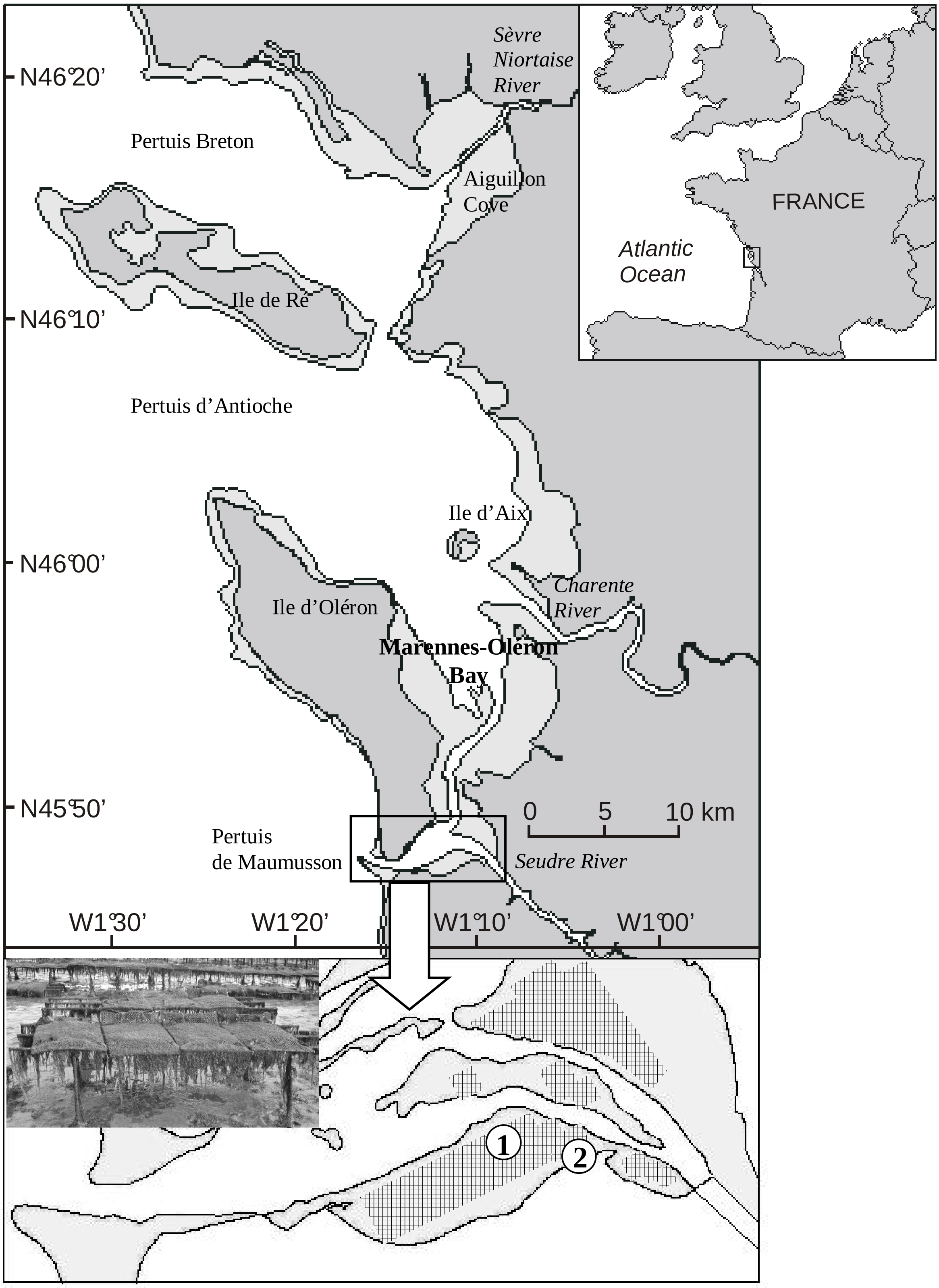
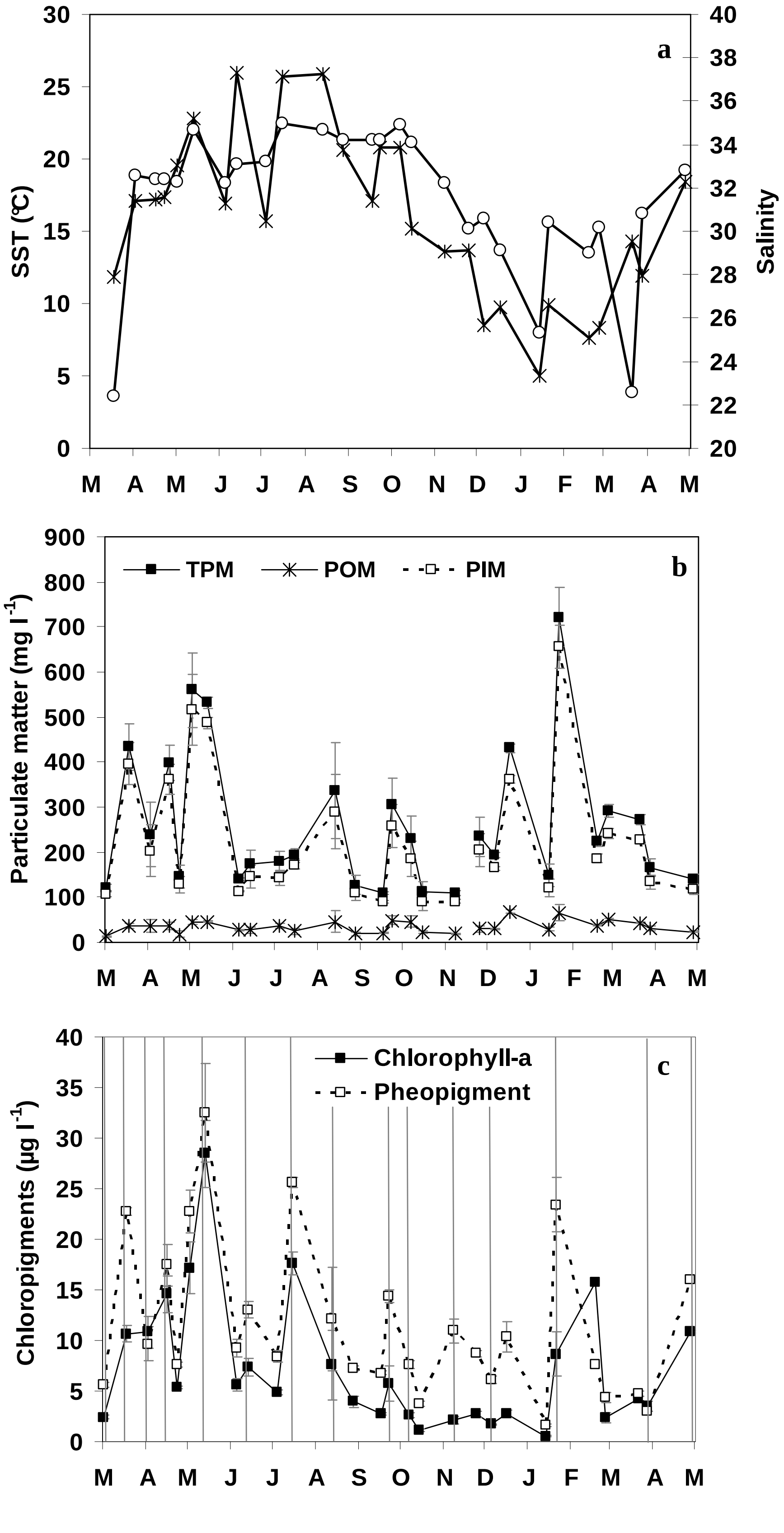


Figure 1: Malet et al.



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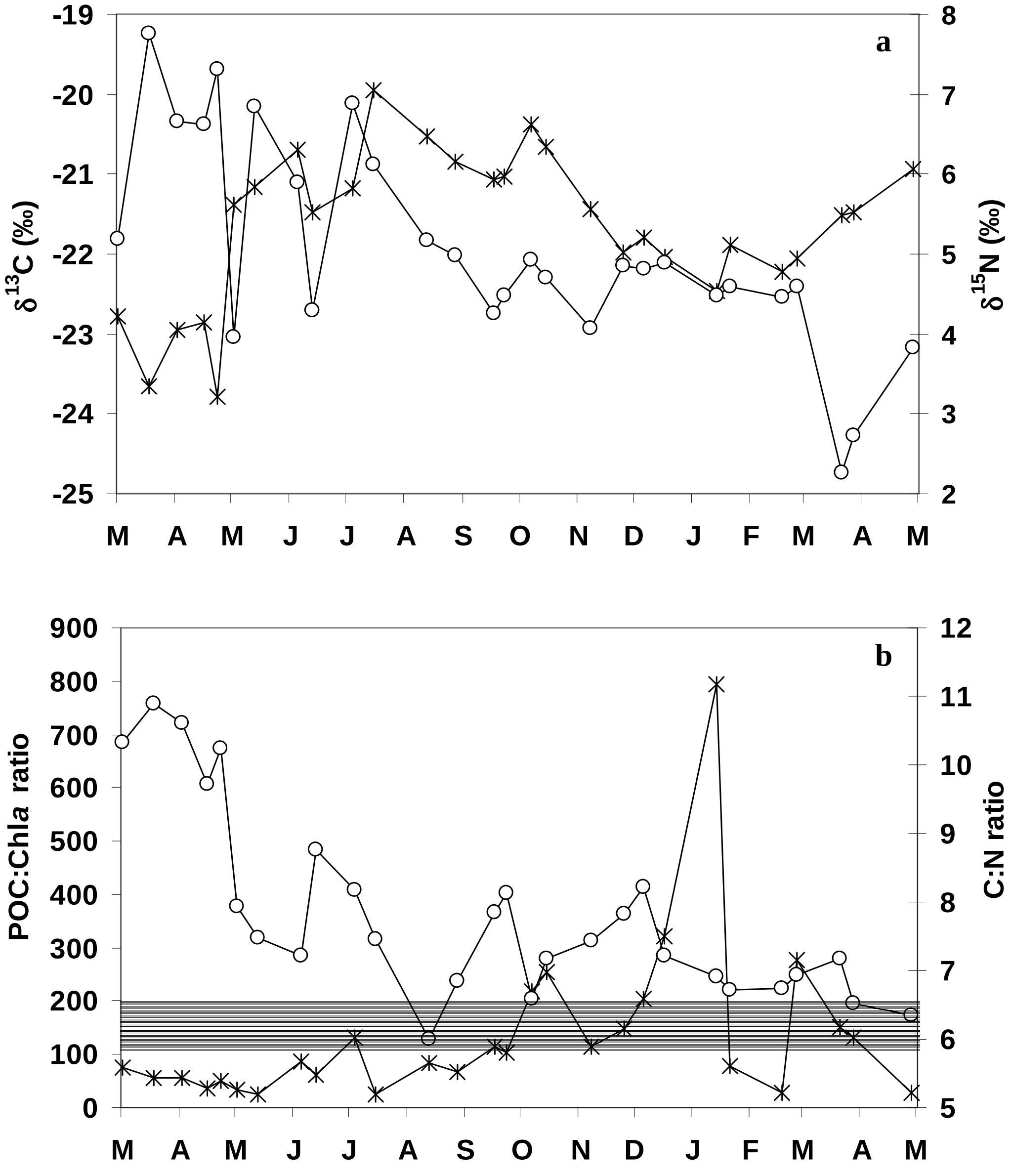

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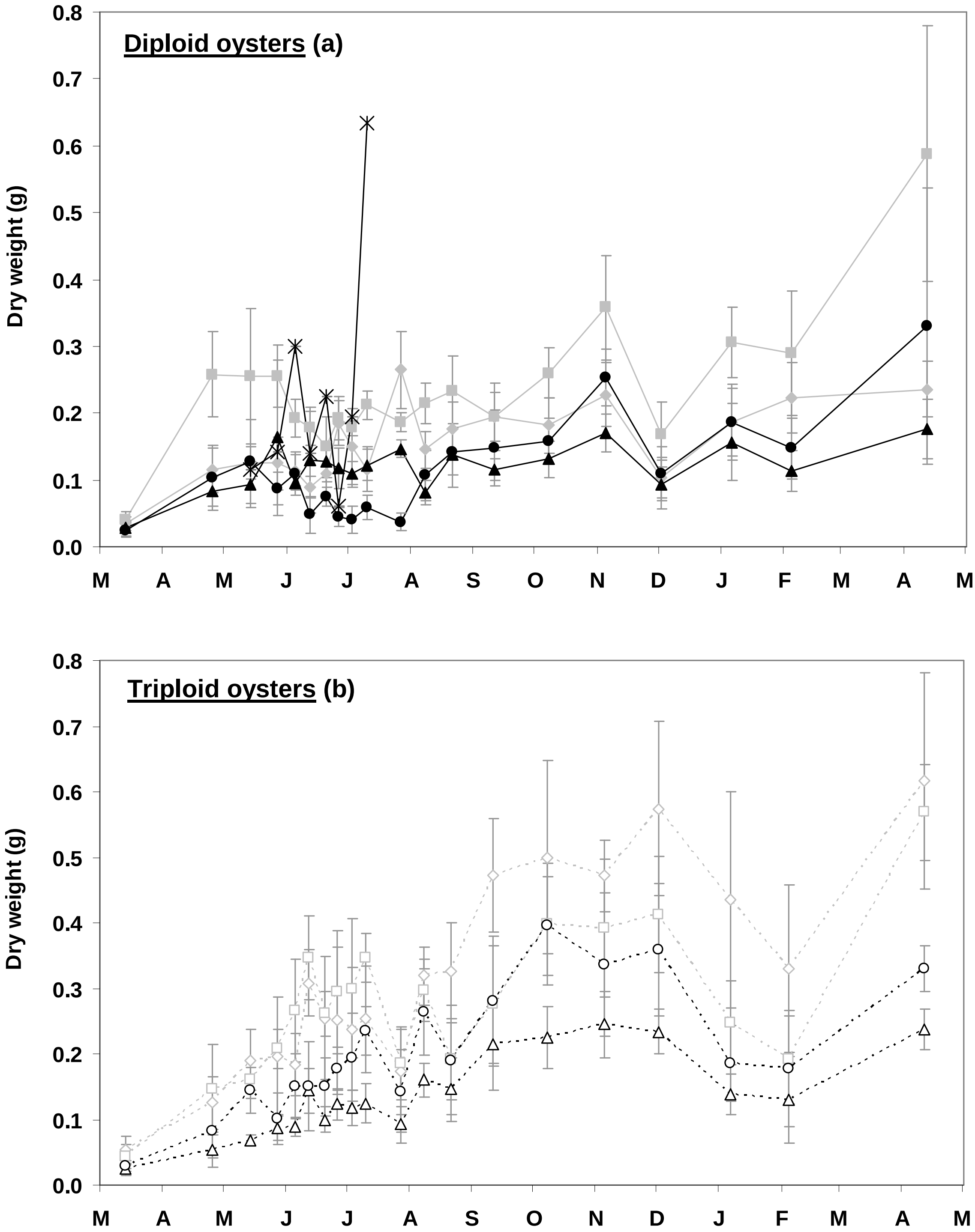

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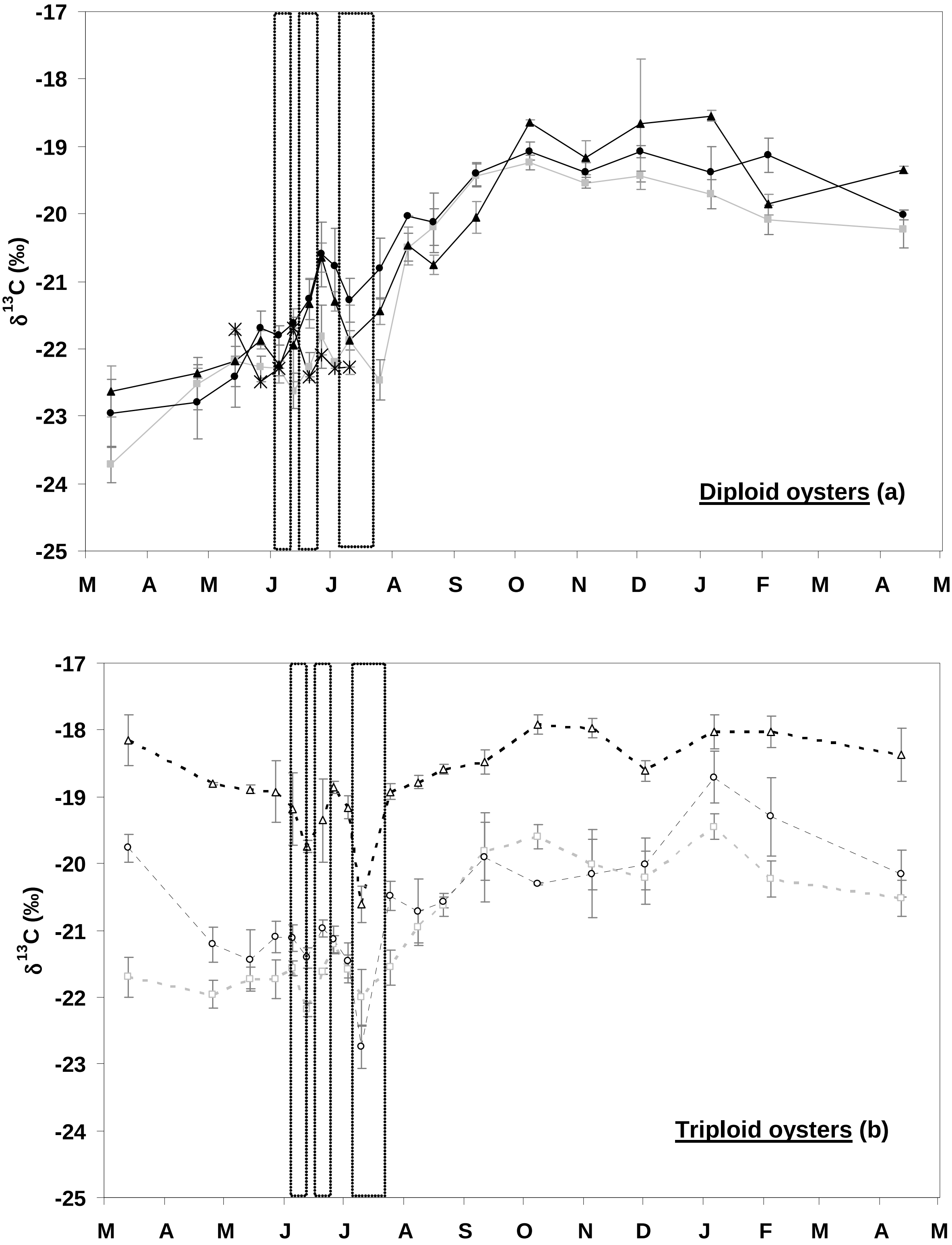

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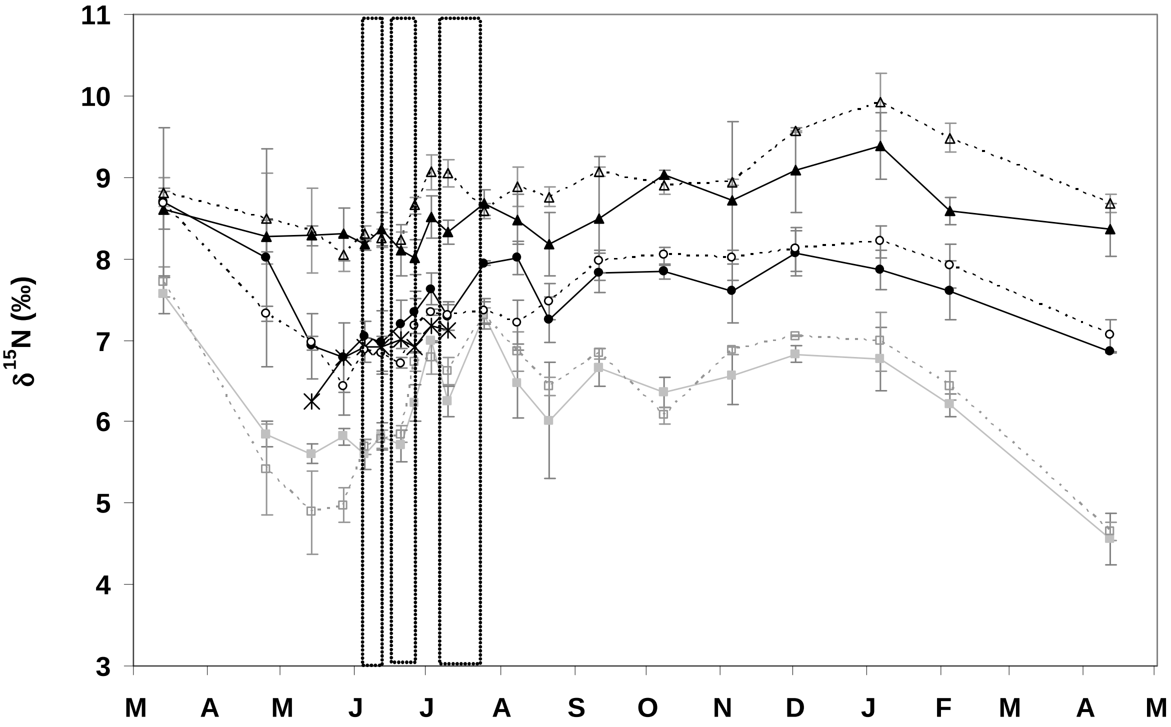

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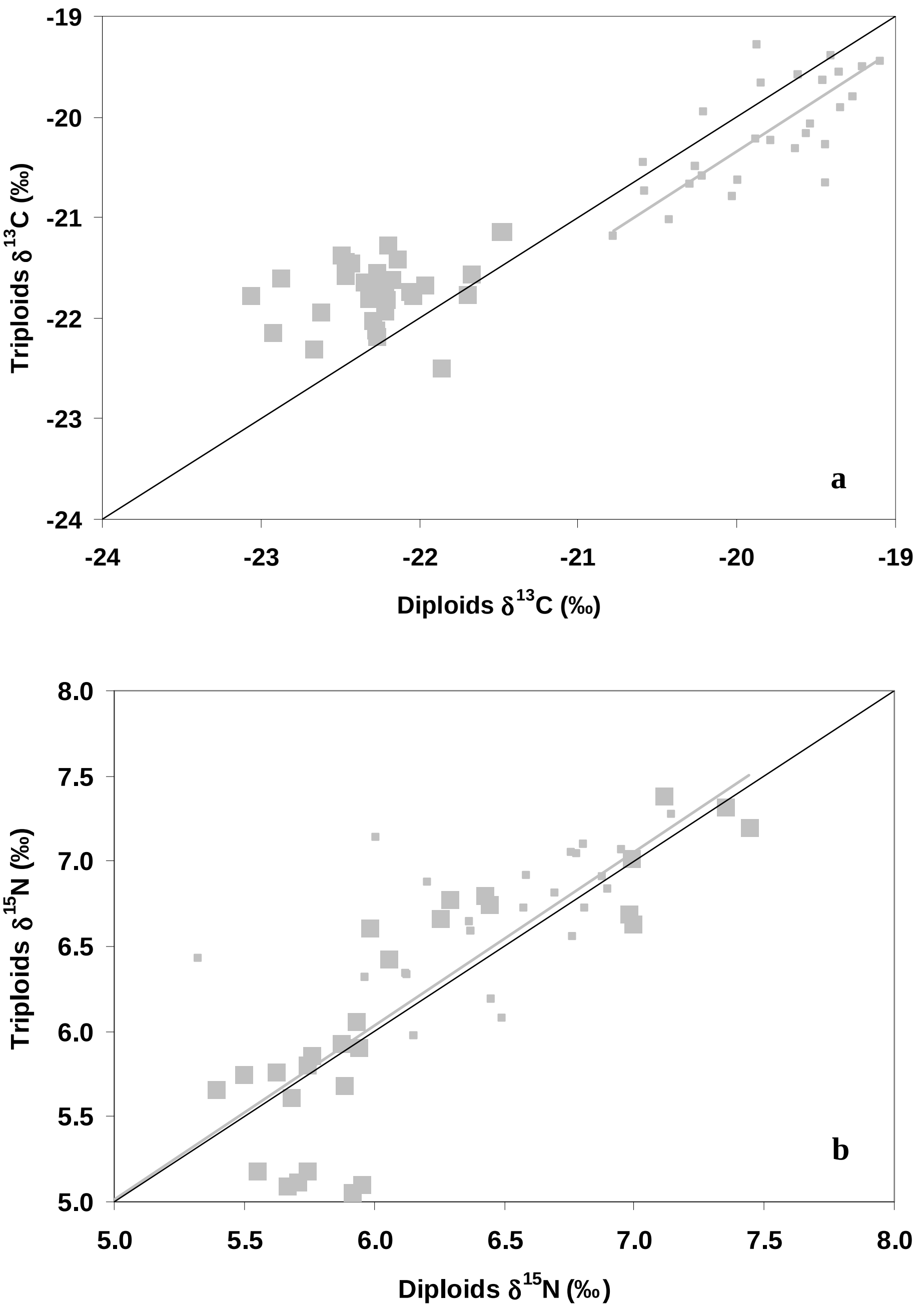

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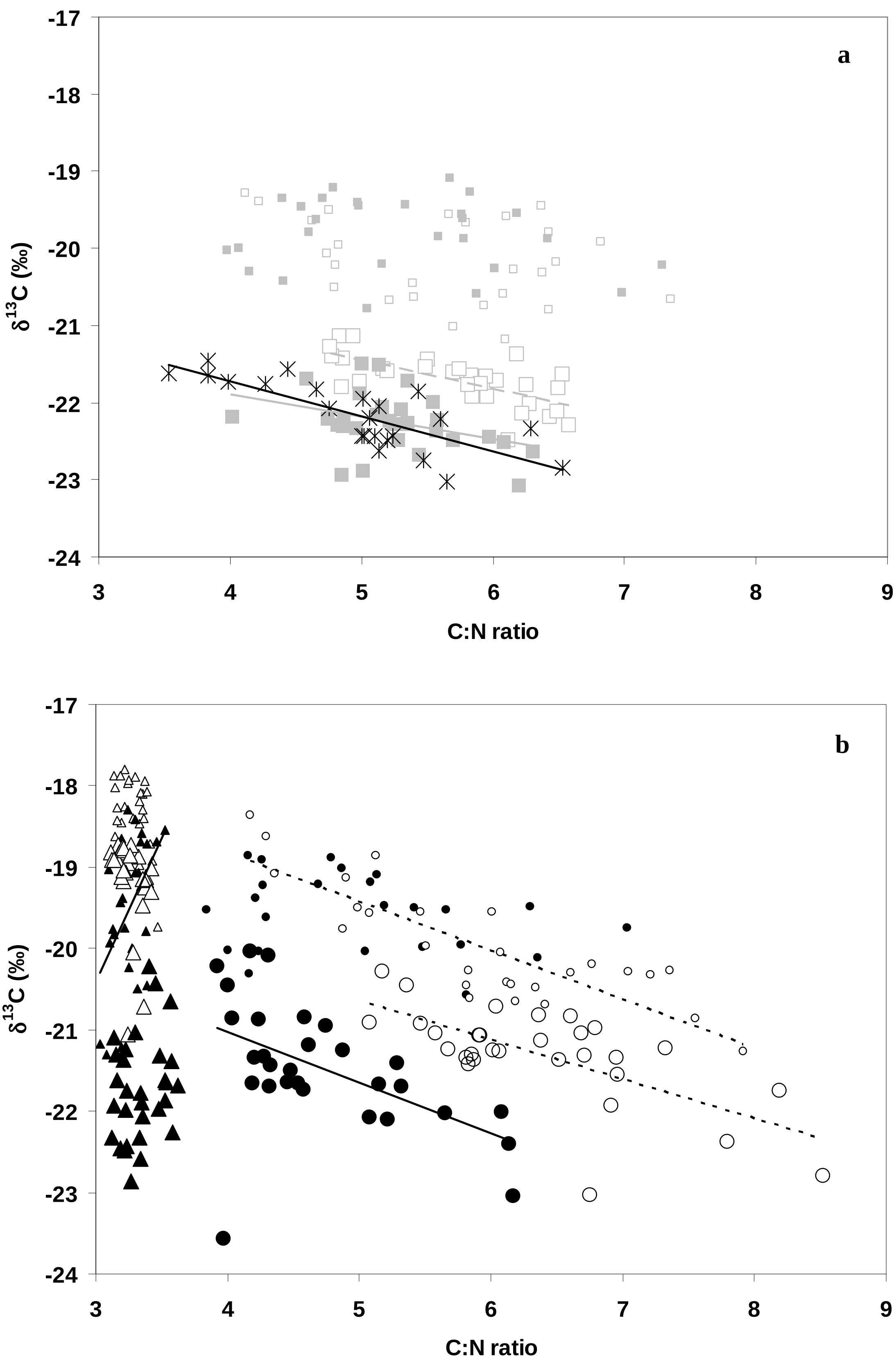

Figure 8: Malet et al.