

# Differential $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among scallop tissues: Implications for ecology and physiology

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## Abstract

There have been several studies where the isotopic composition of organisms has been determined seasonally, but fewer have examined separate organs. In this context, separate organs (*e.g.* gonad, digestive gland and muscle), of a suspension-feeder, the scallop *Pecten maximus*, were used to assess seasonal changes of both stable isotopes and biochemical components. Our study used multiple indicators (stable carbon and nitrogen isotopes ratios, biochemical components and seston Chl *a*) to track nutritive activity and energy allocation in *P. maximus* from the Bay of Brest (France). In addition to seasonal variation in the isotopic composition of *P. maximus* tissues, we found strong differences in the mean isotopic signatures of different organs. This has serious implications for interpretation of animal diets and potential use in animal physiology. Furthermore, we present evidence that seasonal variations of metabolism will cause changes in the isotopic composition not related to changes in the diet. Interpretation of isotopic data may require consideration of values from several separate organs. Finally,  $\delta^{15}\text{N}$  appears powerful to track metabolite fates in the scallop *P. maximus*.

Keywords :  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , diet, metabolite fates, scallops, tracers.

## 1. Introduction

In ecological studies, the use of stable carbon and nitrogen isotopes is now commonly used to trace food webs. Indeed, diet is the primary determinant of animal isotopic composition. It is generally accepted that the  $\delta^{13}\text{C}$  content of an organism reflects the  $\delta^{13}\text{C}$  content of its diet with little (1‰) or no change (see for example DeNiro and Epstein, 1978 ; Fry and Sherr, 1984). The  $\delta^{15}\text{N}$  content of an organism shows a larger enrichment, averaging 3 - 4‰ (see Minagawa and Wada, 1984). This is due to excretion of  $^{15}\text{N}$ -depleted nitrogen (DeNiro and Epstein, 1981, Peterson and Fry, 1987). Stable isotope studies have led to the elucidation of numerous food webs in different environments. In the literature, many studies have used  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as natural tracers with the assumption that these ratios are homogeneously distributed in all tissues of an organism and do not vary temporally. But it is increasingly apparent that interpretation of stable isotope ratios should be made with caution (Gannes, 1998; Peterson, 1999).

Since the early work of Stephenson et al. (1984), several authors have explored seasonal stable isotopic variation in marine organisms (Goering et al., 1990; Riera et al., 1996, Kang et al., 1999; Rolff, 2000). Peterson and Fry (1987) and Peterson (1999) emphasized the limitations of isotopic measures, and the need to do both temporal and spatial sampling to fully understand the observed variations. They also recommend using stable isotopes in combination with other techniques to allow for more robust conclusions. Furthermore, Stephenson et al. (1984) argued that stable isotopic composition of macrophytes differed according to tissue type. In terrestrial animals, Tieszen et al. (1983) also suggested that multiple tissues should be used for dietary analyses. Indeed, isotopic enrichment depends on tissue turn over rates and will consequently vary with tissue type. Tissues with long turnover times such as muscle, integrate dietary isotope ratios over the whole lifetime of the organism. Isotope ratios in tissues with faster turnover rates, such as digestive gland and

gonads, reflect the diet during a shorter period. Isotopic variations among organs may reflect their different metabolic rates as well as their turn-over rates. For marine organisms, the recent stable isotope enrichment study of Raikow and Hamilton (2001) demonstrated that  $\delta^{15}\text{N}$  values of three tissues of unionid mussels reflected short-term and long-term assimilation of nutrients. Differential isotope fractionation occurs among different tissues and establishing a relationship between an organism and its diet is not straightforward (Owens, 1987). In this context, it appears essential to check the uniformity of stable isotopic distribution among organs and to examine their seasonal variations prior to interpretation of food web studies.

Our primary objective in this study was to identify the stable isotopic composition of *Pecten maximus* by a dual isotopic analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in three tissues, and then to assess seasonal variation. Secondly, we used isotopic variations to track energy and nutrient flow through the organism. This assessment of metabolic fluxes by stable isotopic measurement should help to improve knowledge of energy allocation strategy (e.g. reserve storage, somatic vs. gonadal production) in scallops.

## **2. Material and methods**

### *2.1 Study site*

This study was carried out on the Roscanvel bank (30 m depth, 4°30'W, 48°20'N) of the Bay of Brest (France). The Bay of Brest is a macrotidal ecosystem of 180 km<sup>2</sup> that is connected to the Atlantic Ocean by a narrow and deep strait (for a review of the characteristics of the study site, see Chauvaud et al., 2000).

### *2.2 Sample collection*

Hydrobiological parameters were measured approximately twice a week (Chlorophyll-*a*, temperature, salinity) from February to July 2000. Temperature and Salinity

were recorded with a Sea-bird SBE 19 CTD profiler. A classical method was used for bottom chlorophyll-*a* collection and analysis (see Lorrain et al., 2000 for more details). Suspended particulate organic matter (SPOM), considered as a potential food source for scallops, was sampled by filtering 500 ml of water, from one meter above the bottom, through a Whatman GF/F filter. Samples of SPOM were collected approximately twice a week from February to July 2000 and then frozen until analysis. Scallops were sampled under a different schedule: for isotopic measurements and lipid analysis, scallops (*Pecten maximus*) were collected by scuba diving on 15 sampling dates (24 February; 15 and 22 March; 1, 10, 26 April; 3, 12, 17, 19, 30 May; 9, 26 June; 14 September and 13 October 2000). For each collection date, one set of 5 scallops was utilised for isotopic measurements and a second one for lipid analysis. For biometric measurements (see §2.4), another set of 20 scallops was utilised (Sampling dates in Table 1). All scallops collected were in the 3-year age class, which eliminated age-related differences in our samples. We preferred scuba diving to dredging in order to prevent sediment contamination of scallops. Tissue samples were thus free of inorganic carbon, which could have distorted the analyses of  $\delta^{13}\text{C}$ .

When collected, scallops were immediately transferred to the laboratory (maximum 2 hours) and killed by sectioning adductor muscle near the flat valve. Stomachs of dead scallops were then rinsed with a few ml of 0.2- $\mu\text{m}$  filtrated seawater injected gently via the mouth, to purge the digestive track and to limit a phytoplanktonic signature in the digestive gland. This rinsing was processed until complete cleaning of the digestive gland (*i.e.* clear digestive gland's rejections). Muscle, gonad and digestive gland were then dissected from each individual. All samples were frozen at  $-30^{\circ}\text{C}$ , then freeze dried.

### 2.3 Isotopic analysis

Before analysis, filtered SPOM samples were exposed to HCl vapour for four hours at room temperature to remove carbonates. The filters were then folded and placed into tin cups

(9 mm height, 5 mm diameter) and kept in closed vials until analysis. The samples were analysed for C and N content and isotope ratios using a Carlo Erba NA 2100 elemental analyser, configured for C and N analysis. This elemental analyser was coupled to a Finnigan Delta S isotope ratio mass spectrometer.

Scallops samples (gonad, digestive gland and muscle) were ground to an homogeneous, fine powder and 1-mg samples placed into 6×4 mm tin cups for CF-IRMS analysis, using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser with ANCA-NT Solid/Liquid Preparation Module (PDZ Europa Ltd., Crewz, UK). The analytical precision (SD, N=5) was 0.2 ‰ for both N and C, estimated from standards analysed along with the samples. Triplicate analyses performed on some samples confirmed that analytical reproducibility was very good (0.2 ‰ maximum variation). All isotopic data are given in the conventional delta notation in units of parts per thousands (‰) and the standards are Pee Dee Belemnite for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ :

$$\delta^{13}\text{C}_{\text{sample}} \text{ or } \delta^{15}\text{N}_{\text{sample}} = (R_{\text{sample}} / R_{\text{standard}} - 1) * 1000 \text{ where } R = {}^{13}\text{C}/{}^{12}\text{C}, {}^{15}\text{N}/{}^{14}\text{N}$$

#### 2.4 Biometric and lipid measurements

From each collection, 20 scallops were dissected. Wet and dry (100°C for 48 h) weights of the gonad (G), adductor muscle (M), digestive gland (DG) and the remaining tissues (R) were recorded for each individual (Table 1). Weight indices were then calculated for each organ (G, M, DG and R) according to Paulet and Boucher (1991):

$$\text{Index} = \text{Organ dry weight} \times 100 / \text{shell dry weight.}$$

In 1996, the lipid content of the female and male gonads, adductor muscles and digestive glands of individuals of *P. maximus* were determined by the Bligh and Dyer (1959) method. Lipids of the digestive gland were also assessed in 2000.

## 2.5 Estimation of the whole body stable isotopic composition

As the whole body was not analysed for stable isotopes in this study, we simulated a mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for the whole body of *P. maximus* based on a mixing model that uses relative dry weight of each organ (G, M and DG):

$$\delta_{\text{whole body}} = [\sum(\delta_{\text{organ}} * \text{Weight}_{\text{organ}})] / (\sum \text{Weight}_{\text{organ}})$$

We considered this estimation to be representative of the whole body as the organs studied represent more than 75% of the total weight (the gills and the mantle were not analysed).

## 2.6 Statistics

Among-date differences of isotopic means were tested (99% CL) using the Kruskal-Wallis test and the Newman-Keuls multiple range test (pairwise comparison, Statgraphics plus). Linear relationships were tested with simple regression (Statgraphics plus).

## 3. Results

### 3.1 Environmental data

In this study, bottom-water temperature varied between 9°C at the end of January and 16.9°C in mid-September. Rainfall led to minor fluctuations in bottom-water salinity from 33 to 35.2 PSU. The year 2000 was characterised by several phytoplanktonic events (Fig.1). During the spring, six blooms occurred. Stable SPOM carbon isotopes values ranged from -22.1 to -27.4 ‰, and  $\delta^{15}\text{N}$  values, from -2 to 6.7‰, with a mean of -24.4‰ and 2.6‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively. Values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  increased from February to December, with higher values during phytoplankton blooms. In fact, phytoplanktonic events were synchronous with stable isotope increases. High stable isotope values in February, however, were not due to phytoplankton, as the first bloom does not occur until March in the Bay of Brest (see Chauvaud et al., 2000).

### 3.2 *P. maximus* tissue isotopic composition

Strong isotopic differences were observed among the organs (Fig.2A, B). The mean  $\delta^{13}\text{C}$  was -15.2‰, -18.1‰ and -20.1‰ for the muscle, gonad and digestive gland respectively; for  $\delta^{15}\text{N}$ , it was 9.4 , 7.8 and 6.0‰. Furthermore, the values for the different organs fell in the same relative order during the entire sampling period (muscle > gonad > digestive gland). The carbon isotopic differences were the most pronounced with the digestive gland and gonad depleted about 5‰ and 3‰, respectively, relative to the adductor muscle. Discrepancy among organs was of minor importance for  $\delta^{15}\text{N}$ , with a 1.8‰ enrichment from digestive gland to gonad, and 1.6‰ from gonad to muscle.

Seasonal differences existed in stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  composition for all the organs (Fig.2) but the most pronounced were for the digestive gland. The strongest variations were measured in spring. In the digestive gland and the gonad,  $\delta^{15}\text{N}$  values decreased from April to May. Following this decrease, a progressive increase occurred until late June. A parallel and similar evolution can be seen among the organs. For example, the decrease in  $\delta^{15}\text{N}$  values in early march is seen in all the organs, but first in the digestive gland, and then in the gonad and muscle with a little delay. These general trends were reversed and attenuated for  $\delta^{13}\text{C}$ .

### 3.3 *Estimated whole body values*

Our mixing model combined with monthly dry weight data (Table 1) allowed us to estimate the whole body  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at -16.6‰ and 8.5‰, respectively. Seasonal variation of 1.5‰ and 1.2 ‰ were found during all the sampling period for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  respectively (not shown here).

### 3.4 Lipid data

Lipids were assessed for all the organs only in 1996. Nevertheless, they can be compared to  $\delta^{13}\text{C}$  results in 2000 (Fig.3) as this year (1996) is considered normal with respect to lipid content and environmental parameters (Saout, 2000). A negative linear relationship existed between  $\delta^{13}\text{C}$  and the percentage of lipids (Fig.3,  $y = -0,2071x - 14,761$ ,  $R^2 = 0,82$ ,  $p < 0.0001$ ). Values for the three tissue types were tightly grouped, with the digestive gland having the highest lipid and lowest  $\delta^{13}\text{C}$  and the muscle having the lowest lipid and highest  $\delta^{13}\text{C}$ .

In 2000, lipids of the digestive gland showed seasonal variations (Fig.4), but the seasonal  $\delta^{13}\text{C}$  values did not necessarily follow the same pattern. The two showed similar trends during spring (15 March - 9 June), but the patterns diverged in summer, autumn and winter. The digestive gland weight index had its most pronounced increase from 22 March to 1 April (Fig. 4), concomitant to the  $\delta^{13}\text{C}$  increase and to  $\delta^{15}\text{N}$  decrease. The small  $\delta^{13}\text{C}$  peak in March is related to the digestive gland growth, mainly due to protein production during this period (A. Donval, unpublished)

## 4. Discussion

Our isotopic data clearly showed temporal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variations in the three studied tissues of *Pecten maximus*. Furthermore, the differing mean isotopic composition of the tissue types was particularly striking. Isotopic variations among organs reached 1.6 to 5‰, and whole body and muscle  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values differed by more than 1‰. It is now well accepted that the average  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of animal tissues are, respectively, about 3 - 4‰, and 1‰ more positive than those of the animal's diet (DeNiro & Epstein, 1978, 81; Peterson and Fry, 1987). But these enrichment factors are used in the literature irrespectively of the tissue analysed (e.g. muscle or whole body). Our study demonstrates that results on diet

and trophic-group will vary depending on tissue type. For instance, muscle and calculated whole body  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values differed by more than 1‰. So, using classical tissue enrichment factors and average stable isotope values of muscle ( $\delta^{13}\text{C} = -15.2\text{‰}$ ;  $\delta^{15}\text{N} = 9.4\text{‰}$ ) and whole body ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}_{\text{calculated whole body}} = -16.6$  and  $8.5\text{‰}$  respectively), one should expect the isotopic ratios of the filter-feeder's diet to be about -16.2 (muscle) or -17.6‰ (estimated whole body, for  $\delta^{13}\text{C}$ ) and 5.4-6.4‰ or 4.5-5.5‰ (for  $\delta^{15}\text{N}$ ). Our SPOM mean values were -24.4‰ and 2.5‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, in fact very different of expected diet values.

Isotopic relationships between potential food sources and various tissues of *Pecten maximus* in the Bay of Brest are illustrated in figure 5, i.e. muscle values compared with potential food sources as described in the literature; and also digestive gland values. The results showed that muscle values were strongly enriched in  $^{13}\text{C}$  compared to potential food sources. Indeed, our phytoplankton values (=SPOM values during blooms) and those of Savoye (2001, -22.4‰ ( $\pm 1.5\text{‰}$ , n=74) and 4.7‰ ( $\pm 1.7\text{‰}$ , n=74) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were much lower than expected calculated food sources values with classical enrichment factors (black stars, Fig. 5). This discrepancy was mainly for  $\delta^{13}\text{C}$ , as  $\delta^{15}\text{N}$  values were less different. A classical mixing model (see Raikow and Hamilton, 2001) could predict that *Pecten maximus* consume a mixed diet with a minority of phytoplankton, or conversely, that it consume mainly phytoplankton together with a small amount of another food of very enriched  $\delta^{13}\text{C}$  value. As in mussels, the *P. maximus* diet might include dissolved organic carbon (Roditi et al., 2000), deposit material (Raikow and Hamilton, 2001), benthic algae, microheterotrophs and bacteria (Kang et al., 1999; Kreeger and Newell, 2001; Nichols and Garling, 2000). As we did not have stable isotope values of these potential food sources in the Bay of Brest, (but only overall POM) we cannot evaluate the scallop's diet in detail. We can just say that phytoplankton is not the only resource being used by the scallops. One can also argue that

microphytobenthos can be the main source of *P. maximus* diet as its classical average value is -16‰ (Kang et al., 1999). Nevertheless, this hypothesis can be ruled out as the main as we sampled scallops at 30m depth. In this location, a high light extinction coefficient (close to 0.2 m<sup>-1</sup>, Le Pape, 1996) prohibited microphytobenthic production and its ingestion by *Pecten maximus*. But alloctonous production can not be ruled out. Further, scallops (Shumway, 1987, 1997), and more specifically *P. maximus* (Chauvaud, 1998), have been shown to consume mainly phytoplankton. And in this study, the digestive gland value ( $\delta^{13}\text{C} = -20.1\text{‰}$ ) is closest to that of phytoplankton, the presumed diet of *Pecten maximus* (Fig. 5).

Several explanations can be advanced for the high discrepancy between diet and tissue stable isotope values. First, as already mentioned, a selective assimilation of one component of the SPOM with high isotopic value could occur. Indeed, Raikow and Hamilton (2001) postulated that differential, rather than uniform, assimilation of food resource components within the SPOM could occur. A selective assimilation of nutrients from diet could also be an explanation and further work on stable isotopic composition of the different biochemical fractions of the SPOM would be of interest. Dietary proteins, for example could have an enriched isotopic value relative to bulk material.

Moreover, the metabolism of these animals could favour a high isotopic fractionation between diet and tissues. Indeed, selective metabolic fractionation leads to a preferential loss of lighter isotopes during respiration and excretion (De Niro & Epstein, 1978; Tieszen et al, 1983 ). In consequence, the organism tends to be isotopically enriched in <sup>13</sup>C or <sup>15</sup>N (to respect the equilibrium). But classical enrichment factors have been calculated mainly for terrestrial organisms (De Niro & Epstein, 1978, 81) and a large range is found in the literature (Mingawa and Wada, 1984; Raikow and Hamilton, 2001). We therefore believe that there is a need to validate for each species the isotopic fractionation between diet and tissues. *Pecten maximus* could have an enrichment factor superior to the average literature values. Further

experimental and in-situ studies are required to confirm this hypothesis. Particular attention should be paid to isolate the different fractions of the POM (e.g. bacteria, microphytobenthos, microheterotrophs).

The relative contribution of different dietary sources may also shift during the year, associated with both seston composition and the physiology of individual consumers (Kreeger and Newell, 2001). Temporal changes in stable isotopes values of the three tissues showed that seasonal variations were most pronounced in the digestive gland. Indeed, this organ has a rapid turn-over and reflects short-term assimilation (Raikow and Hamilton, 2001). We agree with Raikow and Hamilton (2001), that it would be appropriate to use isotope ratios of this organ to assess true seasonal diets of individuals, since the muscle values reflect more long-term integration of nutrient sources. But then, which enrichment factor can be used to compensate for such different signatures? And what phenomenon can explain the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  enrichment from digestive gland to muscle?

We suggest, in agreement with numerous studies, that the variations in  $\delta^{13}\text{C}$  result from differences in biochemical content among these tissues, especially the lipid content. Indeed, lipids have already been shown to be depleted in  $\delta^{13}\text{C}$  relative to the other biochemical components (Tieszen et al., 1983; Focken and Backer, 1998; Thomson et al., 2000) and our own data (see Fig. 3) clearly reinforced the assumption that high lipid content in the digestive gland (25% vs. 4% in the muscle) can explain its light isotopic  $\delta^{13}\text{C}$  signal. As lipid content is inversely related to  $^{13}\text{C}$  content, it declines along the digestive gland to muscle trajectory. One could claim that lipid bias the signal and should be extracted before analysis.

But further work is needed to confirm that lipids are the main determinant of  $\delta^{13}\text{C}$  variations among the organs: *i.e.* stable isotope composition of the extracted lipid fraction should be measured and also complete biochemical composition of the tissues (e.g. proteins and carbohydrates). Furthermore, differences among the organs could also reflect the

phenomenon of isotopic routing (Gannes et al, 1998), with a differential allocation of dietary elements to specific tissues. Secondary fractionation during physiological processes could also be an explanation.

As lipid can not account for  $\delta^{15}\text{N}$  differences among organs, other biochemical components, especially a different protein content could lead to this  $^{13}\text{C}$  enrichment from digestive gland to muscle. A mechanism of selective  $^{15}\text{N}$  enrichment could also be occurring from digestive gland to muscle because of isotopic routing or metabolic fractionation.

Nevertheless, if we assume that lipid content can explain  $\delta^{13}\text{C}$  patterns among tissues, it could also explain temporal  $\delta^{13}\text{C}$  patterns in the digestive gland. All seasonal isotopic patterns might disappear if lipids were removed from the samples. We can not rule out this hypothesis as it has not been tested in this study. However, we believe that patterns of lipid utilisation alone do not account for seasonal variations. Indeed, the most important  $\delta^{13}\text{C}$  change occurred from 22 March to 1 April (from -20.1 to -18.6‰) and during this period, lipid content did not vary (32.6 vs. 32.3, Fig. 4). Thus, the  $\delta^{13}\text{C}$  increase was not lipid dependant. Newly formed material indicated by the development of the digestive gland (increase of 33% between 22 March and 1 April) was able to reverse the  $\delta^{13}\text{C}$  values in the digestive gland. From 10 April to 30 May, lipid content and  $\delta^{13}\text{C}$  followed the same pattern, whereas they were inversely related for the rest of the study period. Thus lipid content could not explain the  $\delta^{13}\text{C}$  results during April and May, because the two are typically reversed. We believe that physiological processes, in addition to tissue lipid content and food sources, can have profound effects on the isotopic signature of the two stable isotope tracers.

In the literature,  $\delta^{13}\text{C}$  isotopic increases are reported to occur synchronously with spring phytoplankton blooms (Canuel *et al.*, 1995, Rolff, 2000). Here, the high variability of primary production (Chl *a*, fig.1) and seston ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) was not associated with similar variations of the two stable isotopes in the tissues. Further, in our study, the different

organs did not react in the same way. Indeed, *P. maximus* exhibits an annual  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic range as large as 3‰ for the digestive gland, but only 1‰ for the adductor muscle (Fig.2). Furthermore, isotopic values among organs do not vary independently; variations in the gonad follow those in the digestive gland. We suggest that such variation reflects metabolic transfers among organs, and that it is strong enough to mask the association between lipid and  $\delta^{13}\text{C}$ . Previous results of a study on *P. maximus* in the Bay of Brest showed that energy allocation between germinal and somatic compartments exhibited clear seasonal variations (Saout, 2000). Three temporal windows were identified (Fig.6): (1) priority is given to reproduction with a major utilisation of the adductor muscle metabolites; (2) direct transfer of assimilated food to gonad. Excess energy can be stored in the digestive gland, and then in the muscle; (3) restocking of metabolites in the muscle.

A parallel decrease in  $\delta^{13}\text{C}$  values from early May to late June can be seen in all the organs (see Fig. 2A), but differences in lipid content may hide general trends in  $\delta^{13}\text{C}$ . Nitrogen, on the other hand, was not influenced by lipid content and is a better indicator of metabolic fluxes and more appropriate for use in explaining the energy budget. Observed  $\delta^{15}\text{N}$  variations are consistent with the energy-allocation hypothesis proposed by Saout (2000). In winter, the delta values of muscle and gonad are similar because metabolites are transferred from the muscle for gonadal maturation. Thereafter, food ingested and assimilated by the digestive gland is the preferred source of metabolites for gametogenesis, which brings gonadal  $\delta^{15}\text{N}$  values closer to those of the digestive gland and drives them farther from those of the adductor muscle. All values become progressively closer later in the summer and fall as metabolite stores in the muscle are utilised by the gonad.

Herzka et al. (2000), postulated that for poikilotherms such as fishes and crustaceans, the contribution of metabolic turn-over to isotopic changes is poor, whereas in endotherms as mammals and birds, it is high due to their higher basal metabolism (Tieszen et al., 1983;

Hobson & Clark, 1992). However, in *P. maximus*, it seems that the metabolism is important enough to produce such changes in isotopic composition of the various tissues. Tieszen et al. (1983) showed that kinetic changes in isotopic composition of an organ after a diet shift depends on turn-over rates. We can imagine running such experiments with enriched- $^{13}\text{C}$  or  $^{15}\text{N}$  to correctly evaluate carbon and nitrogen turnover.

Information obtained from  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in studies of animals depends on the choice of tissues analysed. The digestive gland best reflects seasonal patterns. Undoubtedly the tissue types analysed in various studies has influenced the evaluation of diets and food web reconstruction (DeNiro & Epstein, 1978; Tieszen *et al.*, 1983). In this study, temporal variation in the  $\delta^{15}\text{N}$  of the digestive gland, gonad and adductor muscle can be understood in terms of the fluxes of metabolites among organs. We conclude that, if possible, seasonal variations and multi-organ studies should be conducted when using stable isotopes to study foodwebs. But as not every study can afford to analyse different organs and different seasons, the choice of the organ and the sampling frequency should be adapted on ecological question asked. For example to assess major sources of C and N, muscle tissues should be sampled rather than whole body, and only a few times per year. But if the question has to do with seasonal variations, then digestive gland might be used together with muscle to correct for the apparent depletion in  $^{13}\text{C}$  of the digestive gland.

But even if seasonal effects and multi-organ variability are incorporated, much uncertainty still remains (e.g. mixed diets, metabolic fractionation, selective assimilation) and an experimental determination of isotopic fractionation between diet and tissues should also be conducted. Stable isotope studies should also consider that metabolism can have profound effects on the isotopic signatures of the two most commonly used stable isotopes tracers. As

many earlier authors have pointed out, we believe that trophic pathways should best be studied through a mix of different strategies.

#### Acknowledgements.

We are grateful to our scuba diving team for dedicated assistance in the collection of *Pecten maximus* samples, the captain and all the crew members of the ships “Côte d’Aquitaine” and “Côte de la Manche” and Per Legal for technical assistance in sampling, Claudie Quéré and Jeanne Moal for their help in preparing tissues samples. We also thank Philippe Soudant for discussion and helpful comments and particularly Susan Ford and anonymous reviewers for English corrections and comments which improved the original manuscript.

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Table 1 : *Pecten maximus* mean dry weights (N = 20 for each date) of the muscle, gonad and digestive gland in 2000.

Sampling dates	Muscle	Gonad	Digestive gland
24-01-00	3,29	0,61	0,62
18-02-00	3,80	1,18	0,66
03-03-00	3,73	1,43	0,66
17-03-00	2,95	0,99	0,53
01-04-00	3,48	1,05	0,81
19-04-00	3,59	1,50	0,95
04-05-00	3,26	1,10	0,87
17-05-00	3,50	0,97	0,87
24-05-00	3,82	1,51	0,95
08-06-00	3,27	1,02	0,73
26-06-00	4,66	1,21	1,25
24-07-00	4,29	0,65	1,07
11-08-00	5,08	1,22	0,97
24-08-00	6,11	1,79	1,32
14-09-00	6,36	0,99	1,46
14-10-00	6,84	1,58	1,46
01-11-00	5,72	1,44	1,09
14-12-00	6,33	1,74	1,17
<b>Mean</b>	<b>4.45</b>	<b>1.22</b>	<b>0.97</b>

## Figure captions

Figure 1: Temporal stable isotope variation (black squares, ‰) of suspended particulate organic matter (SPOM) in relation to chlorophyll *a* (chl *a*) concentration (grey area,  $\mu\text{g.l}^{-1}$ ) in the bottom water. A)  $\delta^{13}\text{C}$ ; B)  $\delta^{15}\text{N}$ .

Figure 2: Mean seasonal isotopic variations ( $N=5$ ;  $\pm$  SD, ‰) in the muscle (M), the digestive gland (DG) and the gonad (G) of *Pecten maximus*, and for suspended particulate organic matter (SPOM). A)  $\delta^{13}\text{C}$ ; B)  $\delta^{15}\text{N}$ .

Figure 3: The  $\delta^{13}\text{C}$  values of the muscle, gonad and digestive gland with respect to their lipid content at the same sampling season (2000 vs. 1996, see explanation in section 3.4): linear relationship,  $R^2 = 0.81$ ,  $p < 0.0001$ .

Figure 4: Three different parameters (mean values) in *Pecten maximus* digestive gland in 2000: ( $\blacktriangle$ ) Lipid content in % of total dry body weight ( $N=5$ ), ( $\blacksquare$ )  $\delta^{13}\text{C}$  ( $N=5$ , ‰), ( $\diamond$ ) Digestive gland index \* 20 (DGI,  $N=20$ )

Figure 5. Natural stable isotope signatures ( $\delta^{15}\text{N}$  vs.  $\delta^{13}\text{C}$ ) of isolated tissues (Digestive gland, Muscle and Gonad) from *Pecten maximus* and potential food sources (Phytoplankton and POM). Tissue data are means of 5 individuals for each of 15 collection dates in 2000. POM data are means for 53 collection dates at the reference station during the year 2000. Phytoplankton data come from Savoye (2001, same reference station in 1998 and 1999,  $n = 74$ ) Error bars are  $\pm$  standard deviation. Black stars are food resources values calculated with classical enrichment factors (see explanation in the text) from muscle (M) and digestive gland (DG) values.

Figure 6. Simplified scheme of energy distribution in *P. maximus* (from results of Saout, 2000). Arrows size corresponds to relative importance of energy transfer. Priority ( $\Rightarrow$ ) and secondary ( $\Rightarrow$ ) processes involved in energy allocation are noted. Shaded arrows ( $\Rightarrow$ ) refer to dietary inputs. Periods 1: October-November to March-April. 2: March-April to June. 3: June to October-November.











