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Influence of food supply on the $\delta^{13}C$ signature of mollusc shells: implications for palaeoenvironmental reconstitutions

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Abstract:

Compared to oxygen isotopes, the carbon isotope composition of biogenic carbonates is less commonly used as proxy for palaeoenvironmental reconstructions because shell $\delta^{13}C$ is derived from both dissolved inorganic (seawater) and organic carbon sources (food), and interactions between these two pools make it difficult to unambiguously identify any independent effect of either. The main purpose of this study was to demonstrate any direct impact of variable food supply on bivalve shell $\delta^{13}C$ signatures, using low/high rations of a ^{13}C-light mixed algal diet fed to 14-month-old (adult) cultured Japanese *Crassostrea gigas* under otherwise essentially identical in vitro conditions during 3 summer months (May, June and July 2003, seawater temperature means at 16, 18 and 20°C respectively) in experimental tanks at the Argenton laboratory along the Brittany Atlantic coast of France. At a daily ration of 12% (versus 4%) oyster dry weight, the newly grown part of the shells (hinge region) showed significantly lower $\delta^{13}C$ values, by 3.5‰ (high ration: mean of −5.8±1.1‰, n=10; low ration: mean of −2.3±0.7‰, n=6; ANOVA Scheffe's test, p<0.0001). This can be explained by an enhanced metabolic activity at higher food supply, raising ^{13}C-depleted respiratory CO_2 in the extrapallial cavity. Based on these $\delta^{13}C$ values and data extracted from the literature, and assuming no carbon isotope fractionation between food and shell, the proportion of shell metabolic carbon would be 26±7 and 5±5% for the high- and low-ration *C. gigas* shells respectively; with carbon isotope fractionation (arguably more realistic), the corresponding values would be 69±14 and 24±9%. Both groups of cultured shells exhibited lower $\delta^{13}C$ values than did wild oysters from Marennes-Oléron Bay in the study region, which is not inconsistent with an independent influence of diet type. Although there was no significant difference between the two food regimes in terms of $\delta^{18}O$ shell values (means of 0.1±0.3 and 0.4±0.2‰ at high and low rations respectively, non-significant Scheffe's test), a positive $\delta^{13}C$ vs. $\delta^{18}O$ relationship recorded at high rations supports the interpretation of a progressive temperature-mediated rise in metabolic activity fuelled by higher food supply (in this case reflecting increased energy investment in reproduction), in terms not only of $\delta^{13}C$ (metabolic signal) but also of $\delta^{18}O$ (seawater temperature signal). Overall, whole-shell $\delta^{18}O$ trends faithfully recorded summer/winter variations in seawater temperature experienced by the 17-month-old cultured oysters.

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Introduction

Compared to oxygen isotopes, the carbon isotope composition of biogenic carbonates is less commonly used as proxy for palaeoenvironmental reconstructions for aquatic systems, a problematic issue recently discussed by, for example, Geist et al. (2005, 2006), Schöne et al. (2006) and McConnaughey and Gillikin (2008). Shell $\delta^{13}C$ is derived from both dissolved inorganic carbon (DIC) and organic carbon sources (food), the latter being incorporated into the shell via the metabolic pathway (e.g. Killingley and Berger 1979; Arthur et al. 1983; Krantz et al. 1987; McConnaughey 1989; Wefer and Berger 1991; Klein et al. 1996; McConnaughey et al. 1997). Complex interactions between these two pools make it difficult to unambiguously identify any independent effect of either in the formation of biogenic carbonates (Fig. 1). Thus, δ^{13}CDIC is controlled by the ratio between photosynthesis and the rate of oxidation of organic matter, whereby the former favours 12C incorporation into algal biomass, leading to a higher δ^{13}CDIC in seawater (McKenzie 1985; Hellings et al. 2000, 2001). Other important interactions are with atmospheric carbon dioxide (e.g. Dettman et al. 1999) and continental and/or oceanic run-off (e.g. Salomons and Mook 1986; Surge et al. 2001).

For marine mollusc shells, models exist for the incorporation of dissolved inorganic carbon (Arthur et al. 1983; Kirby et al. 1998; Khim et al. 2000; Surge et al. 2001) as well as of CO_2 derived from metabolism (Tanaka et al. 1986; Geist et al. 2005; Gillikin et al. 2007). Although the major contribution of $\delta^{13}C_{DIC}$ is undeniable, this cannot explain metabolically induced shell isotopic changes—so-called vital effects—for example, due to intrinsic control anchored in ontogenesis (e.g. Wefer and Berger 1991). As shell growth slows with increasing age, accompanied by an enhanced investment into reproduction, there is commonly an increased incorporation of isotopically light metabolic carbon (C_{meta}) into the shell (cf. total metabolism would increase with age/size), which results in a decrease in shell $\delta^{13}C$ (Krantz et al. 1987; McConnaughey 1989; Mitchell et al. 1994; Gillikin et al. 2007). McConnaughey et al. (1997), Lorrain et al. (2004) and Gillikin et al. (2006) have reported that C_{meta} is typically less than 10% in the shells of marine molluscs but, more recently, Gillikin et al. (2007, 2009) found much higher values of 5-37% for *Mercenaria mercenaria* and of 15-35% for the freshwater mussel *Pyganodon cataracta*, in both cases associated with marked ontogenetic increases in C_{meta} and decreases in $\delta^{13}C$ in the shells.

Contrary to $\delta^{13}C$, the oxygen isotope signatures ($\delta^{18}O$) are not correlated with organism age or shell height (McConnaughey and Gillikin 2008; Gillikin et al. 2009), and vital effects have not been reported to control $\delta^{18}O$ signatures (Wefer and Berger 1991). Shell $\delta^{18}O$ changes rather reflect seawater temperature and salinity regimes (Epstein and Mayeda 1953; Epstein et al. 1953; Killingley and Berger 1979; Jones et al. 1989; Kirby et al. 1998; Pierre 1999; Andrews and Crowe 2000; Surge et al. 2001, 2003; Schöne et al. 2003, 2004; Dettman et al. 2004; Gillikin et al. 2005; Lartaud 2007).

Turner (1982), then Wefer and Berger (1991) have reported the impact of shell growth as such on the $\delta^{13}C$ shell signal. This "kinetic effect" (Fig. 1) results in a preferential incorporation of lighter carbon and also oxygen isotopes, which conducts to a combined decrease in $\delta^{13}C$ and $\delta^{18}O$ (McConnaughey 1989; Mitchell et al. 1994). Unlike for other phylogenetic taxa, such as corals or some foraminifers (Berger et al. 1978; Emiliani et al. 1978; Duplessy et al. 1981; McConnaughey 1989), carbon and oxygen incorporation into mollusc shells does not seem to be strongly influenced by kinetic effects, which could be suppressed by the lower pH and carbonic anhydrase at the calcification site (McConnaughey et al. 1997; McConnaughey and Gillikin 2008).

Metabolic activity is also modulated by extrinsic (environmental) factors potentially influencing $\delta^{13}C_{shell}$ fingerprints, notably food availability (Fig. 1). To date, however, most

100 interest has focused on food type, rather than on the amount of food available (food supply as
101 such).
102 Using a food tracer (C_3/C_4 plants), De Niro and Epstein (1978), Goodfriend and Hood (1983),
103 Stott (2002) and Metref et al. (2003) identified contributions by carbon derived from different
104 trophic pathways in freshwater molluscs and terrestrial gastropods. Feeding on $\delta^{13}C$-light C_3
105 plants reduces $\delta^{13}C$ in shells. Likewise, isotopic characterisation by Aucour et al. (2003)
106 showed an influence of food source (phytoplankton and particulate organic matter) on the
107 $\delta^{13}C$ signature of freshwater bivalve shells. Such dietary impacts are less documented for the
108 shells of marine organisms, particularly in coastal areas (but see, e.g. Botello et al. 1980; Latal
109 et al. 2006), although much work has dealt with their soft body tissues (e.g. De Niro and
110 Epstein 1978; Riera and Richard 1996; Malet 2005; Paulet et al. 2006). Investigations of the
111 link between food supply and shell $\delta^{13}C$ include those of Stott (2002) on the pulmonate snail
112 *Helix aspera*, Aucour et al. (2003) on the freshwater mussels *Dreissena polymorpha* and
113 *Corbicula fluminea*, Geist et al. (2005) on the freshwater mussel *Margaritifera margarititfera*,
114 and Vander Putten et al. (2000) on *Mytilus edulis*.
115 For the Japanese oyster *Crassostrea gigas* (Thunberg, 1793), Riera and Richard (1996)
116 investigated the influence of food quality (type) on soft tissue isotope signatures along the
117 Atlantic French coast (Marennes-Oléron Bay).
118 Within the context presented above, the main purpose of this study was to demonstrate any
119 direct impact of variable food supply on bivalve shell $\delta^{13}C$ signatures, using low/high rations
120 of a ^{13}C-light mixed algal diet fed to 14-month-old cultured *C. gigas* under otherwise
121 essentially identical in vitro conditions during 3 summer months (May-July 2003) in
122 experimental tanks at the Argenton laboratory along the Brittany Atlantic coast of France.
123
124 <heading1>Materials and methods
125 <heading2>*Crassostrea gigas* cultures and diets
126 The oyster's breeding and culture conditions have been reported in detail in Delaporte et al.
127 (2006). In brief, oysters were produced in March 2002 in the IFREMER hatchery at La
128 Tremblade (Charente-Maritime, Atlantic coast of France), from wild broodstock collected in
129 Marennes-Oléron Bay (Figs. 2 and 3). Over the spring/summer of 2002 (April-September),
130 the spat was reared at the Bouin IFREMER station (Vendée), ca. 200 km north along the
131 coast. Juveniles were held in the "claires" of Marennes (Marennes-Oléron Bay) during the
132 following winter (October 2002-April 2003). Claires are open-sky, shallow ponds
133 traditionally used for oyster culture and final conditioning in this area (Goulletquer and Héral

1997). They communicate with the bay through channels, and function as an open system with exchange of water for a few days each spring tide (Audemard et al. 2004). During the remainder of the tidal cycle, they function as a closed system.

At the Bouin station, cultural conditions are carefully controlled to promote spat growth (cf. a summer-like high temperature of at least 17°C). Diet phytoplankton (*Skeletonema costatum)* is produced in drill water rich in manganese (Pirastru 1994; Hussenot and Buchet 1998), in order to internally mark the shells with a manganese spike which can later be identified by means of cathodoluminescence (CL) analysis (Langlet et al. 2006; Lartaud 2007; Barbin et al. 2008). Although no environmental parameters were monitored in the Marennes ponds during our study period, Audemard et al. (2004) reported late winter water temperatures ranging from 8 to 10°C in March 1999 and values lower than 3°C in mid-November 1999, which is consistent with Lartaud's (2007) measurements in other ponds nearby (minimum of -0.4°C in December 2005). Monitoring of salinity in Marennes-Oléron Bay coastal waters since 1977 showed a general rainfall-induced decrease in salinity below 25 PSU during winter months (Struski 2005). Bioavailable food comprises a mixture of estuarine macroalgae and marine particulate organic matter (Riera and Richard 1996).

From the Marennes ponds, 14-month-old sexually ripe oysters were transferred to and cultured at the IFREMER shellfish laboratory in Argenton (Finistère) over spring-early summer 2003 (May to July; Figs. 2 and 3), in two 700 l tanks with 20 µm-filtered seawater. In one tank, the algal ration was maintained at 4% oyster dry weight in algal dry weight per day (CN1 ration, corresponding to 1 l of food diluted in 100 l of seawater supplied per hour); in the other tank, the corresponding value was 12% (CN3 ration, 3 l of food in 100 l of seawater). The concentration of chlorophyll *a* around the oysters was about 5 µg chy a l^{-1} low-ration and 15 µg chlo a l^{-1} under high-ration conditions (Enriquez-Diaz 2004). Oysters were fed with a mixture of three microalgae (*Isochrysis* aff. *galbana*, *Chaetoceros calcitrans* and *Tetraselmis chui*) provided in equal biomass proportions. A mixed diet was preferred because, compared to a single-species diet, this promotes healthy growth and reduces perturbation in tissue production (Utting and Millican 1997; Robert and Gérard 1999). The unicellular algae are cultured in bubbling CO_2, which results in a very low $\delta^{13}C_{diet}$ signature of -47.4‰, associated with $\delta^{13}C_{organic\ tissues}$ of -19.2‰ (at a ration of 8% oyster dry weight in algal dry weight per day; Paulet et al. 2006).

The two tanks were thoroughly cleaned each 48 h and the water exchanged four times per day (Delaporte et al. 2006). It therefore is highly unlikely that oxidation of organic matter by

bacterial activity would have produced any measurable change in $^{13}C_{DIC}$, which was not monitored (but see below). The Argenton laboratory is situated further north along the coast, near Brest; so, during the experiment, the average photoperiod and seawater temperature of the Marennes-Oléron Bay area (16°C mean in May, 18°C mean in June and 20°C mean in July) was artificially applied. Water salinity and oxygenation were maintained constant at 34 PSU and 90-100% respectively. Thus, it seems reasonable to assume that any effect of oyster respiration on $\delta^{13}C_{DIC}$ would have been negligible.

At the end of the feeding experiment (July 2003), CN1 and CN3 specimens had mean shell lengths of 7.1±0.4 cm (n=10) and 7.5±0.6 cm (n=9), i.e. very similar for the two experimental groups. This is consistent with the previous findings of Enriquez-Diaz (2004) who demonstrated that shell growth did not differ measurably between CN1 and CN3 specimens cultured under our Argenton laboratory feeding experiment conditions (mean shell growth rates of low- and high-ration specimens over 12 months were 2.3±0.2 and 2.0±0.3 cm year^{-1} respectively). By contrast, Enriquez-Diaz (2004) and Delaporte et al. (2006) reported that CN3 specimens showed a markedly higher mass of soft body tissues and, by implication, higher metabolic activity than low-ration CN1 oysters of similar size (mass visceral growth was only 0.79±0.26 g year^{-1} for low-ration and 3.31±2.25 g year^{-1} for high-ration specimens). *C. gigas* reaches sexual maturity at an age of ca. 12 months. By this time, somatic growth (e.g. shell) has markedly slowed and energy investment is largely in reproduction. Gametogenesis starts when the seawater temperature increases above 10°C. Energy investment in reproduction increases gradually until spawning, which takes place when the temperature exceeds 19°C (Deslous-Paoli and Héral 1988; Chavez-Villalba et al. 2002). At the end of the feeding experiment in July 2003, three CN1 and three CN3 specimens were selected for stable isotope analyses of carbonate shells (cf. below).

<heading2>Stable isotope analyses of carbonate shells and dissolved inorganic carbon in experimental tanks

For the experimental oysters, the shell sampling for isotope analyses was achieved by mechanical drilling (0.5 mm drill bit diameter) along the maximum growth axis, on polished hinge sections of six oyster shells. Based on Langlet (2002), Lartaud (2007) and Lietard and Pierre (2008), the foliated internal area immediately under the ligament zone was drilled along a sclerochronological profile deduced via cathodoluminescence (cf. Fig. 4). Thin sections were viewed under an optical microscope coupled with a cold cathode (catodine Opea, 15 kV and 200 µA mm^{-2}, pressure 0.1 Torr). A numerical Nikon D70 (800 ASA)

camera was used for luminescence image acquisition (exposure time 10 s), and mounted photographs to generate luminescence spectra by means of JMicrovision software. Note that luminescence analyses are only semi-quantitative, so that luminescence intensity is expressed in arbitrary units (AU). The oldest part of the shell (Bouin station phase) can clearly be distinguished from the Mn-rich algae used as food for the spat (Lartaud 2007; Fig. 4). Moreover, the umbo length at the time of transfer from the winter phase in the Marennes ponds to the spring/summer phase in the Argenton experimental tanks can be deduced from the "natural" seasonal luminescence pattern (cf. luminescence is higher during summer; Langlet et al. 2006; Lartaud et al. 2006; Lartaud 2007). Powdered samples were digested in 100% H_3PO_4 at 50°C and the CO_2 produced analyzed using a mass spectrometer (VG Micromass 602). Isotopic data are reported in conventional delta (δ) notation relative to the Vienna Pee Dee Belemnite (VPDB). An internal standard was calibrated to the NBS-19 reference standard. Precision for both $\delta^{18}O$ and $\delta^{13}C$ is ±0.10‰.

In the Argenton experimental tanks, spot analyses of $\delta^{13}C_{DIC}$ were carried out in September-October 2008 using the protocol outlined by Gillikin and Bouillon (2007), yielding a value of -2.4‰ (*n*=3). For these measurements, seawater maintained at 35 PSU was sampled in tanks containing bivalves (the clam *Ruditapes philippinarum*) with ^{13}C-depleted food, representing physicochemical conditions close to those of our experiment of May-July 2003. The oxygen isotope signature of seawater was not monitored in the Argenton tanks. Note that the Argenton laboratory is far from any river, the tanks are indoors, i.e. protected from any rain, and tank seawater salinity (but not temperature) was in any case maintained constant during the 3-month feeding experiment.

<heading1>Results

<heading2>$\delta^{18}O$ and $\delta^{13}C$ of experimental *Crassostrea gigas* shells

The complete isotope dataset for the six experimental *C. gigas* shells is reported in Table 3 in the electronic supplementary material available online for this article. For the 17-month study period (March 2002-July 2003), extending over three phases at summer-like, winter and then spring-summer temperature regimes, the mean maximum seasonal range in $\delta^{18}O$ signatures is 0.7±0.3‰. Shell-specific luminescence spectra and $\delta^{18}O$ profiles are in generally good agreement (Fig. 5). An oldest shell part (0 to 4-9 mm umbo length) corresponding to the initial summer growth period (T>17°C) at the Bouin station can be distinguished in terms of relatively low $\delta^{18}O$ values (-0.6±0.2‰ for the combined dataset), followed by younger shell material corresponding to subsequent winter growth (T=3-10°C) in the Marennes ponds,

showing heavier isotopic composition (0.7±0.2‰), and then the youngest part of the shell grown under more summer-like temperatures (T=16-20°C) in the Argenton experimental tanks, again with relatively low $\delta^{18}O$ values (0.2±0.2‰), albeit less markedly. The difference between the summer-like Bouin and winter Marennes $\delta^{18}O$ signatures is statistically significant (ANOVA Scheffe's test, $p<0.0001$, Table 1), as is that between the winter Marennes and the summer Argenton $\delta^{18}O$ signatures ($p=0.0052$), but also between the summer-like Bouin and the summer Argenton $\delta^{18}O$ signatures ($p<0.0001$).

For the youngest part of the shell, the low-(CN1) and high-ration (CN3) specimens cultured under summer temperatures in the Argenton tanks have similarly low $\delta^{18}O$ values of 0.4±0.2 and 0.1±0.3‰ respectively, which do not differ statistically (Table 1). The summer CN1 and CN3 $\delta^{18}O$ values are both lower than the winter Marennes signature (although the difference is significant only for CN3). Compared to the summer Bouin signature, those for the summer CN1 and CN3 specimens are both significantly less depleted.

The mean $\delta^{13}C$ of the shells from the Argenton experimental tanks (−4.48±1.1‰) is lower than the mean $\delta^{13}C$ of the oldest shell part corresponding to the Bouin growth phase (-2.3±0.1‰) and of the shell material corresponding to the Marennes pond growth phase (-1.5±0.2‰). No statistical difference is observed (ANOVA Scheffe's test, $p=0.2880$, Table 1) between the summer-like Bouin and winter Marennes mean $\delta^{13}C$ signatures. On the contrary, the difference is statistically significant ($p<0.0001$) between the summer-like Bouin and the summer Argenton $\delta^{13}C$ signatures, as is the case between the winter Marennes and the summer Argenton $\delta^{13}C$ signatures.

$\delta^{13}C$ is clearly different between the low- (CN1) and high-ration (CN3) shells (Fig. 6). The CN1 specimens have higher isotopic values (mean of -2.3±0.7‰), close to those for the summer-like Bouin and the winter Marennes pond growth phases (ANOVA Scheffe's test, $p=0.9980$ and $p=0.2650$ respectively, Table 1). By contrast, the CN3 specimens are characterized by markedly lower $\delta^{13}C$ signatures (mean of -5.8±1.1‰), which is statistically from the mean values of all other growth phases and conditions (Table 1). As revealed by the $\delta^{13}C$ profile of each shell (Fig. 5), the decrease in isotopic values is gradual and not correlated with the beginning of the feeding experiment in the Argenton tanks.

For the youngest part of the shell (Argenton tanks), there is a positive linear correlation between $\delta^{13}C$ and $\delta^{18}O$ for the high-ration CN3 shells ($\delta^{13}C=3.16\times\delta^{18}O-6.14$, $R^2=0.79$, $n=10$), but not for the low-ration CN1 shells ($R^2=0.01$, $n=6$, see Fig. 6).

<heading1>Discussion and conclusions

<heading2>$\delta^{18}O$ in cultured *Crassostrea gigas* shells

The finding that both shell-specific luminescence spectra and $\delta^{18}O$ profiles are in generally good agreement with the "seasonal" variations in seawater temperature at which *C. gigas* was cultured is consistent with the strong impact of physicochemical conditions reported for shell isotopes in oysters, and other bivalves, notably temperature. Even a cursory glance at Fig. 6 shows a distinct separation between the summer-like Bouin growth period at high temperatures exceeding 17°C, with most negative $\delta^{18}O$ values, and winter growth in the Marennes ponds at distinctly lower temperatures of 3-10°C, with most positive $\delta^{18}O$ values. Between these two extremes, the broadly intermediate grouping of the low- and high-ration specimens is not inconsistent with the relatively warm temperatures of 16-20°C used in the Argenton experimental tanks but, nevertheless, more difficult to interpret, also because of lack of accompanying data on $\delta^{18}O$ seawater.

Considerably overlap between the summer low-ration CN1 and the winter Marennes shell $\delta^{18}O$ values (non-significant Scheffe's test) could at least in part be explained by a decrease in salinity in the Marennes ponds, leading to lower seawater $\delta^{18}O$ (whereas salinity was maintained constant in the Argenton experimental tanks). Effects of freshwater and evaporation on the oxygen isotope composition of seawater and, thereby, shells are well known and, in fact, Struski (2005) reported a rainfall-induced decrease in salinity below 25 PSU during winter months in coastal waters of the Marennes-Oléron Bay since 1977. There is free exchange between the open bay waters and the ponds during a few days each spring tide (see above). At other times of the tidal cycle when the ponds function as a closed system, the impact of direct rain input would be particularly strong, especially during the rainy winter season.

An additional explanation could be that the Argenton tank temperature regime spanned both cooler spring (16°C) and warmer summer (20°C) temperatures. Thus, one would expect any effect of seawater temperature to be more blurred than that demarcating the summer Bouin and winter Marennes growth phases. This would largely account also for only minimal overlap between the summer low-ration CN1 and the summer Bouin $\delta^{18}O$ shell signals (Fig. 6). By contrast, more substantial overlap between the summer high-ration CN3 and the summer Bouin $\delta^{18}O$ shell signals incorporates a positive linear relationship between $\delta^{18}O$ and $\delta^{13}C$ for the CN3 shells, spanning a larger range also of more negative values for both signatures. One plausible interpretation would be that, as tank seawater temperature increased from 16°C in May to 18°C in June and then to 20°C in July 2003, there was a progressive temperature-mediated stimulation of metabolic activity supported by the high ration fed to the

CN3 specimens, associated with progressive decreases in both $\delta^{18}O$ (seawater temperature signal) and $\delta^{13}C$ (metabolic signal) at the growing shell margin (cf. two of the three specimens in Fig. 5, and also see below).

<heading2>$\delta^{13}C$ in cultured *Crassostrea gigas* shells

The similar shell $\delta^{13}C$ values recorded during the Bouin station, Marennes pond, and Argenton low-ration CN1 growth phases (-2.3±0.1, -1.5±0.2 and -2.3±0.7‰ respectively) could be explained by a lack of strong differences in local $\delta^{13}C_{DIC}$ signatures between these sites. $\delta^{13}C_{DIC}$ of -2.5‰ has been recorded in May 1993 at the mouth of the Charente estuary, close to the Marennes-Oléron Bay (Riera and Richard 1996). In other ponds close to Marennes, with physicochemical conditions similar to those of the present study, Lartaud (2007) documented $\delta^{13}C_{DIC}$ of -2.3±1.4‰ and $\delta^{13}C_{oyster\ shells}$ of -2.3±0.2‰, interpreted as reflecting major control of seawater carbon isotope composition on the shell fingerprint of this species. Furthermore, $\delta^{13}C_{DIC}$ was -2.4‰ in the Argenton laboratory tanks during the feeding experiment (see Materials and methods). Due to the lack of $\delta^{13}C_{DIC}$ monitoring data over our 17-month study period, however, we deem it inappropriate to speculate further on this aspect. Likewise, the finding that both low-ration CN1 and high-ration CN3 shells exhibit lower $\delta^{13}C$ values than do wild oysters from the Marennes-Oléron Bay (Lartaud 2007), which feed on ^{13}C-enriched algae ($\delta^{13}C$ of -22.6±0.7‰; Riera and Richard 1996), and also from Normandy (Lartaud 2007; Fig. 7) is not inconsistent with an influence of the ^{13}C-depleted algal diet (-47.4‰; Paulet et al. 2006) fed to adult oysters during the Argenton feeding experiment but, because of lacking isotopic data on the different algal diets used for the rearing of spat and juveniles, we cannot comment on why the Bouin station and the Marennes pond signatures should also be lower.

Despite the lack of $\delta^{13}C_{DIC}$ monitoring during the Argenton feeding experiment, and as argued in the Materials and methods, it is likely that $\delta^{13}C_{DIC}$ did not differ measurably between the low- and high-ration treatments. We therefore contend that the significant decrease in shell $\delta^{13}C$—by -3.5‰—recorded at high rations (CN3) of one and the same diet is due largely to an enhanced metabolic activity, superimposed on a possible effect of diet as such. This is consistent with the long-known effect of metabolism on the uptake of shell ^{13}C derived from organic carbon, and the findings of Baldwin and Newell (1995), Enriquez-Diaz (2004), Delaporte (2005), Bayne and Svensson (2006), Delaporte et al. (2006) and Paulet et al. (2006) who showed that an increase in food availability promotes the ecophysiological activity of oysters (cf. higher somatic growth, reproduction and respiration). Metabolic carbon (C_{meta})

incorporation causes decrease in shell $\delta^{13}C$ (Tanaka et al. 1986): respiratory CO_2, poor in ^{13}C, is hydroxylated and transferred to the carbonate-secreting fluid in the extrapallial cavity (Mc Connaughey 1989; Wefer and Berger 1991; Klein et al. 1996; McConnaughey et al. 1997). C_{meta} can be assessed by an equation developed after Tanaka et al. (1986) and modified by McConnaughey et al. (1997):

$$C_{meta} \times \delta^{13}C_{meta} + (1-C_{meta})\delta^{13}C_{DIC} = \delta^{13}C_{shell} - \varepsilon_{CaCO3\text{-}HCO3^-}$$

where C_{meta} is the proportion (%) of metabolic (respired) carbon in the shell, $\delta^{13}C_{meta}$ and $\delta^{13}C_{DIC}$ are the isotope compositions of HCO_3^- derived from metabolism and dissolved inorganic carbon respectively, and $\varepsilon_{CaCO3\text{-}HCO3^-}$ is the enrichment factor between calcite and bicarbonate (1‰ in Romanek et al. 1992). Within the constraints identified above, we attempt a rough estimation of C_{meta} for *C. gigas* cultured at low- and high-rations in the Argenton laboratory, for both treatments using $\delta^{13}C_{DIC}$ of -2.4‰ and $\delta^{13}C_{organic\ tissues}$=-19.2‰ (Paulet et al. 2006; cf. Materials and methods) as a measure of $^{13}C_{meta}$ (cf. Tanaka et al. 1986). Assuming no carbon isotope fractionation between food and shell (arguably unrealistic), the proportions of shell metabolic carbon would be 5±5 and 26±7% for low- and high-ration *C. gigas* shells respectively. With carbon isotope fractionation (arguably more realistic; see Balakrishnan and Yapp 2004), the corresponding values would be 24±9% and an impressive 69±14% (Table 2).

We caution that these inferred values need independent confirmation. Nevertheless, and in contrast with the findings of McConnaughey et al. (1997), Lorrain et al. (2004) and Gillikin et al. (2006), the impact of metabolic activity on shell organic carbon sequestration could be higher than expected in bivalves, in the present case fuelled by increased food supply. This contributes to the mounting evidence that C_{meta} can substantially exceed 10% in both marine and freshwater molluscs (Gillikin et al. 2007, 2009). However, not only intrinsic control of shell growth during ontogeny but also extrinsic constraints imposed by variable food supply can evidently modulate the influence of metabolism on the $\delta^{13}C$ fingerprints of bivalves.

In the case of the Argenton low- and high-ration experimental adult oysters, inferred variations in metabolic activity would result largely from corresponding changes in reproductive, rather than somatic growth (see Materials and methods). In addition to food-induced effects, there is independent evidence of increasing seawater temperature promoting reproduction in oysters (e.g. Deslous-Paoli and Héral 1988; Chavez-Villalba et al. 2002), consistent with the positive $\delta^{13}C$ vs. $\delta^{18}O$ relationship observed in the high-ration shells. Evidently, the combined carbon and oxygen isotopic patterns of shells can be modulated by key environmental drivers of reproduction. By implication, the role of gametogenesis in

interpreting proxy records needs more attention than has been the case to date, notably for $\delta^{13}C$.

Geochemical records in mollusc shells are notoriously "corrupted" by physiology and, as discussed by Schöne (2008), physiology and shell growth are inextricably intertwined. Shells record ambient environmental conditions only when growing; when not, this causes, for example, oxygen isotope-derived temperature records to be incomplete and temperature amplitudes often truncated (e.g. Goodwin et al. 2003; Ivany et al. 2003). In addition to well-known physiology-dictated isotopic changes with ontogenesis, the present paper has demonstrated that the amount of food can be a major factor influencing the carbon isotope composition of shells. By implication, more work on such extrinsic control of $\delta^{13}C$ is urgently needed before shells can be fully used for palaeoenvironmental reconstitutions. Although it seems that this will not be a simple task, there is a huge potential for integrating this aspect into high-resolution studies of ancient environments using shells.

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References

Andrus CFT, Crowe DE (2000) Geochemical analysis of *Crassostrea virginica* as a method to determine season of capture. J Archaeol Sci 27:33-42

Arthur MA, Williams DF, Jones DS (1983) Seasonal temperature-salinity changes and thermocline in the mid-Atlantic Bight as recorded by the isotopic composition of bivalves. Geology 11:655-659

Aucour AM, Sheppard SMF, Savoye R (2003) $\delta^{13}C$ of fluvial mollusk shells (Rhône River): a proxy for dissolved inorganic carbon? Limnol Oceanogr 48:2186-2193

405 Audemard C, Sajus MC, Barnaud A, Sautour B, Sauriau PG, Berthe FJC (2004) Infection
406 dynamics of *Marteilia refrigens* in flat oyster *Ostrea edulis* and copepod *Paracartia grani* in
407 a claire pond of Marennes-Oléron Bay. Diseases Aquat Organisms 61:103-111
408 Balakrishnan M, Yapp CJ (2004) Flux balance models for the oxygen and carbon isotope
409 compositions of land snails shells. Geochim Cosmochim Acta 68:2007-2024
410 Baldwin BS, Newell RIE (1995) Feeding rate responses of oyster larvae (*Crassostrea*
411 *virginica*) to seston quantity and composition. J Exp Mar Biol Ecol 189:77-91
412 Barbin V, Ramseyer K, Elfman M (2008) Biological record of added manganese in seawater:
413 a new efficient tool to mark in vivo growth lines in the oyster species *Crassostrea gigas*. Int J
414 Earth Sci 97:193-199
415 Bayne BL, Svensson S (2006) Seasonal variability in feeding behaviour, metabolic rates and
416 carbon and nitrogen balances in the Sydney oyster, *Saccostrea glomerata* (Gould). J Exp Mar
417 Biol Ecol 332:12-26
418 Berger WH, Killingley JS, Vincent E (1978) Stable isotopes in deep-sea carbonates: box core
419 ERDC-92, West Equatorial Pacific. Oceanol Acta 1:203-216
420 Botello AV, Mandelli EF, Macko S, Parker PL (1980) Organic carbon isotope ratios of
421 Recent sediments from coastal lagoons of the Gulf of Mexico, Mexico. Geochim Cosmochim
422 Acta 44:557-559
423 Chavez-Villalba J, Barret J, Mingant C, Cochard JC, Le Pennec M (2002) Autumn
424 conditioning of the oyster *Crassostrea gigas*: a new approach. Aquaculture 210:171-186
425 Delaporte M (2005) Modulation des paramètres hémocytaires par la nutrition chez l'huître
426 creuse *Crassostrea gigas*. Implication dans les mortalités estivales. PhD Thesis, Université
427 Rennes 1
428 Delaporte M, Soudant P, Lambert C, Moal J, Pouvreau S, Samain JF (2006) Impact of food
429 availability on energy storage and defense related hemocyte parameters of the Pacific oyster
430 *Crassostrea gigas* during an experimental reproductive cycle. Aquaculture 254:571-582
431 De Niro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in
432 animals. Geochim Cosmochim Acta 42:495-506
433 Deslous-Paoli JM, Héral M (1988) Biochemical composition and energy value of *Crassostrea*
434 *gigas* (Thunberg) cultured in the bay of Marennes-Oléron. Aquat Living Resources 1:239-249
435 Dettman DL, Reische AK, Lohmann KC (1999) Controls on the stable isotope composition of
436 seasonal growth bands in aragonitic fresh-water bivalves (Unionidae). Geochim Cosmochim
437 Acta 63 (7/8):1049-1057
438 Dettman DL, Flessa KW, Roopnarine PD, Schöne BR, Goodwin DH (2004) The use of

63
64
65

439 oxygen isotope variation in shells of estuarine mollusks as a quantitative record of seasonal
440 and annual Colorado River discharge. Geochim Cosmochim Acta 68:1253-1263
441 Duplessy JC, Blank PL, Bé AWH (1981) Oxygen-18 enrichment of planktonic foraminifera
442 due to gametogenic calcification below the euphotic zone. Science 213:1247-1250
443 Emiliani C, Hudson JH, Shinn EA, George RY (1978) Oxygen and carbon isotopic growth
444 record in a reef coral from the Florida Keys and a deep-sea coral from Blake Plateau. Science
445 202:627-629
446 Enriquez-Diaz MR (2004) Variabilité et bioénergétique de la reproduction chez l'huître creuse
447 *Crassostrea gigas*. PhD Thesis, Université Bretagne Occidentale
448 Epstein S, Mayeda T (1953) Variation of ^{18}O content of waters from natural sources.
449 Geochim Cosmochim Acta 4:213-224
450 Epstein S, Buchsbaum R, Lowenstam HA, Urey HC (1953) Revised carbonate-water isotopic
451 temperature scale. Geol Soc Am Bull 64:1315-1326
452 Geist J, Auerswald K, Boom A (2005) Stable carbon isotopes in freshwater mussel shells:
453 environmental record or marker for metabolic activity? Geochim Cosmochim Acta
454 69:3545-3554
455 Geist J, Auerswald K, Boom A (2006) Response to the comment by B. Schöne et al. (2006)
456 on "Stable carbon isotopes in freshwater mussel shells: environmental record or marker for
457 metabolic activity?". Geochim Cosmochim Acta 70:2662-2664
458 Gillikin DP, Bouillon S (2007) Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved
459 inorganic carbon using a simple modification of an elemental analyzer-isotope ratio mass
460 spectrometer (EA-IRMS): an evaluation. Rapid Commun Mass Spectrom 21:1475-1478
461 Gillikin DP, De Ridder F, Ulens H, Elskens M, Keppens E, Baeyens W, Dehairs F (2005)
462 Assessing the reproducibility and reliability of estuarine bivalve shells (*Saximodus giganteus*)
463 for sea surface temperature reconstruction: implication for paleoclimate studies. Palaeogeogr,
464 Palaeoclimatol, Palaeoecol 228:70-85
465 Gillikin DP, Lorrain A, Bouillon S, Willenz P, Dehairs F (2006) Stable carbon isotopic
466 composition of *Mytilus edulis* shells: relation to metabolism, salinity, $\delta^{13}C$ DIC and
467 phytoplankton. Org Geochem 37:1371-1382
468 Gillikin DP, Lorrain A, Meng L, Dehairs F (2007) A large metabolic carbon contribution to
469 the $\delta^{13}C$ record in marine aragonitic bivalve shells. Geochim Cosmochim Acta 71:2936-2946
470 Gillikin DP, Hutchinson KA, Kumai Y (2009) Ontogenic increase in metabolic carbon in
471 freshwater mussel shells (*Pyganodon cataracta*). J Geophys Res 114 G01007. doi:
472 10.1029/2008JG000829

63
64
65

473 Goodfriend GA, Hood DG (1983) Carbon isotope analysis of land snail shells: implications
474 for carbon sources and radiocarbon dating. Radiocarbon 25:810-830
475 Goodwin DH, Schöne BR, Dettman DL (2003) Resolution and fidelity of oxygen isotopes as
476 paleotemperature proxies in bivalve mollusk shells: models and observations. Palaios
477 18:110-125
478 Goulletquer P, Héral M (1997) Marine molluscan production trends in France: from fisheries
479 to aquaculture. NOAA Tech Rep NMFS 129:137-164
480 Hellings L, Van Den Driessche K, Baeyens W, Keppens E, Dehairs F (2000) Origin and fate
481 of dissolved inorganic carbon in interstitial waters of two freshwater intertidal areas: a case
482 study of the Scheldt Estuary, Belgium. Biogeochemistry 51:141-160
483 Hellings L, Dehairs F, Van Damme S, Baeyens W (2001) Dissolved inorganic carbon in a
484 highly polluted estuary (the Scheldt). Limnol Oceanogr 46:1406-1414
485 Hussenot J, Buchet V (1998) Marais maritimes et aquaculture. Activités durables pour la
486 préservation et l'exploitation des zones humides littorales. Quae, Versailles
487 Ivany CL, Wilkinson BH, Jones DS (2003) Using stable isotopic data to resolve rate and
488 duration of growth throughout ontogeny: an example from the surf clam, *Spisula solidissima*.
489 Palaios 18:126-137
490 Jones DS, Arthur MA, Allards DJ (1989) Sclerochronological records of temperature and
491 growth from shells of *Mercenaria mercenaria* from Narragansett Bay, Rhode Island. Mar
492 Biol 102:225-234
493 Khim BK, Woo KS, Je JG (2000) Stable isotope profiles of bivalve shells: seasonal
494 temperature variations, latitudinal temperature gradients and biological carbon cycling along
495 the east coast of Korea. Cont Shelf Res 20:843-861
496 Killingley JS, Berger WH (1979) Stable isotopes in a mollusk shell: detection of upwelling
497 events. Science 205(13):186-188
498 Kirby MX, Soniat TM, Spero HJ (1998) Stable isotope sclerochronology of Pleistocene and
499 Recent oyster shells (*Crassostrea virginica*). Palaios 13:560-569
500 Klein RT, Lohmann KC, Thayer CW (1996) Sr/Ca and $^{13}C/^{12}C$ ratios in skeletal calcite of
501 *Mytilus trossulus*: covariation with metabolic rate, salinity, and carbon isotopic composition
502 of seawater. Geochim Cosmochim Acta 60 (21):4207-4221
503 Krantz DE, Williams DF, Jones DS (1987) Ecological and paleoenvironmental information
504 using stable isotope profiles from living and fossil molluscs. Palaeogeogr Palaeoclimatol
505 Palaeoecol 58:249-266
506 Langlet D (2002) Enregistrement haute fréquence des conditions environnementales par les

63
64
65

tests de bivalves. Application des techniques de marquage, cathodoluminescence, et chimie à l'huître *Crassostrea gigas* de l'étang de Thau (Hérault, France). PhD Thesis, UPMC Paris 06

Langlet D, Alunno-Bruscia M, Rafélis M, Renard M, Roux M, Schein E, Buestel D (2006) Experimental and natural manganese-induced cathodoluminescence in the shell of the Japanese oyster *Crassostrea gigas* (Thunberg, 1793) from Thau Lagoon (Hérault, France): ecological and environmental implications. Mar Ecol Prog Ser 317:143-156

Lartaud F (2007) Les fluctuations haute fréquence de l'environnement au cours des temps géologiques. Mise au point d'un modèle de référence actuel sur l'enregistrement des contrastes saisonniers dans l'Atlantique nord. PhD Thesis, UPMC Paris 06

Lartaud F, Langlet D, De Rafelis M, Emmanuel L, Renard M (2006) Description of seasonal rythmicity in fossil oyster shells *Crassostrea aginensis*, Tournouer, 1914 (Aquitanian) and *Ostrea bellovacina*, Lamarck, 1806 (Thanetian). Cathodoluminescence and sclerochronological approaches. Geobios 39:845-852

Latal C, Piller WE, Harzhauser M (2006) Shifts in oxygen and carbon isotope signals in marine molluscs from the Central Paratethys (Europe) around the Lower/Middle Miocene transition. Palaeogeogr Palaeoclimatol Palaeoecol 231:347-360

Lietard C, Pierre C (2008) High-resolution isotopic records ($\delta^{18}O$ and $\delta^{13}C$) and cathodoluminescence study of lucinid shells from methane seeps of the Eastern Mediterranean. In: Gröcke DR, Gillikin DP (eds) Advances in mollusc sclerochronology and sclerochemistry: tools for understanding climate and environment. Geo-Mar Lett SI 28(5/6):195-203. doi: 10.1007/s00367-008-0100-z

Lorrain A, Paulet YM, Chauvaud L, Dunbar R, Mucciarone D, Fontugne M (2004) $\delta^{13}C$ variation in scallop shells: increasing metabolic carbon contribution with body size? Geochim Cosmochim Acta 68(17):3509-3519

Malet N (2005) Ecologie alimentaire de l'huître *Crassostrea gigas*: dynamiques des compositions isotopiques naturelles. PhD Thesis, Université La Rochelle

McConnaughey T (1989) ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. Geochim Cosmochim Acta 53:151-162

McConnaughey TA, Gillikin DP (2008) Carbon isotopes in mollusk shell carbonates. In: Gröcke DR, Gillikin DP (eds) Advances in mollusc sclerochronology and sclerochemistry: tools for understanding climate and environment. Geo-Mar Lett SI 28(5/6):287-299. doi: 10.1007/s00367-008-0116-4

McConnaughey T, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: respiration and photosynthesis. Geochim Cosmochim Acta 61 (3):611-622

McKenzie JA (1985) Carbon isotopes and productivity in the lacustrine and marine environment. In: Stumm W (ed) Chemical processes in lakes, Wiley, New York, pp 99-118

Metref S, Rousseau DD, Bentaleb I, Labonne M, Vianey-Liaud M (2003) Study of the diet effect on $\delta^{13}C$ of shell carbonate of the land snail *Helix aspersa* in experimental conditions. Earth Planet Sci Lett 211:381-393

Mitchell L, Fallick AE, Curry GB (1994) Stable carbon and oxygen isotope compositions of mollusc shells from Britain and New Zealand. Palaeogeogr Palaeoclimatol Palaeoecol 111:207-216

Paulet YM, Lorrain A, Richard J, Pouvreau S (2006) Experimental shift in diet $\delta^{13}C$: a potential tool for ecophysiological studies in marine bivalves. Org Geochem 37:1359-1370

Pierre C (1999) The oxygen and carbon isotope distribution in the Mediterranean water masses. Mar Geol 153:41-55

Pirastru L (1994) The Bay of Bourgneuf underground salt water: physicochemical characteristics, bioavailability of phosphates and potential fertility for *Skeletonema costatum* (Grev.) Cleve. PhD Thesis, Université Nantes

Riera P, Richard P (1996) Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine Bay of Marennes-Oleron. Estuarine Coastal Shelf Sci 42:347-360

Robert R, Gérard A (1999) Bivalve hatchery technology: the current situation for the Pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France. Aquat Living Resources 12:121-130

Romanek CS, Grossman EL, Morse JW (1992) Carbon isotopic fractionation in synthetic aragonite and calcite: effects of temperature and precipitation rate. Geochim Cosmochim Acta 56:419-430

Salomons W, Mook WG (1986) Isotope geochemistry of carbonates in the weathering zone. In: Fritz P, Fontes JC (eds) Handbook of Environmental Isotope Geochemistry, vol 2. Elsevier, Amsterdam, pp. 239-270

Schöne BR (2008) The curse of physiology—challenges and opportunities in the interpretation of geochemical data from mollusk shells. In: Gröcke DR, Gillikin DP (eds) Advances in mollusc sclerochronology and sclerochemistry: tools for understanding climate and environment. Geo-Mar Lett SI 28(5/6):269-285. doi: 10.1007/s00367-008-0114-6

Schöne BR, Tanabe K, Dettman DL, Sato S (2003) Environmental controls on shell growth rates and $\delta^{18}O$ of the shallow-marine bivalve mollusk *Phacosoma japonicum* in Japan. Mar Biol 142:473-485

575 Schöne BR, Freyre Castro AD, Friebig J, Houk SD, Oschmann W, Kröncke I (2004) Sea
576 surface water temperatures over the period 1884-1983 reconstructed from oxygen isotope
577 ratios of a bivalve mollusk shell (*Arctica islandica*, southern North Sea). Palaeogeogr
578 Palaeoclimatol Palaeoecol 212:215-232
579 Schöne BR, Rodland DL, Surge DM, Fiebig J, Gillikin DP, Baier SM, Goewert A (2006)
580 Comment on "Stable carbon isotopes in freshwater mussel shells: environmental record or
581 marker for metabolic activity?" by J. Geist et al. (2005). Geochim Cosmochim Acta
582 70:2658-2661
583 Stott LD (2002) The influence of diet on the $\delta^{13}C$ of shell carbon in the pulmonate snail *Helix*
584 *aspersa*. Earth Planet Sci Lett 195:249-259
585 Struski C (2005) Modélisation des flux de matières dans la baie de Marennes-Oléron:
586 couplage de l'hydrodynamisme, de la production primaire et de la consommation par les
587 huîtres. PhD Thesis, Université La Rochelle
588 Surge D, Lohmann KC, Dettman DL (2001) Controls on isotopic chemistry of the American
589 oyster, *Crassostrea virginica*: implications for growth patterns. Palaeogeogr Palaeoclimatol
590 Palaeoecol 172:283-296
591 Surge DM, Lohmann KC, Goodfriend GA (2003) Reconstructing estuarine conditions: oyster
592 shells as recorders of environmental change, Southwest Florida. Estuarine Coastal Shelf Sci
593 57:737-756
594 Tanaka N, Monaghan MC, Rye DM (1986) Contribution of metabolic carbon to mollusc and
595 barnacle shell carbonate. Nature 320:520-523
596 Turner JV (1982) Kinetic fractionation of $\delta^{13}C$ during calcium carbonate precipitation.
597 Geochim Cosmochim Acta 46:1183-1191
598 Utting SD, Millican PF (1997) Techniques for the hatchery conditioning of bivalve
599 broodstocks and the subsequent effect on egg quality and larval viability. Aquaculture
600 155:47-56
601 Vander Putten E, Dehairs F, Keppens E, Baeyens W (2000) High resolution distribution of
602 trace elements in the calcite shell layers of modern *Mytilus edulis*: environmental and
603 biological controls. Geochim Cosmochim Acta 64(6):997-1011
604 Wefer G, Berger WH (1991) Isotope paleontology: growth and composition of extant
605 calcareous species. Mar Geol 100: 207-248
606

63
64
65

Fig. 1 Schematic representation of the main sources and flows of carbon incorporated into mollusc shells. Note existing knowledge of ca. 5-40% metabolic carbon incorporation (based on McConnaughey et al. 1997; Gillikin et al. 2007, 2009; McConnaughey and Gillikin 2008)

Fig. 2 Map of the Atlantic coast of France, showing the locations of various study sites dealt with in this paper: *1* La Tremblade IFREMER hatchery, *2* Bouin IFREMER station, *3* Marennes marine ponds close to the Marennes-Oléron Bay and *4* Argenton IFREMER laboratory

Fig. 3 Overview of cultivation history of *C. gigas* spanning the 14 months of growth in the hatchery, Bouin (spat) and Marennes ponds (juveniles), followed by 3-months experimental growth at different food rations in the Argenton experimental tanks

Fig. 4 Photograph showing the location of the hinge region sampled for isotopic analyses, based on pre-examination by cathodoluminescence (CL) to determine those parts of the shell corresponding to the summer (Bouin)/winter (Marennes ponds)/summer (Argenton) growing stages. Example taken from shell AGcn1-15 (low ration). *AU* Arbitrary units

Fig. 5 Cathodoluminescence (*grey line*), $\delta^{18}O$ (*continuous black lines*) and $\delta^{13}C$ (*dashed lines*) of six *C. gigas* shells from the low-ration CN1 group (AGcn1-7, AGcn1-11, AGcn1-15) and high-ration CN3 group (AGcn3-1, AGcn3-10, AGcn3-14). Isotope values are ‰ VPDB; note the inverted $\delta^{18}O$ scales. The growth periods at the Bouin station (a, summer), Marennes ponds (b, winter) and Argenton laboratory (c, summer, CN1 and CN3 treatments) can be distinguished. CL (*AU*) cathodoluminescence (arbitrary units)

Fig. 6 Cross plot of carbon and oxygen isotope compositions of six shells cultured at summer temperatures at the Bouin station (spat), then winter temperatures in the Marennes ponds (juveniles), followed by spring/summer temperatures with either low or high food rations at the Argenton laboratory (CN1 4%, CN3 12% oyster dry weight in algal ration dry weight per day). Note the positive $\delta^{13}C$ vs. $\delta^{18}O$ relationship at high rations

Fig. 7 Comparison of the $\delta^{13}C_{shell}$ signatures (mean and ±95% confidence intervals) of *C. gigas* cultured at the Bouin station, in the Marennes ponds, and at the Argenton laboratory at low and high experimental rations of ^{13}C-depleted algae ($\delta^{13}C_{diet}$ of -47.4‰), cultured *C. gigas* from L'Houmeau ponds (Charente-Maritime) and coastal waters in Normandy (English Channel) and the Marennes-Oléron Bay (Bay of Biscay), feeding on normal algae ($\delta^{13}C_{diet}$ of -21.7‰).

Table 1 ANOVA Scheffe's test at 5% significance level (S significant) of the differences in isotopic signal recorded between *C. gigas* shells grown during an initial summer period in the

Bouin station (March-September 2002), followed by a winter period in the Marennes ponds (November 2002-April 2003), and then a summer period with either low (CN1) or high (CN3) food rations in the Argenton experimental tanks (May-July 2003)

	Mean difference	Critical difference	*p* value
$\delta^{18}O$ (‰VPDB), CN1 and CN3 combined			
Summer CN1+CN3 vs. summer Bouin	0.811	0.351	<0.0001 S
Summer CN1+CN3 vs. winter Marennes	–0.477	0.351	0.0052 S
Summer Bouin vs. winter Marennes	–1.288	0.351	<0.0001 S
$\delta^{13}C$ (‰VPDB), CN1 and CN3 combined			
Summer CN1+CN3 vs. summer Bouin	–2.229	1.121	<0.0001 S
Summer CN1+CN3 vs. winter Marennes	–2.938	1.121	<0.0001 S
Summer Bouin vs. winter Marennes	–0.708	1.121	0.2880
$\delta^{18}O$ (‰VPDB), CN1 and CN3 not combined			
Summer CN1 vs. summer CN3	0.246	0.586	0.6859
Summer CN1 vs. summer Bouin	0.964	0.543	0.0001 S
Summer CN1 vs. winter Marennes	–0.324	0.543	0.4019
Summer CN3 vs. summer Bouin	0.718	0.457	0.0006 S
Summer CN3 vs. winter Marennes	–0.570	0.457	0.0089 S
$\delta^{13}C$ (‰VPDB), CN1 and CN3 not combined			
Summer CN1 vs. summer CN3	3.454	1.149	<0.0001 S
Summer CN1 vs. summer Bouin	–0.071	1.065	0.9981
Summer CN1 vs. winter Marennes	–0.779	1.065	0.2650
Summer CN3 vs. summer Bouin	–3.525	0.897	<0.0001 S
Summer CN3 vs. winter Marennes	–4.233	0.897	<0.0001 S

Table 2 Inferred proportion of metabolic carbon (C_{meta}) in *C. gigas* shells grown during summer 2003 (May-July) with either low (CN1) or high (CN3) food rations in the Argenton experimental tanks, either without or with carbon isotope fractionation between food and shell (isotopic data are given in ‰ VPDB; see text for more details).

	CN1	CN3

$\delta^{13}C_{shell}$	–2.3±0.7	–5.8±1.1
$\varepsilon_{CaCO3\text{-}HCO3^-}$	1.0	1.0
Without food/shell fractionation		
$\delta^{13}C_{meta}$	–19.2	–19.2
$\delta^{13}C_{DIC}$	–2.4	–2.4
C_{meta}	5±5%	26±7%
With food/shell fractionation		
$\delta^{13}C_{meta}$	–9.2	–9.2
$\delta^{13}C_{DIC}$	–1.0	–1.0
C_{meta}	24±9%	69±14%

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Figure 1

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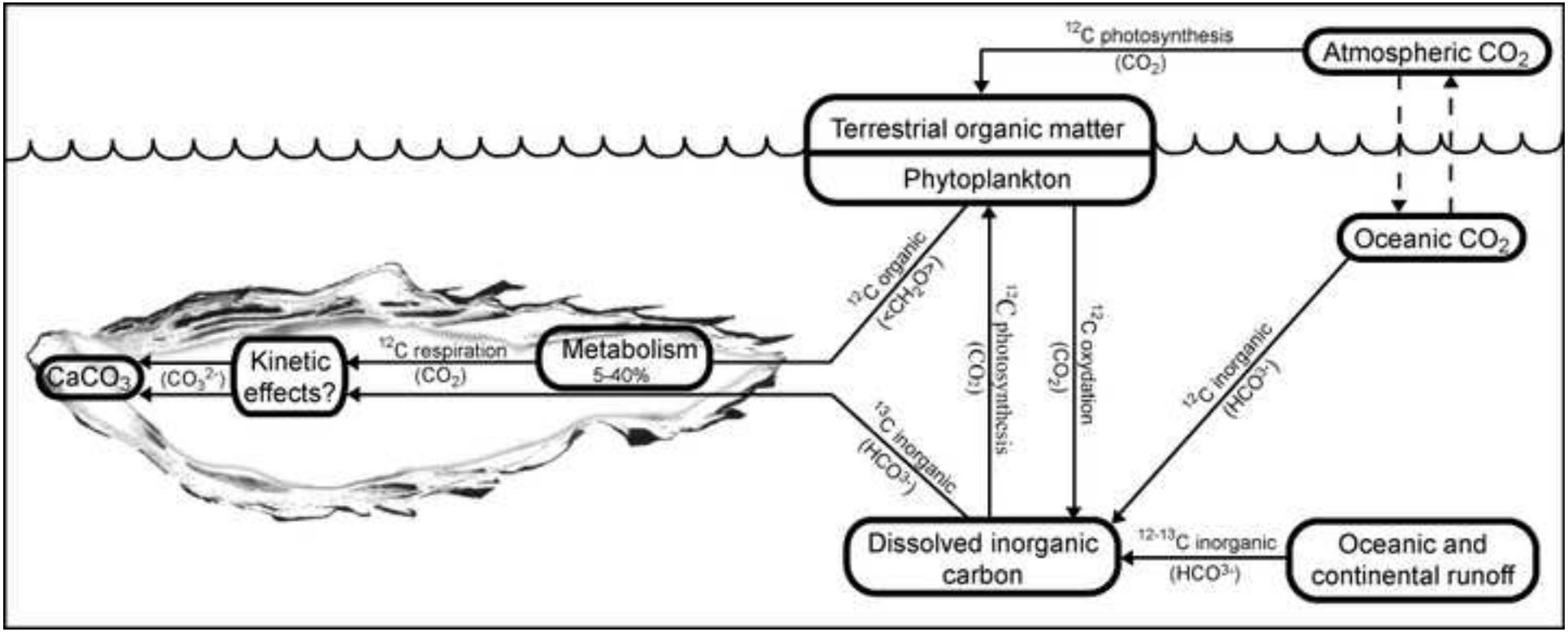

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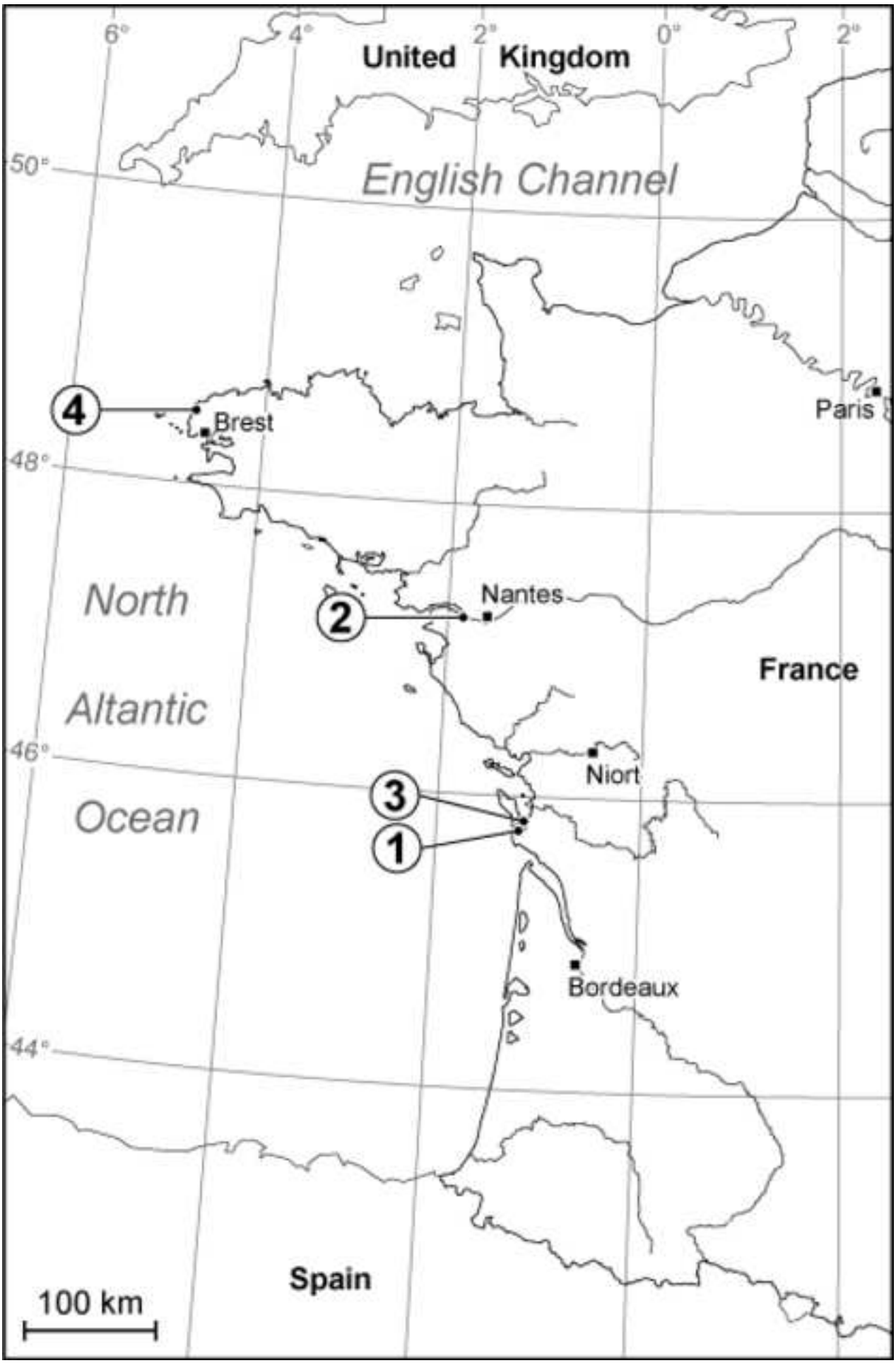

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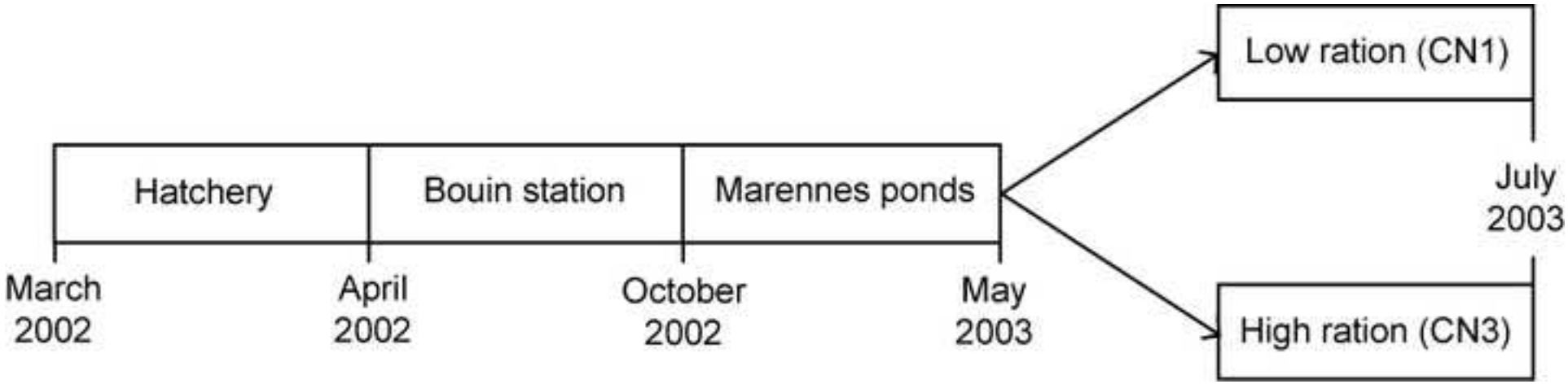

Figure 4

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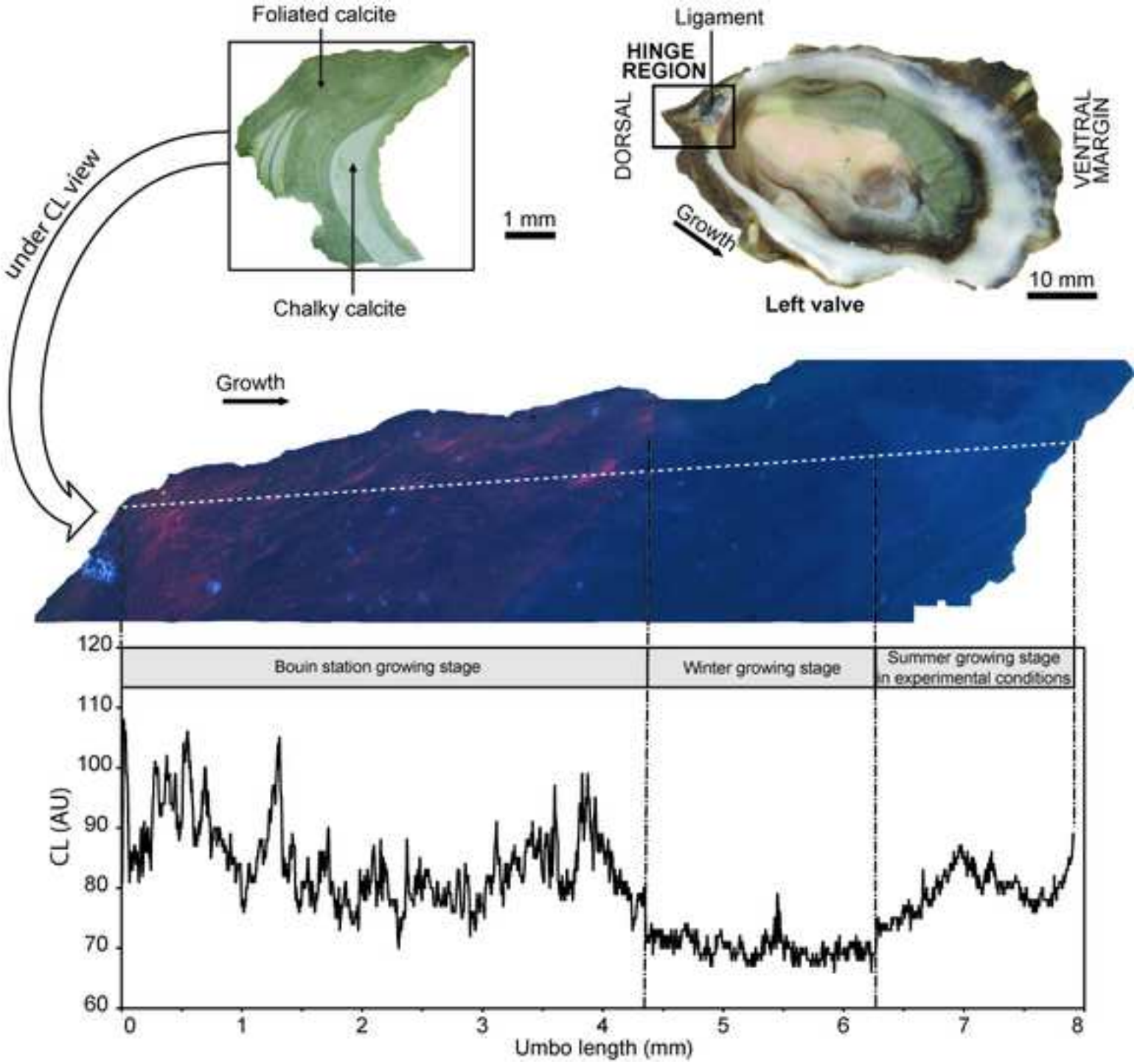

Figure 5

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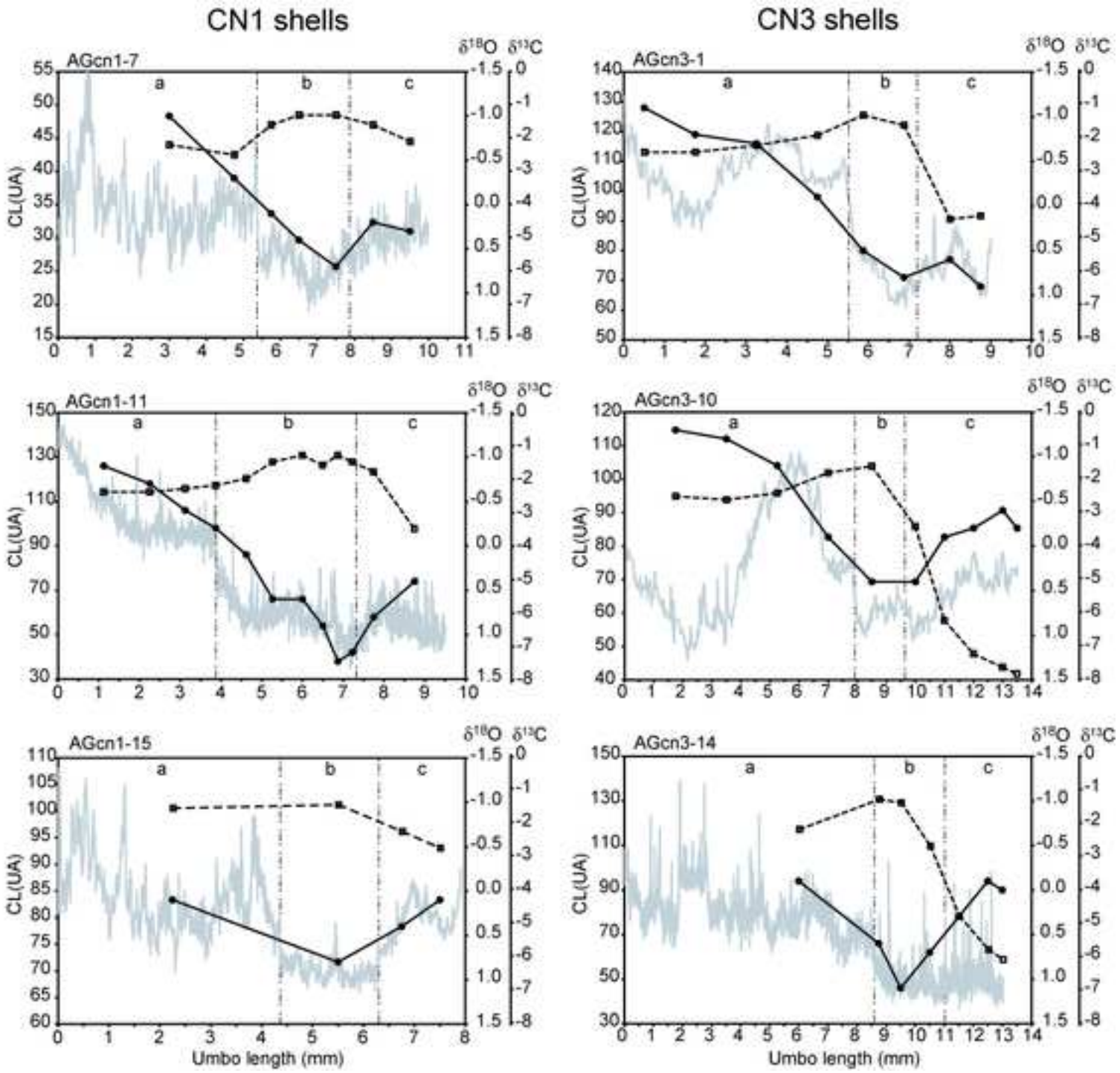

Figure 6

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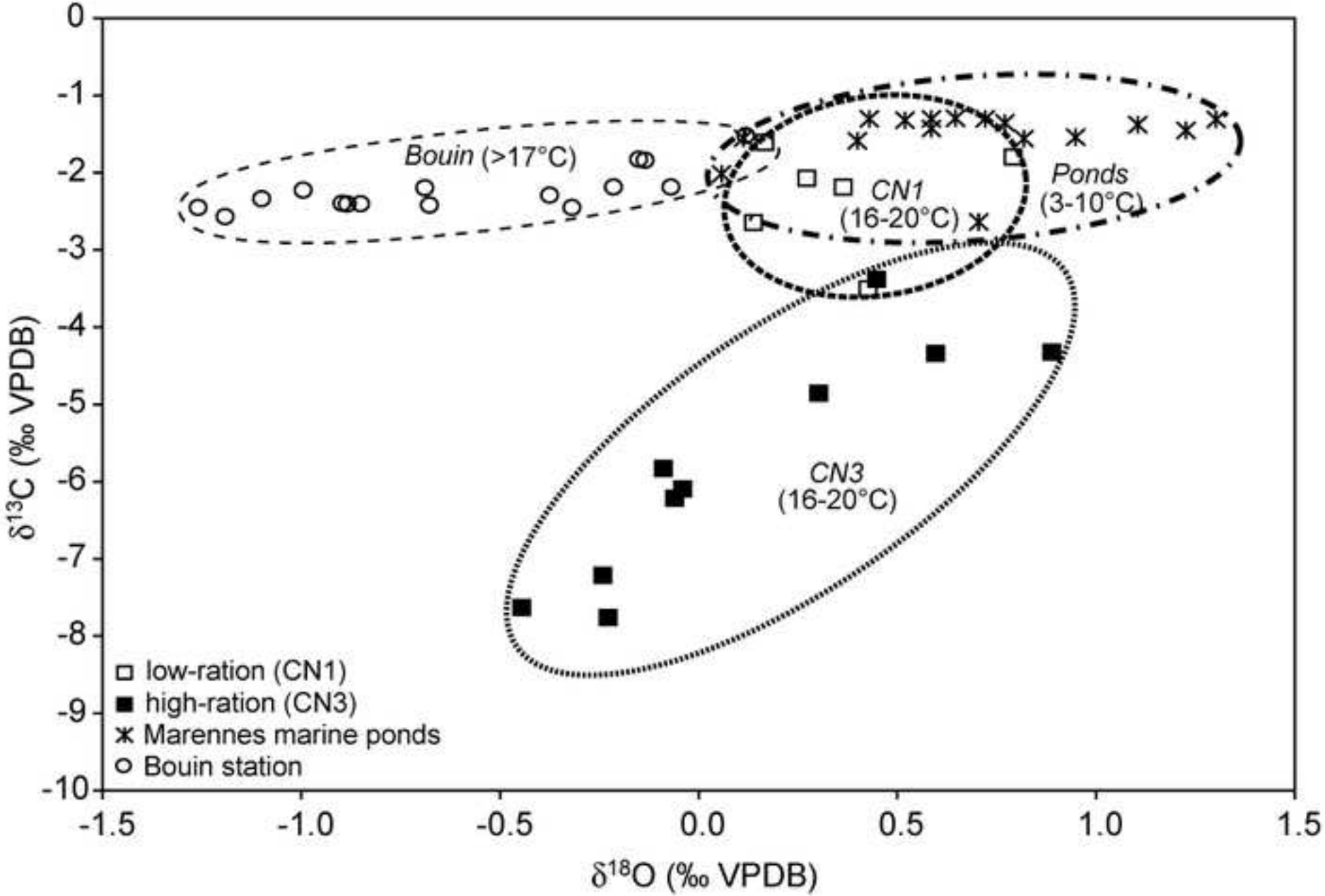

Figure 7

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