New insights into the seasonal feeding ecology of *Pecten maximus* using pigments, fatty acids and sterols analyses

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ABSTRACT: We combined the use of pigments, fatty acids and sterols as biomarkers of the seasonal variation in food sources of the great scallop *Pecten maximus*. From March to October 2011, on a biweekly to twice-weekly basis, scallops and seawater from the water column and the water–sediment interface were collected in the Bay of Brest (Brittany, France). Pigment compositions in the seawater and in the stomach and rectum content of the scallops were analyzed by HPLC. Fatty acids and sterols from digestive gland (DG) tissue were analyzed by gas chromatography. Potential relationships between the temporal proportion of each marker in the environment and in the digestive tract were tested using multivariate analysis. Proportions of diatoms and Dinophyceae biomarkers alternated in the digestive tract of *P. maximus*. This switch of feeding was confirmed by the polar lipid composition of the DG. Peridinin, characterizing Dinophyceae, occurred in high proportions in the digestive tract compared to the low ambient concentration, suggesting a selection of this microalgae group by the scallop. Chlorophyceae and green macroalgae tracers were found in low proportions, suggesting they were not actually ingested. Markers of Prymnesiophyceae were also observed at significant levels. Cyanobacteria tracers showed that this microalgae class was not ingested by the scallops during monitoring but may be of higher importance during winter. Switching from one food source to another as well as selectivity in feeding are discussed relative to the season.

KEY WORDS: Trophic ecology · Food sources · Pigments · Fatty acids · Trophic marker · Phytoplankton · *Pecten maximus*

INTRODUCTION

Scallops, such as *Pecten maximus* (Linnaeus, 1758), are sessile, suspension-feeding animals living half-buried on the sea floor. They rely on the availability of trophic resources in the nearby water to obtain their food. In coastal environments, factors such as temperature, light, riverine inputs, salinity, tide and currents influence seawater primary production. The availability of food depends on the strong seasonality of these hydrological and biochemical conditions; therefore, suspension-feeding bivalves must have evolved to develop a plastic trophic niche (Rossi et al. 2004, Nérot et al. 2012). Scallops may thus feed on pelagic and benthic microalgae, protozoans, microzooplankton, dissolved organic carbon and detrital organic matter (Shumway et al. 1987, Lorrain et al. 2002, MacDonald et al. 2006, Lavaud et al. 2014, Aya &

Kudo 2017). A recent study has confirmed this diversified diet (Nérot et al. 2012), yet only qualitatively.

Studies aiming at tracing the potential trophic sources of an organism use various chemical, biological, biochemical or physiological indicators of the prey items in the environment and inside the organisms. Among these indicators, stable isotopes are often used (Rossi et al. 2004, Bode et al. 2006, Marín Leal et al. 2008). Although they are able to discriminate between taxa at different trophic levels (benthic vs. planktonic microalgae for example), their low turnover is sometimes insufficient to distinguish short-term changes in the diet of the consumer. Pigments and lipids (fatty acids [FAs] and sterols), on the other hand, have long been used to characterize the class, genus and even species of microalgae (Jeffrey 1974, 1997, Pastoureaud et al. 1995, Loret et al. 2000, Louda et al. 2008, Sanz et al. 2015 for pigments; Parrish et al. 1995, Soudant et al. 1996, Bachok et al. 2003, 2009 for FAs; and Volkman 1986, Napolitano et al. 1993, Soudant et al. 1998a for sterols). These biomarkers have been extensively used in foraging ecology and food web studies. Statistical methods are in development to assess the contribution of different food sources in the diet, e.g. CHEMTAX for pigments (Mackey et al. 1996) and for FA analysis (Dijkman & Kromkamp 2006) or quantitative fatty acid signature analysis (QFASA) in FA studies (Iverson et al. 2004). However, their distribution is complex, with few unambiguous biomarkers. It is therefore important that other techniques are used to identify the food types present (Wright & Jeffrey 2006). In this context, combining pigment and lipid compositions of tissues could prove advantageous to assess seasonal variability of food sources (Hurtado et al. 2012).

The present study describes the temporal qualitative and quantitative variations in trophic sources consumed by *P. maximus*, in an innovative approach combining 3 well established trophic markers: pigments, FAs and sterols, in the digestive gland and rectum contents. The coupling of these 3 biomarkers is expected to provide a full understanding of both high-frequency and long-term patterns in the feeding ecology of the great scallop and to help trace different food sources from various origins.

MATERIALS AND METHODS

Study area and sampling protocol

The study was conducted in the Bay of Brest (Brittany, France). *Pecten maximus* were dredged in the bay in November 2010 and then relocated to Lanvéoc (48° 17' N, 4° 27' W, average depth: 12 m). From 14 March 2011 until 24 October 2011, 3 individuals were collected by SCUBA divers every 2 wk. During April and May, corresponding to a period of high phytoplankton productivity, scallops were sampled twice a week. Only animals over 3 yr old (identified by yearly shell growth rings; Mason 1957), and thus fully mature, were selected for this study. The digestive gland (DG) content, hereafter referred to as the stomach content, was collected in 2 ml Eppendorf tubes by squeezing the DG. In the same way, the content of the posterior part of the intestine (branch outside the gonad) was obtained by pressing the rectum. Stomach fraction was used as a proxy of ingested food, and rectum fraction was considered as the digested part of food that was not assimilated. A total of 2 aliquots of DG tissue were encapsulated into aluminium cups. DG content and tissue samples were frozen in liquid nitrogen and stored at −80°C until sample treatment.

Seawater was also sampled using a 5 l Niskin bottle at 2 m below the surface during the entire study period. Sampling of the water–sediment interface was carried out by SCUBA divers from early May, using a 450 ml syringe to collect water at approximately 3 cm above the sea bottom. Sampling was performed as far as possible at medium tidal coefficient and around mid-tide. Phytoplankton species composition and abundance were determined in samples both in the water column and at the water–sediment interface. A volume of 250 ml of each sample was fixed with acetic Lugol's solution. Quantitative and qualitative analyses were carried out from settled cells using an inverted phase microscope. Vacuum filtration of seawater was conducted on GF/F filters (0.7 μm) previously heated for 6 h at 450°C. Between 500 ml and 1.5 l of sub-surface seawater were filtered at each sampling time. The volume of filtered bottom water ranged from 50 to 150 ml, depending on filter clogging. Two filtrations were carried out for water column samples and 2 for water–sediment interface samples, for pigment and lipid analysis. Filtrates were stored in sealed aluminium folds at −80°C until sample treatment. Simultaneous analyses of pigment composition of the seston from the water column and from the water–sediment interface were conducted whenever possible as they were not available for the first 2 mo of the study. Nevertheless, the occurrence, timing and intensity of spring phytoplankton at the bottom were well documented in a study carried out at the same time and the same location (Chatterjee et al. 2013). Pigment and lipid composition of all samples (DG

content, DG tissue of scallops and filtrates) were analyzed as described below.

Pigment analysis

The procedure used to identify and quantify pigments in the filters and in the DG contents was the method described by Claustre & Ras (2009). The extraction of pigments was achieved from 200 µl of stomach contents, 50 µg of rectum contents and seawater filtrates from the 2 depths, in 100% methanol enriched with vitamin E acetate (used as internal standard; Sigma-Aldrich). To improve extraction yield, samples were disrupted by sonication using an S-4000 sonicator (Misonix) and stored at −20°C. Sonication was repeated after 2 and 4 h of extraction. Samples were centrifuged for 10 min at 3000 rpm (805 × g; 4°C) and passed through 13 mm syringe filters (Puradisc, 0.2 µm, PTFE; Fischer). Pigment analyses were carried out on a complete Agilent Technologies 1200 series HPLC system, equipped with a ZORBAX Eclipse XDB-C$_8$ silica column (3 × 150 mm, 3.5 mm particle size) and a diode array detector, which permits automatic pigment identification based on absorption spectra. The elution was run at a flow rate of 0.55 ml min^{-1} using solvent A (tetrabutylammonium acetate and methanol, 30:70 v/v) and solvent B (methanol) in the following elution procedure (min, solv. A, solv. B): (0, 90, 10), (22, 5, 95), (27, 5, 95), (28, 90, 10) and (33, 90, 10). Pigment optical densities were monitored at 450 nm (chloropigments and carotenoids) and at 667 nm (chlorophyll a [chl a] and derived pigments), and automatically compared to the retention times of 15 pigment standards (DHI and Sigma-Aldrich) previously calibrated. The injection precision of the method was estimated at 0.4%, and the effective limits of quantification for most pigments were rather low: 2.89 10^{-5} µg ml^{-1} for chl a and 3.98 10^{-5} µg ml^{-1} for carotenoids. Chemstation software was used for verification and eventual correction of the peak integrations in each chromatogram.

Lipid analysis

Chemicals

HPLC-grade solvents were purchased from VWR International. Boron trifluoride (BF3; 14% by weight in methanol), tricosanoic acid (C23:0), cholestane and 37-component fatty acid methyl ester (FAME) mix were obtained from Sigma-Aldrich. Silica gel 60

(63−230 µm mesh) was purchased from Merck. Gas chromatography (GC) capillary column was a DBWAX (30 m × 0.25 mm i.d., 0.25 µm thickness) for FAME analysis and a Rtx65 (15 m × 0.25 mm i.d., 0.25 µm thickness) for sterol analysis and were obtained from Agilent and Restek, respectively.

Lipid extraction

Lipid extraction was conducted by resuspension of the filtrates or of 200 mg aliquots of DG previously grounded by ball milling under liquid nitrogen. Samples were then put in glass tubes containing 6 ml of chloroform-methanol (2:1, v/v) and stored at −20°C before analysis.

Separation

After centrifugation (2 min, 1000 rpm), lipid analyses were carried out on 1 ml of DG lipid extracts and on 5 ml of filtrate lipid extracts (due to the small amount of material in the seawater when compared to the DG). Lipid extracts were then evaporated to dryness under nitrogen, recovered with 3 chloroform-methanol (98:2, v/v) washings of 500 µl each and deposited at the top of a silica gel microcolumn (Pasteur pipette of 5 mm i.d., plugged with glass wool and filled with silica gel 60, both previously heated for 6 h at 450°C and deactivated with 6% water by weight). Neutral lipids (NL), including triglycerides, free FAs and sterols, were eluted with 10 ml of chloroform-methanol (98:2 v/v) and polar lipids (PL), containing glycolipids and phospholipids, were eluted with 20 ml of methanol; both were collected in 20 ml vials.

Transesterification

After evaporation to dryness under nitrogen, PL and NL fractions were recovered and transferred in 8 ml vials with 3 chloroform-methanol (98:2, v/v) washings of 1 ml each. The NL fraction was then equally divided into 2 vials. All of the PL fraction and half of NL fraction were dedicated to FA analysis; the other part of the NL fraction was dedicated to sterol analysis. As internal standards, 2.3 µg of C23:0 was added to the FA vials and 2.5 µg of cholestane to the sterols vials. After evaporation to dryness under nitrogen, FAMEs were obtained by adding 800 µl of methanol-BF3 to FA vials, vortexing and heating for 10 min at 100°C. For sterol analysis, 2 ml of a sodium

methoxyde solution (NaOH, 27 µg µl⁻¹ in methanol) was added to the sterol vials, which were maintained under agitation for 90 min at room temperature. Before GC analysis, organic phases containing FAME or sterols were washed 3 times with 1 ml of distilled water and 800 µl of hexane and centrifuged (1 min at 1000 rpm) before eliminating the aqueous phase each time. Finally, organic phases were transferred into tapering vials and stored at −20°C.

GC analysis

FAMEs were analyzed in an Agilent 6890 gas chromatograph equipped with an on-column injector and a flame-ionization detector, with hydrogen as a carrier gas. They were identified by their retention times with reference to those of a standard 37-component FAME mix and other known standard mixtures from marine bivalves (Soudant et al. 1995) and designated following the formula C:X(n-Y) where C is the number of carbon atoms, X is the number of double bonds and Y is the position of the first double bond counted from the CH terminal. The sterols were separated by GC, in a Chrompack 9002 equipped with an on-column injector and a flame-ionization detector, with hydrogen as a carrier gas. Sterols were identified by comparison of their retention time with standards as described in Soudant et al. (1998a).

Data analysis

As chl *a* is present in all marine algae species and because the goal of this study was to identify specific patterns in the dynamics of several algae classes, chl *a* was removed from the total amount of pigments; each pigment quantity was therefore expressed as its contribution to the total amount of pigment without chl *a*. This was applied to all samples (seawater and digestive tract contents).

Statistical analyses were carried out to determine significant temporal variations in the different food proxies, analyzed using R v.3.3.2 (R Core Team 2013). Potential correlations between pigment concentrations in the 2 water compartments and the stomach contents were investigated through simple linear regressions. A canonical correspondence analysis (CCA) integrating all food proxy variables in the environment (phytoplankton counts and pigments in the water column and at the water–sediment interface) and the proportion of pigments, FAs and sterols in the stomach contents over time was performed using the package 'vegan' (Oksanen et al. 2017). Degraded pigments (pheophytin and pheophorbide) as well as pigments, FAs and sterols whose proportions averaged less than 0.5% were excluded. Because no water samples were available at the water–sediment interface until May, the CCA was performed for sampling points after 2 May. First trials included all variables at the same time, and were not successful in identifying clear relationships, likely due to the elevated number of variables. Therefore 2 separate analyses were performed, both using phytoplankton counts as variables and time series of pigment proportions in the water and the stomach contents or lipid proportions in the DG tissues.

RESULTS

Seston pigment concentration and composition

A general pattern of phytoplankton abundance during the study period can be seen in Fig. 1, showing the dynamics of chl *a* concentration in the seston from the water column as well as at the water–sediment interface. The first sign of primary production recovery after winter in the water column occurred at the end of March, but the first significant phytoplankton bloom was observed at the water–sediment interface in mid-April. Microalgae grew in the water column in early May. In June, another bloom occurred in the water column while pigment concentrations in the bottom water decreased. During the summer, few blooms
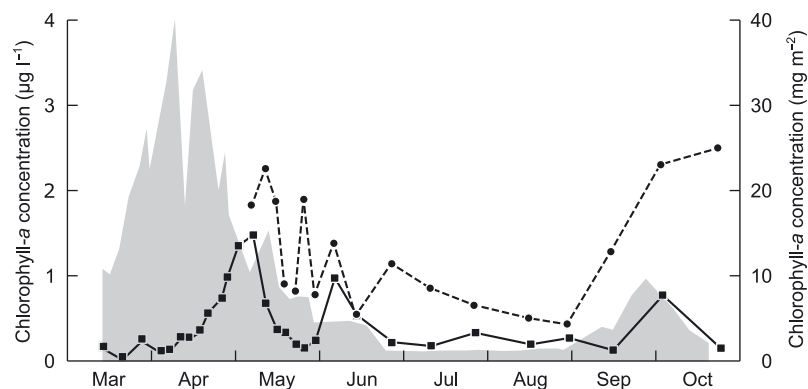

Fig. 1. Chl *a* concentration in the water column (solid line, black squares) and in the water–sediment interface (dashed line, black dots) in the Bay of Brest, France, in 2011. Additional data for chl *a* concentration (grey area, right axis) at the sea floor are from Chatterjee et al. (2013)

were recorded in the water column, while low pigment amounts were found at the bottom. In autumn, phytoplankton biomass increased in both water compartments. Pigment concentration in the water column seemed to decline rapidly in August, while bottom production reached high levels in September, comparable to those observed in spring.

Detailed phytoplankton composition in the water column and the water–sediment interface are presented in Table 1. Although identification has been performed to species level, data are presented by microalgae classes. The water from the sea bottom was mainly composed of diatoms (Bacillariophyceae), of which the highest abundance was recorded in early March. In the water column, the first observed bloom occurred in late March and was mainly composed of Dinophyceae and Cryptophyceae, with 276 800 and 2 810 000 cells l^{-1} respectively (mostly *Heterocapsa triquetra* and *Gyrodinium flagellare*). A synchronous elevation of alloxanthin and peridinin was observed in the water column (Fig. 2b,e). The pigment composition of seston in late April showed increasing proportions of 19′-hexanoyloxyfucoxanthin (19′HF) and 19′-butanoyloxy-fucoxanthine (19′BF), up to 15 and 10 % respectively in the water column (Fig. 2d,f). At the same time, the highest abundances of Prymnesiophyceae and Chrysophyceae were recorded (Table 1). Diatoms were highly abundant from the beginning but showed a surge in early May (mostly *Chaetoceros* sp., *Cerataulina pelagica* and *Dactiliosolen fragilissima* in the water column, and *Navicula* sp. and *Fragillaria* sp. at the water–sediment interface), accompanied by peaks of fucoxanthin in both water compartments (Fig. 2a), contributing 77 % of the total pigments. Almost all pigment proportions increased during this bloom, except for peridinin, which only showed several short peaks (around 10 % of the total pigment) during the study period. The second major microalgae bloom was mostly due to diatoms, and occurred in early June with up to 2 747 400 cells l^{-1}. A sharp increase of fucoxanthin concentration was again observed in the water. From then on, fucoxanthin concentrations remained at lower levels both in the water column and at the water–sediment interface, whereas the proportion of chl *b* started to increase from about 10 % to more than 20 % in the water column in June (Fig. 2e). During this period, only trace amounts of green microalgae (Chlorophyceae) were observed. The same occurred at the bottom from July, also accompanied by a similar increase of peridinin and zeaxanthin concentrations. Alloxanthin and 19′HF accounted for about 5 % of the total pigments from mid-summer until the end of the study. High

numbers of Cryptophyceae and Prymnesiophyceae were observed during these last months. Finally, in October, a last bloom of diatoms was recorded at the water–sediment interface while seston pigment composition was dominated by fucoxanthin (up to 40 %) and chl *b* (50 %). Degradation pigments such as pheophorbide-*a* and pheophytin-*a* were detected at very low amounts in the water during the whole study period. Other pigments were detected in this study (probably degraded forms of the main pigments already described) but were not taken into account as we did not have the specific standards for their identification at our disposal.

Pigment composition of the digestive tract contents

Over the duration of the study, the quantity of pigments measured in the stomach content seemed to match the quantity found in the seston only during certain periods of the study (Fig. 3). This is reflected by a weak correlation between the pigment concentration in the stomach content and the pigment concentration in the seston from both the water column and the water–sediment interface (r^2 = 0.3074 and r^2 = 0.1322 respectively; p < 0.01) when considering the whole sampling period. However, these correlations are greatly improved when the time series is split into 2 periods (spring and summer/fall). A very strong correlation between the concentration in the stomach contents and in the water column was found from June until the end of the study (r^2 = 0.9394, p < 0.01). On the other hand, the correlation between the concentration in the stomach contents and in the water–sediment interface was stronger before June (r^2 = 0.4943, p < 0.01).

Fucoxanthin was, after chl *a*, the major pigment found both in stomach and rectum (up to 55 and 50 % respectively; Table 2). Peridinin had a maximum occurrence of 43 % in the stomach and 28 % in the rectum. The dynamics of these 2 predominant pigments varied in an opposite manner, as shown in Fig. 4. Fucoxanthin in the stomach fluctuated strongly during spring (Fig. 2a), with sharp increases from 10–20 % up to 50–55 % in late April, mid-May, late May and early June. The minimum was observed on 30 May (11.4 %) and the maximum on 28 April at 53.6 %. As observed in the water, a significant increase was registered in autumn. The proportion of fucoxanthin in the rectum was very similar to what was observed in the stomach, as shown by a linear regression coefficient of r^2 = 0.82 (p < 0.01). Peridinin showed a similar pattern, with a fluctuating spring period followed by low proportions

Table 1. Phytoplankton composition of the water column (2 m under the surface) and at the water–sediment interface (3 cm above bottom) in the Bay of Brest in 2011 (in no. of cells l⁻¹)

Water column

Date (dd/mo)	Bacillario-phyceae	Dino-phyceae	Chloro-phyceae	Chryso-phyceae	Crypto-phyceae	Cyano-phyceae	Prymnesio-phyceae
10/03	26 950	23 300	0	0	0	0	25 750
17/03	21 260	53 130	0	5 000	236 100	0	2 500
25/03	11 370	276 800	0	0	2 810 000	0	16 100
31/03	138 635	24 920	0	2 700	122 000	0	0
08/04	184 940	35 740	0	0	171 000	0	600
14/04	17 630	18 750	0	16 900	143 200	0	15 800
23/04	116 560	115 060	0	12 050	211 000	0	91 530
29/04	601 820	48 960	0	75 400	27 630	0	32 950
05/05	956 290	54 800	0	9 540	105 500	0	47 480
13/05	640 910	76 891	7 780	0	291 060	0	0
23/05	7 225	124 000	47 730	2 500	445 450	0	7 500
30/05	91 220	10 330	0	0	13 190	0	0
06/06	2 747 400	32 780	0	0	55 260	0	13 190
13/06	232 060	86 780	0	4 900	82 900	0	0
21/06	28 230	46 120	0	1 900	231 100	0	5 200
30/06	789 730	55 720	9 100	1 000	226 080	0	40 250
08/07	126 310	18 810	0	0	30 140	0	15 500
12/07	627 150	79 980	0	0	105 500	2 080	12 560
21/07	19 120	90 930	0	0	288 900	0	12 640
28/07	158 690	4 590	0	0	37 680	0	0
06/08	170 770	38 860	0	0	145 700	0	21 700
11/08	23 520	76 810	0	0	158 260	0	0
19/08	18 920	52 860	0	0	384 340	0	1 000
26/08	221 600	6 360	0	0	60 290	0	0
04/09	1 520	200	0	0	11 000	0	0
10/09	5 140	23 540	0	0	263 760	0	0
16/09	7 840	36 700	0	0	146 850	0	4 500
24/09	137 700	15 460	0	0	133 140	0	0
03/10	155 300	95 730	0	5 000	562 690	0	55 260
10/10	30 400	63 880	0	4 340	183 270	0	14 100
17/10	1 000	34 280	0	0	450 210	0	67 960
23/10	1 160	7 820	0	0	77 870	0	15 070

Water–sediment interface

| Date (dd/mo)	Bacillario-phyceae	Dino-phyceae	Chloro-phyceae	Chryso-phyceae	Crypto-phyceae	Cyano-phyceae	Prymnesio-phyceae
10/03	3 042 490	0	0	0	0	0	0
21/03	0	40	0	0	0	0	0
24/03	1 080	0	0	2 100	75 500	0	0
28/03	127 110	20	0	0	0	0	0
31/03	63 540	240	0	0	10 100	0	0
04/04	59 190	280	0	0	4 050	0	0
07/04	23 520	0	0	0	4 000	0	0
11/04	124 140	4 110	0	0	8 050	0	0
14/04	19 170	80	0	0	0	0	0
18/04	74 760	120	0	0	1 000	0	0
21/04	113 050	20 500	0	4 000	217 510	0	0
26/04	34 910	2 280	0	0	12 100	0	0
28/04	89 860	40	0	0	33 720	0	0
02/05	75 400	600	0	0	0	0	0
09/05	301 860	600	0	0	0	0	0
12/05	176 870	1 240	0	0	16 900	0	0
16/05	250 460	12 780	0	0	0	0	0
19/05	1 760	400	0	0	2 000	0	0
23/05	69 020	800	0	0	0	0	0
26/05	600	2 100	0	0	0	0	0
30/05	76 085	1 280	0	0	0	0	0
06/06	720	1 280	0	0	1 000	0	0
14/06	40	2 280	0	0	10 100	0	0
20/06	23 460	10 300	0	0	500	0	0
27/06	180	20	0	0	0	0	0
19/07	0	0	0	0	0	0	0
27/07	0	0	0	0	0	0	0
01/08	3 120	40	0	0	0	0	0
09/08	5 800	100	0	0	0	0	0
16/08	3 480	440	0	0	12 100	0	0
22/08	9 600	200	0	0	0	0	0
30/08	61 500	0	0	0	0	0	0
05/09	2 800	0	0	0	0	0	0
13/09	2 000	0	0	0	0	0	0
19/09	57 550	0	0	90 630	0	0	0
26/09	49 060	0	0	20 130	0	0	0
03/10	51 620	6 040	0	20 140	10 000	0	0
10/10	889 770	3 200	0	6 050	0	0	0
17/10	78 410	4 500	2 000	2 000	0	0	0
24/10	2 000	2 000	0	0	0	0	0
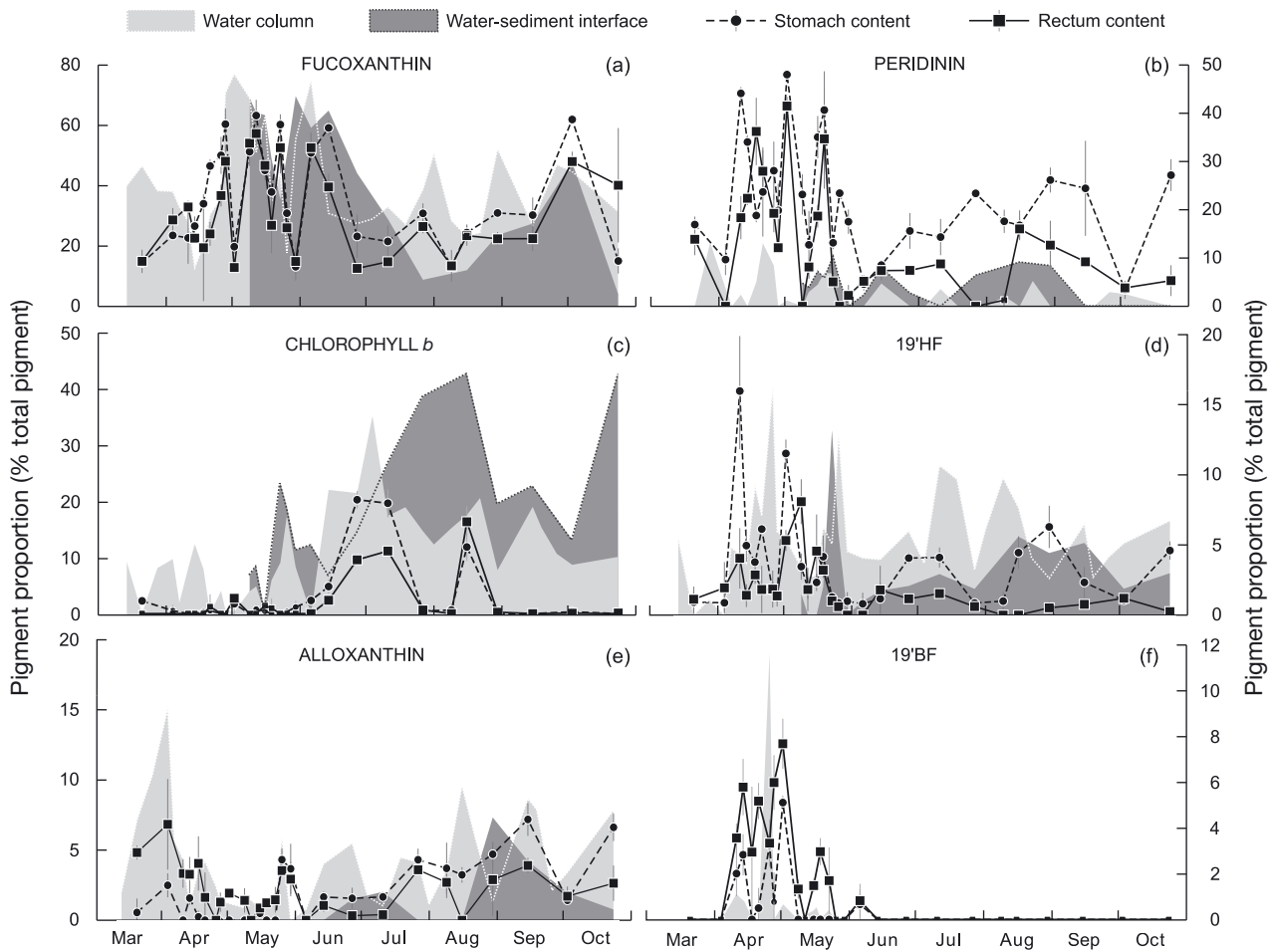
Fig. 2. Proportions of (a) fucoxanthin, (b) peridinin, (c) chl *b*, (d) 19'HF, (e) alloxanthin and (f) 19'BF in the water column (light grey area), at the water–sediment interface (dark grey area) and in the stomach (dashed dark line, black dots) and rectum content (solid dark line, black squares) of *Pecten maximus* in the Bay of Brest in 2011. Chl *a* quantity was removed from total pigment amount before calculating the proportion of each pigment. Standard deviations are indicated by vertical grey lines

in early summer and medium levels until autumn, when it dropped to its minimum (2.4%) on 3 October. The maximum was reached on 2 May at 42.3%. Compared to fucoxanthin, the linear regression between the proportion of peridinin in the rectum and in the stomach was less significant ($r^2 = 0.52$, p < 0.01). For instance, at the end of May as well as in July and August, the discrepancy between the 2 compartments was 20% between the entance to and the exit of the digestive tract (Fig. 2b).

The occurrence of chl *b* was not observed in the digestive tract of the scallops before an important peak of 13.5% during June and July and a shorter one in mid-August (12%; Fig. 2c). In the rectum, proportions of chl *b* were found to be lower during the first increase (10.5%) and higher during the second peak (16%). Nevertheless, the correlation between the 2 compartments was rather elevated ($r^2 = 0.76$).

Two smaller 19'HF peaks of 16 and 11% were recorded in the stomach on 11 April and 2 May, respectively; another increase was reported during August, although this only reached 5% (Fig. 2d). During the first peak and the late summer increase, the proportion of this pigment in the rectum did not change, whereas it increased up to 8% just after the second spring peak. A higher proportion of alloxanthin was present in the rectum than in the stomach until mid-May (Fig. 2e). The proportion of this pigment then increased up to 5% in the stomach in late May. From June to October a regular increase was registered in the digestive tract of *Pecten maximus*. Finally, several events of increased proportions of 19'BF up to 5% were observed in the stomach as well as in the rectum during April and the first week of May, whereas only one significant peak occurred in the seawater at the end of April (Fig. 2f).
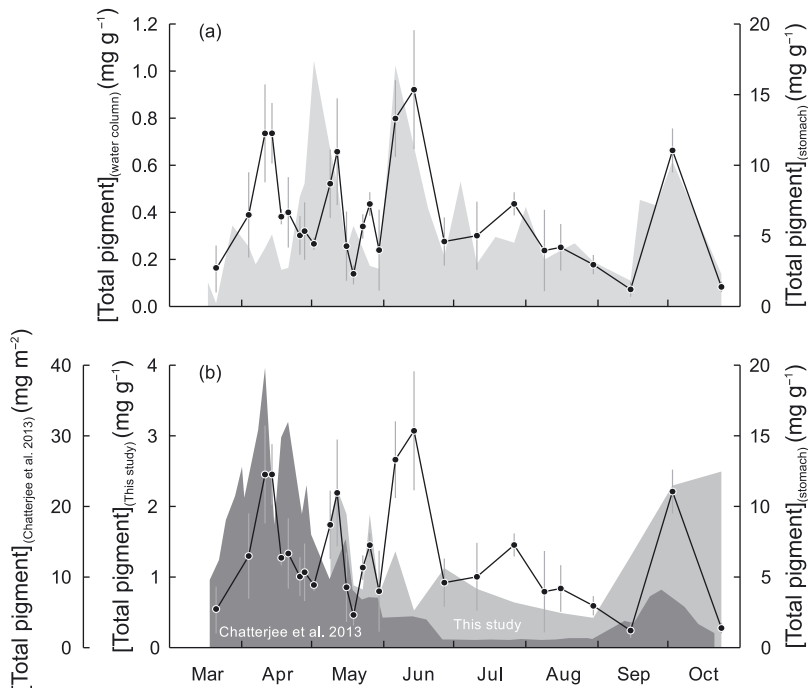
Fig. 3. (a,b) Total quantity of pigment, including chl *a*, measured in the stomach content of *Pecten maximus* (dark line in [a,b]), water column (light grey area in [a]), and at the water–sediment interface (grey area in [b]) in the Bay of Brest in 2011. Standard deviations are indicated by vertical grey lines. Data from the water–sediment interface from the study of Chatterjee et al. (2013) are also plotted (dark grey area in [b]) to complete the present data set

Degraded pigments, namely pheophorbide-*a* and pheophytin-*a*, were also observed in both stomach and rectum (Fig. 5). High proportions (17 to 24%) of these 2 pigments were already present in the stomach at the beginning of the study and dropped down to zero on 11 April. A brief increase of pheophytin-*a* to 24% was noted on 18 April. A peak of degraded pigment was reached between 26 May and 6 June and a second event occurred between 27 July and 30 August. In the rectum, the level of degraded pigments very closely followed the pattern described for the stomach. Pheophytin-*a*, however, fluctuated less from June and varied between 6 and 16%. Moreover, when compared to the total pigment found in the stomach (Fig. 5b), the proportions of degraded pigments seemed rather disconnected from the periods of increased ingestion. During the 4 periods of high total pigment concentration in the digestive tract, proportions of degraded pigments did not show any strong dynamics. Conversely, the 3 periods of high levels of degraded pigments identified in the gut of the scallops occurred after periods of low feeding activity and just before the ingestion peaks.

FA and sterol composition of the DG

First of all, the GC analysis of FAs and sterols in the seawater filters revealed extremely low quantities in the samples. Only the most prominent FAs could be detected, showing only small peaks which could not be accurately quantified. Several trials to concentrate the samples did not solve this detection threshold issue. Nevertheless, the absence of usable FA and sterol data for water samples should not be crippling for the study, as FA markers of microalgae classes observed here are well established in the literature. Moreover, data on quantitative microalgae compositions from optical and/ or pigment analyses should constitute a sufficiently robust base for deciphering scallop FA profiles (see 'Discussion' for details).

The total amount of FAs and sterols in the DG of *P. maximus* showed particularly high variations during spring (Fig. 6f). Total FA content of the DG increased from 45 to 75 µmol mg^{-1} between the start and end of the study, with the highest value of 172 µmol mg^{-1} reached in mid-June (Fig. 6f). The main FAs found in the polar fraction were 20:5(n-3) (eicosapentaenoic acid; EPA), 22:6(n-3) (docosahexaenoic acid; DHA) and 16:0, each accounting for about 15% of total FAs (Table 3), 18:4(n-3), 20:4(n-6), around 5% and to a lesser extent 18:2(n-6), 18:3(n-3), 18:1(n-9), 20:4(n-3) and *iso*17:0, which accounted for less than 2%. In the neutral fraction, 20:5(n-3) was the main FA (25%; Table 4), 16:0 accounted for about 15%, 14:0, 16:1(n-7) and 22:6(n-3) ranged between 6 and 12%, 20:4(n-6) was found around 3.5% and 18:1(n-9), 18:3(n-3), 18:4(n-3) and 18:5(n-3) each accounted for about 2%. Within the polar fraction, the ratio between EPA and DHA showed 2 periods of increase in late April/early May and at the beginning of June (Fig. 6e). Lower levels were observed at the beginning of April, at the end of May and in August. An analogous tendency was observed when comparing the proportions of 16:1(n-7) and 18:5(n-3) (in the neutral fraction). A peak of 18:4(n-3) was observed at the end of April, its proportion reaching 5% of total FA and increasing regularly from June to October (Fig. 6c). Similar patterns were observed for 18:2(n-6) and 18:3(n-3), which also exhibited an important in-

Table 2. Pigment proportions expressed as percentage of total pigments in the stomach and rectum of *Pecten maximus* in the Bay of Brest in 2011. Chl *a* was removed from total pigment amount before calculating the proportion of each pigment. PHB: pheophorbide-*a*; PER: peridinin; BFU: 19′BF; FUC: fucoxanthin; HFU: 19′HF; ALO: alloxanthin; ZEA: zeaxanthin; LUT: lutein; CHB: chl *b*; PHT: pheophytin-*a*. Standard deviations are in brackets

Date (dd/mo)	PHB	PER	BFU	FUC	HFU	ALO	ZEA	LUT	CHB	PHT
Stomach										
21/03	18.7 (4.6)	16.9 (1.6)	0.0 (0.0)	14.4 (1.4)	0.9 (0.8)	0.7 (1.2)	2.7 (1.6)	1.5 (0.7)	2.3 (0.3)	27.1 (3.3)
04/04	31.1 (7.2)	9.7 (3.1)	0.0 (0.0)	23.6 (0.8)	0.9 (1.5)	3.1 (1.0)	0.0 (0.0)	0.0 (0.1)	0.5 (0.9)	23.1 (6.3)
11/04	0.0 (0.0)	44.1 (1.2)	2.0 (1.0)	22.7 (8.4)	16.0 (4.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
18/04	7.9 (1.1)	18.8 (1.3)	0.0 (0.0)	34.0 (0.4)	3.8 (0.4)	0.3 (0.5)	0.0 (0.1)	0.1 (0.2)	0.3 (0.5)	28.9 (1.3)
26/04	5.2 (1.1)	28.1 (5.9)	3.2 (0.5)	50.1 (6.0)	1.7 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.1 (1.6)
28/04	0.0 (0.0)	19.8 (5.4)	0.7 (1.3)	60.4 (5.0)	1.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	9.1 (0.9)
02/05	0.0 (0.0)	48.0 (0.1)	5.1 (0.3)	19.8 (2.2)	11.5 (1.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.8 (1.8)	0.2 (0.3)
09/05	0.0 (0.5)	25.6 (4.1)	0.0 (0.0)	56.6 (4.5)	3.8 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.2 (1.2)
12/05	0.0 (0.9)	13.3 (6.9)	0.0 (0.0)	66.0 (5.0)	2.0 (0.7)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.8 (0.8)	1.8 (1.7)
16/05	0.0 (0.0)	35.0 (4.3)	0.0 (0.0)	45.1 (3.1)	2.3 (0.6)	0.6 (0.1)	0.0 (0.0)	0.0 (0.0)	0.4 (0.7)	3.9 (0.5)
19/05	0.0 (0.0)	40.7 (7.9)	0.0 (0.0)	37.9 (6.2)	4.2 (1.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.9 (2.2)
23/05	0.8 (1.2)	13.2 (1.2)	0.0 (0.0)	60.3 (3.2)	1.3 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.7 (0.1)
26/05	22.7 (1.2)	23.4 (0.9)	0.0 (0.0)	30.9 (3.2)	0.9 (0.0)	5.4 (0.9)	0.2 (0.1)	0.4 (0.2)	0.3 (0.3)	3.9 (0.9)
30/05	41.1 (2.0)	17.5 (2.4)	0.0 (0.0)	13.2 (2.0)	1.0 (0.6)	4.6 (2.2)	0.0 (0.0)	0.0 (0.0)	1.2 (1.0)	15.5 (3.6)
06/06	0.0 (0.0)	4.6 (1.3)	0.7 (0.6)	50.9 (3.4)	0.8 (0.8)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	2.4 (0.6)	26.0 (6.7)
14/06	2.4 (2.5)	8.5 (0.3)	0.0 (0.0)	59.2 (2.4)	1.2 (1.1)	2.1 (0.5)	0.0 (0.0)	0.1 (0.1)	4.9 (1.2)	5.7 (1.7)
27/06	5.7 (1.9)	15.6 (3.6)	0.0 (0.0)	23.2 (7.0)	4.1 (0.3)	2.0 (0.9)	0.0 (0.0)	2.1 (0.6)	20.6 (1.5)	14.0 (3.6)
11/07	5.9 (0.8)	14.3 (3.7)	0.0 (0.0)	21.6 (5.2)	4.1 (0.7)	2.1 (0.2)	0.0 (0.0)	2.5 (0.2)	19.9 (2.5)	16.0 (3.7)
27/07	22.7 (1.3)	23.4 (0.9)	0.0 (0.0)	30.9 (3.2)	0.9 (0.0)	5.4 (1.0)	0.2 (0.1)	0.4 (0.2)	0.5 (0.1)	3.9 (0.9)
16/08	29.1 (3.0)	19.1 (3.0)	0.0 (0.0)	27.8 (2.5)	5.0 (1.1)	4.6 (0.6)	0.0 (0.0)	3.8 (0.4)	0.0 (2.4)	5.4 (0.9)
30/08	8.2 (1.2)	26.2 (2.5)	0.0 (0.0)	31.0 (0.5)	6.3 (1.5)	5.9 (1.1)	0.0 (0.0)	3.7 (1.5)	0.0 (0.0)	28.3 (1.8)
05/09	10.2 (9.5)	24.4 (9.7)	0.0 (0.0)	30.3 (5.7)	2.3 (1.1)	9.0 (1.4)	0.0 (0.0)	4.3 (0.3)	0.0 (0.0)	5.8 (5.1)
03/10	4.3 (2.6)	3.4 (1.8)	0.0 (0.0)	62.0 (1.1)	1.1 (0.4)	1.8 (0.2)	0.1 (0.0)	0.0 (0.1)	0.3 (0.6)	9.5 (4.4)
24/10	18.7 (1.2)	27.2 (3.2)	0.0 (0.0)	15.1 (4.0)	4.6 (0.6)	8.3 (1.2)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	6.0 (0.2)
Rectum										
21/03	31.4 (8.8)	13.9 (3.2)	0.0 (0.0)	15.0 (3.7)	1.1 (2.0)	6.0 (0.6)	1.1 (0.4)	1.2 (0.8)	0.0 (0.0)	14.2 (0.8)
04/04	31.1 (8.1)	0.0 (0.0)	0.0 (0.0)	28.7 (3.8)	1.9 (3.4)	8.6 (4.0)	0.4 (0.2)	0.6 (0.3)	0.0 (0.0)	11.0 (1.9)
11/04	6.5 (2.1)	18.4 (4.4)	3.6 (0.6)	33.0 (2.2)	4.0 (2.1)	4.2 (1.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	10.8 (0.8)
18/04	0.0 (0.0)	36.2 (6.9)	2.9 (2.8)	19.5 (17.5)	2.9 (0.6)	5.1 (2.4)	0.3 (0.3)	1.4 (0.8)	0.0 (0.0)	7.9 (7.7)
26/04	15.5 (1.4)	19.3 (3.8)	3.3 (1.0)	36.8 (3.5)	1.9 (1.3)	0.0 (0.0)	0.2 (0.2)	0.1 (0.2)	0.0 (0.0)	6.4 (0.8)
28/04	0.0 (0.0)	12.1 (1.0)	6.0 (1.2)	48.1 (5.3)	1.4 (0.7)	1.6 (0.8)	0.1 (0.1)	0.1 (0.1)	0.0 (0.0)	17.0 (5.2)
02/05	1.5 (0.4)	41.5 (1.8)	7.7 (1.1)	12.9 (0.9)	5.3 (0.7)	2.4 (0.2)	0.4 (0.1)	0.4 (0.1)	2.8 (0.4)	4.6 (0.7)
09/05	12.4 (5.2)	0.0 (0.0)	1.3 (0.4)	54.1 (0.9)	8.1 (1.6)	1.8 (1.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	11.5 (1.9)
12/05	7.8 (1.8)	8.1 (1.1)	0.0 (0.0)	57.3 (0.9)	1.8 (1.5)	0.0 (0.0)	0.2 (0.1)	0.1 (0.1)	0.0 (0.0)	6.6 (0.8)
16/05	4.2 (1.9)	18.7 (2.3)	1.5 (0.3)	46.7 (2.4)	4.6 (2.6)	1.1 (0.2)	0.2 (0.1)	0.1 (0.1)	1.4 (1.3)	7.8 (1.4)
19/05	6.5 (4.9)	34.7 (10.2)	3.0 (0.6)	26.9 (9.1)	3.2 (0.1)	1.6 (0.1)	0.2 (0.0)	0.1 (0.0)	1.0 (1.7)	6.6 (0.6)
23/05	3.3 (1.2)	5.1 (1.4)	1.7 (1.4)	52.6 (8.4)	1.0 (0.4)	1.8 (1.1)	0.1 (0.1)	0.1 (0.1)	0.0 (0.0)	20.1 (4.2)
26/05	35.5 (4.4)	0.0 (0.0)	0.0 (0.0)	26.1 (1.8)	0.6 (0.2)	4.4 (0.3)	0.2 (0.2)	0.3 (0.3)	0.0 (0.0)	15.5 (2.6)
30/05	50.5 (8.9)	2.3 (2.1)	0.0 (0.0)	14.9 (6.2)	0.0 (0.0)	3.7 (1.5)	0.1 (0.2)	0.2 (0.4)	0.0 (0.0)	14.8 (1.3)
06/06	0.0 (0.0)	5.2 (1.2)	0.8 (0.7)	52.6 (4.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	26.6 (5.8)
14/06	6.6 (1.6)	7.4 (1.9)	0.0 (0.0)	39.6 (4.2)	1.8 (1.7)	1.3 (0.5)	0.0 (0.0)	0.7 (0.1)	2.4 (0.8)	20.2 (3.4)
27/06	9.8 (0.9)	7.4 (0.6)	0.0 (0.0)	12.6 (0.8)	1.2 (0.1)	0.4 (0.1)	0.0 (0.0)	7.9 (1.3)	9.7 (0.5)	19.7 (2.8)
11/07	11.7 (0.6)	8.8 (0.8)	0.0 (0.0)	14.8 (1.4)	1.5 (0.3)	0.5 (0.1)	0.0 (0.0)	9.2 (1.6)	11.3 (0.5)	21.6 (3.8)
27/07	33.9 (3.8)	0.0 (0.0)	0.0 (0.0)	26.5 (2.1)	0.6 (0.2)	4.5 (0.2)	0.2 (0.2)	0.3 (0.3)	0.7 (0.6)	15.7 (2.2)
16/08	0.0 (0.0)	16.0 (2.1)	0.0 (0.0)	23.5 (3.2)	0.0 (0.0)	0.0 (0.0)	3.0 (1.4)	10.2 (1.7)	16.6 (2.7)	9.3 (0.5)
30/08	22.5 (7.3)	12.7 (5.0)	0.0 (0.0)	22.4 (2.1)	0.5 (0.5)	3.6 (3.2)	2.9 (2.2)	1.1 (0.2)	0.3 (0.4)	9.2 (2.8)
05/09	20.2 (8.5)	9.2 (0.3)	0.0 (0.0)	22.5 (4.0)	0.8 (0.1)	4.9 (0.6)	1.6 (0.1)	1.2 (0.4)	0.0 (0.0)	19.1 (1.5)
03/10	10.4 (4.0)	3.8 (0.6)	0.0 (0.0)	48.0 (3.1)	1.2 (0.0)	2.1 (0.8)	0.2 (0.3)	0.1 (0.2)	0.1 (0.1)	14.7 (3.2)
24/10	10.1 (4.8)	5.3 (3.1)	0.0 (0.0)	40.2 (18.8)	0.2 (0.1)	3.3 (1.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.9 (2.9)

crease of twice their initial percentage in late May (Fig. 6a). The proportion of 20:4(n-3) only varied between 0.3 and 0.7%, but presented 2 important peaks in April and in late May and gradually in-creased throughout the summer until the end of the study (Fig. 6b). Finally, *iso*17:0 FA found in the PLs presented interesting dynamics as it decreased from March to the end of May before rising again during
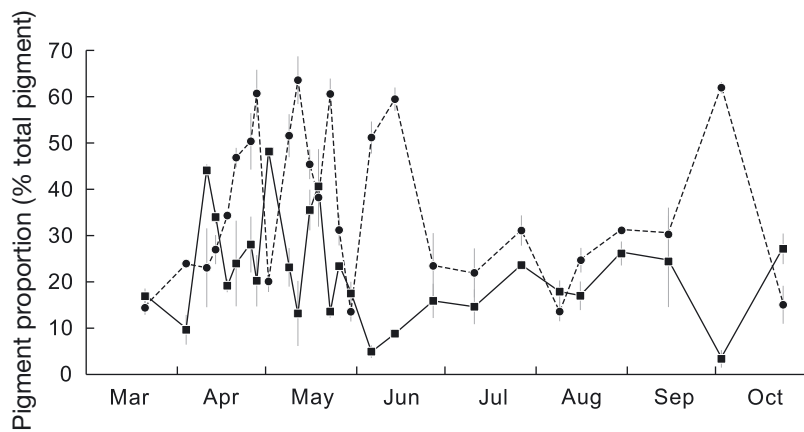
Fig. 4. Proportions of fucoxanthin (dots, dashed line) and peridinin (squares, solid line) in the stomach content of *Pecten maximus* in the Bay of Brest in 2011. Chl *a* quantity was removed from total pigment amount before calculating the proportion of each other pigment. Standard deviations are indicated by vertical grey lines
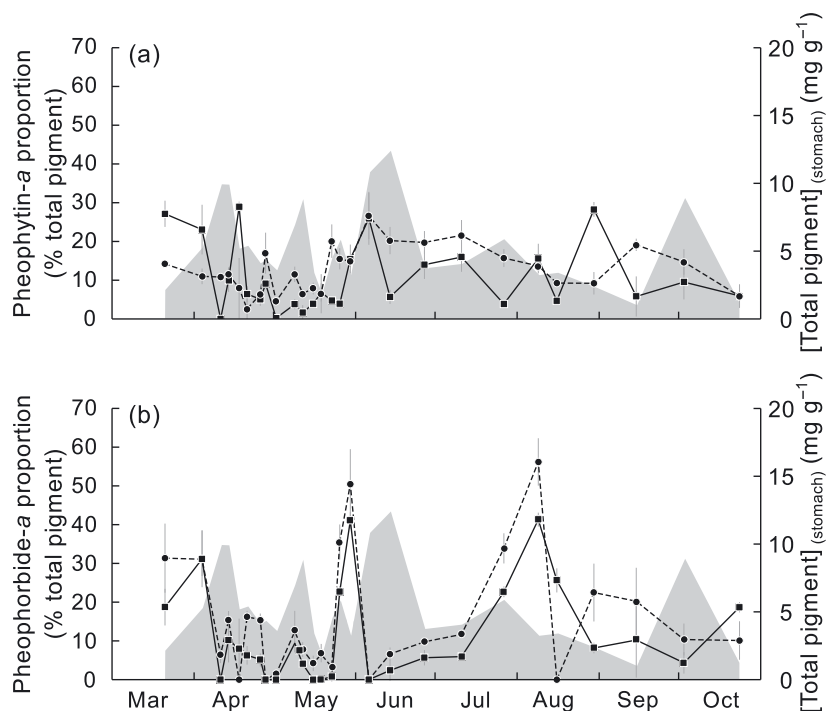

Fig. 5. Total pigment concentration, including chl *a*, in the stomach content of *Pecten maximus* (grey area, right axis) and proportions of (a) pheophorbide-*a* and (b) pheophytin-*a* in the stomach content (solid line, black squares) and the rectum content (dashed lines, black dots) of *P. maximus* in the Bay of Brest in 2011. Standard deviations are indicated by vertical grey lines

summer and reaching a median value at the end of monitoring. Interestingly, our results suggest that most FAs assimilated as reserve compounds (neutral fraction), except 18:5(n-3), were hardly related to the short-term seasonal patterns observed through the analysis of pigments and PLs.

Sterol content in the DG increased from 35.9 to 105.3 µg g^{-1} with a peak at 183.0 µg g^{-1} on 19 May

(Table 5). Cholesta-5-e(n-3)β-ol (cholesterol) was, on average, the most common sterolic compound (24%), followed by 5β-cholesta(n-3)β-ol (coprostanol; 21.6%), 24β-methylene-cholesta-5, 24(28)-die(n-3)β-ol (24 methylene cholesterol; 15.8%), 24β-methylcholesta-5, 22-die(n-3)β-ol (brassicasterol; 11.8%) and 24β-ethylenecholesta-5-e(n-3)β-ol (β-sitosterol; 5.4%). Cholesterol decreased slowly from 29.5 to 20.5% during the study period, without any strong variation (Fig. 7). The evolution of 24 methylene cholesterol followed the same dynamics as fucoxanthin in the stomach content, and EPA or 16:1(n-7) in the DG. A first increase was observed in May, lasting for 2 wk with a maximum at 22%. It was followed by a second, more intense peak in early June, up to 24%, and a final increase in late September to about the same level as the first spring peak. Brassicasterol showed an inverse dynamic: it decreased to 10% from mid-April to mid-May, then increased at the end of the month before reaching a plateau in summer and finally slightly decreasing again from mid-August until the end of the study.

Multivariate analysis

The CCA shown in Fig. 8 validates the observations made through the analyses of the different biochemical markers used in this study, even though the eigenvalues of the CCA axes are not very high (10 to 40.8% of variance explained). Only the most prominent markers identified previously were selected and used in this analysis, since the high number of markers, their variability and the fact that some are less specific of certain microalgae produced results that were hardly interpretable in early trials. The best explained dispersion occurs along the first axis in the pigment analysis (Fig. 8a), differentiating between diatom markers on the left-hand side of the axis and markers of other food sources on the opposite side.
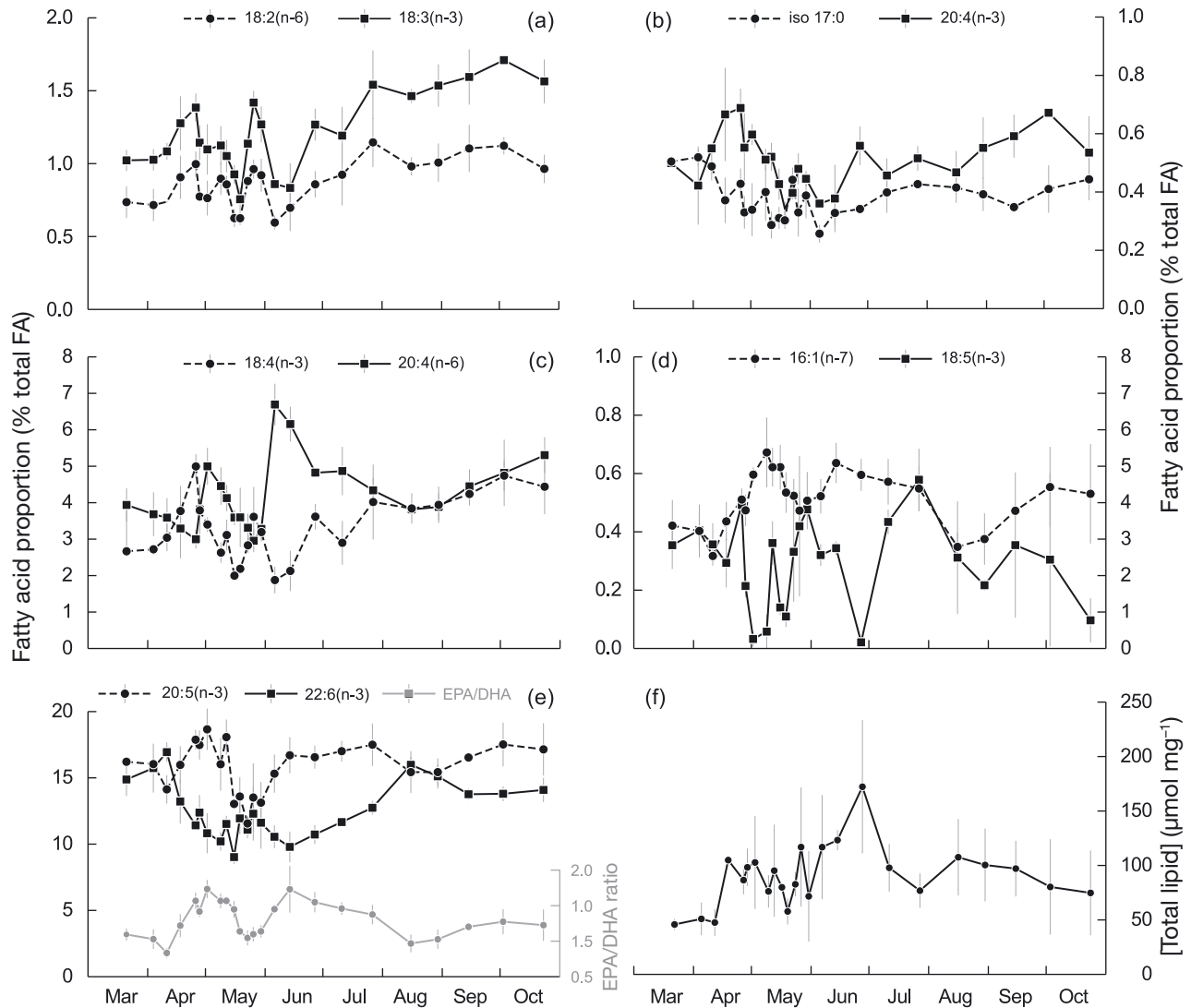
Fig. 6. Proportions of 10 major fatty acids in the digestive gland of *Pecten maximus* (expressed as fatty acid molar percentage of total) in the Bay of Brest in 2011. (a) 18:2(n-6) (dashed line, black dots); 18:3(n-3) (solid line, black squares) in polar lipids; (b) *iso*17:0 (dashed line, black dots) and 20:4(n-3) (solid line, black squares) in polar lipids; (c) 18:4(n-3) (solid line, black dots) and 20:4(n-6) (solid line, black squares) in polar lipids; (d) 16:1(n-7) (dashed line, black dots) in polar lipids and 18:5(n-3) (solid line, black squares) in neutral lipids; (e) 20:5(n-3) (dashed line, black dots) and 22:6(n-3) (solid line, black squares) in polar lipids and their ratio (grey line, grey dots): 20:5(n-3)(EPA):22:6(n-3)(DHA); (f) total fatty acid concentration (polar plus neutral fractions, expressed in μmol mg^{-1}). Standard deviations are indicated by grey vertical lines

Another distinction arises along the second axis, showing markers for diatoms and Dinophyceae in the upper part and other microalgae markers in the lower part. The same trend emerged from the CCA conducted on lipid markers (Fig. 8b), with diatom markers, as well as, to a lesser extent Dinophyceae, clearly distributed away from others. Another observation common to both plots is the clear distinction between microalgae from the water column and those from the water–sediment interface, again confirming the temporal shift reported earlier.

DISCUSSION

Validation of pigments as biomarkers

To our knowledge, the present study is the first to combine the use of pigments, FAs and sterols as food source biomarkers in a seasonal trophic study of a marine suspension-feeding invertebrate. The only other study presenting similar data is by Hurtado et al. (2012), who focused on seasonal dynamics of pigments, FAs and sterols in relation to the reproductive

Table 3. Fatty acid (FA) composition of the polar lipids in the digestive gland of *Pecten maximus* (expressed as FA molar percentage of total) in the Bay of Brest in 2011. Standard deviations are in brackets. TMTD: 4,8,12-trimethyltridecanoic acid; SAFA: sum of saturated FA; MUFA: sum of monounsaturated FA; PUFA: sum of polyunsaturated FA; DMA: dimethyl acetals; (n-3): sum of n-3 polyunsaturated FA; (n-6): sum of n-6 polyunsaturated FA (Table continued on next page)

Date (dd/mo)	TMTD	14:0	16:0	*iso*17:0	18:0	16:1(n-7)	18:1(n-9)	18:1(n-7)	18:2(n-6)	18:3(n-3)
21-Mar	8.3 (7.8)	3.0 (0.1)	11.5 (0.7)	0.5 (0.0)	8.6 (1.1)	3.4 (0.7)	1.3 (0.1)	1.0 (1.0)	0.7 (0.1)	1.0 (0.1)
04-Apr	5.2 (9.0)	3.2 (0.4)	11.3 (0.7)	0.5 (0.0)	8.7 (1.1)	3.2 (0.7)	1.2 (0.2)	1.4 (1.2)	0.7 (0.1)	1.0 (0.1)
11-Apr	1.9 (3.3)	3.2 (0.3)	13.3 (0.9)	0.5 (0.1)	8.9 (0.6)	2.5 (0.1)	1.0 (0.1)	2.0 (0.2)	0.7 (0.0)	1.1 (0.1)
18-Apr	7.1 (6.4)	3.9 (0.5)	12.2 (1.0)	0.4 (0.1)	7.8 (1.4)	3.5 (0.5)	1.3 (0.0)	2.1 (0.2)	0.9 (0.1)	1.3 (0.2)
26-Apr	8.0 (1.8)	3.9 (0.1)	14.0 (1.0)	0.4 (0.1)	7.9 (0.3)	4.1 (0.3)	1.7 (0.1)	2.5 (0.2)	1.0 (0.1)	1.4 (0.1)
28-Apr	6.0 (5.3)	4.0 (0.6)	11.9 (0.8)	0.3 (0.1)	8.2 (0.3)	3.8 (0.4)	1.2 (0.1)	2.6 (0.2)	0.8 (0.0)	1.1 (0.1)
02-May	6.6 (2.1)	4.3 (0.3)	13.0 (1.3)	0.3 (0.1)	5.3 (4.6)	4.8 (0.2)	1.2 (0.2)	3.5 (0.2)	0.8 (0.1)	1.1 (0.2)
09-May	4.9 (2.1)	4.0 (1.3)	14.9 (1.6)	0.4 (0.1)	9.0 (0.2)	5.4 (0.9)	1.5 (0.1)	4.7 (0.3)	0.9 (0.1)	1.1 (0.1)
12-May	5.9 (0.8)	4.0 (0.5)	14.3 (1.2)	0.3 (0.0)	5.4 (4.7)	5.0 (0.5)	1.3 (0.1)	5.3 (0.4)	0.9 (0.1)	1.1 (0.1)
16-May	10.1 (0.6)	4.5 (0.3)	14.7 (0.2)	0.3 (0.0)	10.6 (0.5)	5.0 (0.6)	1.4 (0.2)	4.5 (0.3)	0.6 (0.1)	0.9 (0.1)
19-May	7.0 (6.1)	4.3 (0.5)	15.2 (0.9)	0.3 (0.0)	9.4 (0.5)	4.3 (0.5)	1.3 (0.1)	4.2 (0.1)	0.6 (0.0)	0.8 (0.1)
23-May	8.8 (1.3)	4.6 (0.2)	15.8 (0.5)	0.4 (0.0)	9.8 (0.2)	4.2 (0.4)	1.8 (0.1)	3.5 (0.2)	0.9 (0.0)	1.1 (0.0)
26-May	6.9 (1.3)	3.9 (0.8)	15.3 (1.3)	0.3 (0.0)	9.5 (0.6)	3.8 (0.5)	2.0 (0.2)	3.1 (0.6)	1.0 (0.1)	1.4 (0.1)
30-May	8.3 (1.4)	4.6 (0.8)	15.1 (0.9)	0.4 (0.1)	8.9 (0.6)	4.1 (0.8)	1.8 (0.3)	2.9 (0.4)	0.9 (0.1)	1.3 (0.1)
06-Jun	7.6 (0.0)	5.7 (0.4)	12.4 (0.9)	0.3 (0.1)	8.1 (0.6)	4.2 (0.5)	1.2 (0.1)	2.5 (0.1)	0.6 (0.0)	0.9 (0.0)
14-Jun	6.1 (1.5)	5.4 (0.3)	13.6 (1.4)	0.3 (0.0)	8.4 (0.3)	5.1 (0.5)	1.4 (0.1)	2.8 (0.2)	0.7 (0.2)	0.8 (0.2)
27-Jun	6.0 (1.4)	4.9 (0.6)	14.1 (1.4)	0.3 (0.0)	5.2 (4.5)	4.8 (0.4)	1.5 (0.1)	2.9 (0.2)	0.9 (0.1)	1.3 (0.1)
11-Jul	5.8 (1.7)	4.7 (0.4)	14.8 (0.7)	0.4 (0.0)	8.5 (0.6)	4.6 (0.6)	1.7 (0.3)	2.7 (0.2)	0.9 (0.2)	1.2 (0.2)
27-Jul	4.7 (1.3)	4.1 (0.1)	15.5 (0.8)	0.4 (0.0)	7.7 (0.5)	4.4 (0.6)	2.1 (0.4)	2.7 (0.1)	1.1 (0.2)	1.5 (0.2)
16-Aug	4.7 (0.3)	3.2 (0.3)	13.8 (0.2)	0.4 (0.1)	7.7 (0.6)	2.8 (0.4)	1.4 (0.1)	2.4 (0.1)	1.0 (0.1)	1.5 (0.0)
30-Aug	4.4 (1.2)	3.3 (0.5)	14.2 (1.2)	0.4 (0.1)	7.6 (0.4)	3.0 (0.7)	1.5 (0.2)	2.5 (0.1)	1.0 (0.1)	1.5 (0.1)
05-Sep	3.2 (0.3)	3.8 (0.1)	15.2 (1.1)	0.4 (0.0)	7.7 (0.3)	3.8 (0.1)	1.6 (0.2)	2.7 (0.1)	1.1 (0.2)	1.6 (0.2)
03-Oct	3.3 (0.4)	2.8 (1.9)	15.6 (0.1)	0.4 (0.1)	7.2 (1.4)	4.4 (1.1)	1.8 (0.2)	2.7 (0.1)	1.1 (0.1)	1.7 (0.0)
24-Oct	4.1 (0.5)	2.7 (1.9)	14.6 (1.2)	0.4 (0.1)	7.2 (1.5)	4.2 (1.4)	1.8 (0.2)	2.6 (0.2)	1.0 (0.1)	1.6 (0.1)

Table 4. Fatty acid (FA) composition of the neutral lipids in the digestive gland of *Pecten maximus* (expressed as FA molar percentage of total) in the Bay of Brest in 2011. Standard deviations are in brackets. TMTD: 4,8,12-trimethyltridecanoic acid; SAFA: sum of saturated FA; MUFA: sum of monounsaturated FA; PUFA: sum of polyunsaturated FA; DMA: dimethyl acetals; (n-3): sum of n-3 polyunsaturated FA; (n-6): sum of n-6 polyunsaturated FA (Table continued on next page)

Date (dd/mo)	TMTD	14:0	16:0	16:1(n-7)	18:1(n-9)	18:1(n-7)	18:2(n-6)	18:3(n-3)	18:4(n-3)	18:5(n-3)
21-Mar	1.5 (0.2)	2.1 (0.4)	6.5 (2.5)	8.8 (0.4)	2.8 (0.0)	3.8 (0.1)	2.1 (0.0)	2.2 (0.0)	6.5 (0.0)	0.4 (0.1)
04-Apr	2.0 (0.4)	2.1 (0.1)	7.9 (2.3)	8.0 (0.8)	2.5 (0.0)	4.0 (0.1)	2.1 (0.0)	2.5 (0.0)	7.9 (0.0)	0.4 (0.1)
11-Apr	1.6 (0.4)	2.3 (0.4)	9.9 (0.9)	7.5 (0.8)	2.1 (0.3)	3.3 (0.0)	2.3 (0.2)	2.8 (0.4)	9.9 (2.3)	0.4 (0.1)
18-Apr	1.8 (0.2)	7.5 (0.5)	16.5 (0.2)	8.1 (0.6)	1.8 (1.3)	3.6 (0.1)	1.9 (0.4)	2.1 (0.7)	8.3 (1.2)	0.3 (0.1)
26-Apr	1.8 (0.2)	8.1 (0.7)	14.3 (4.2)	8.2 (1.7)	2.8 (0.2)	2.9 (0.1)	2.2 (0.2)	2.8 (0.2)	8.4 (0.8)	0.5 (0.0)
28-Apr	1.8 (0.3)	7.6 (0.9)	14.2 (2.2)	9.4 (0.4)	1.7 (1.0)	3.8 (0.2)	2.4 (0.3)	2.3 (1.0)	8.9 (2.9)	0.2 (0.0)
02-May	1.6 (1.4)	10.4 (1.6)	11.7 (4.8)	7.2 (3.2)	2.8 (0.2)	3.6 (0.2)	1.6 (0.2)	2.6 (0.4)	7.7 (1.4)	0.0 (0.0)
09-May	1.1 (1.0)	10.5 (3.2)	13.4 (3.4)	9.8 (3.5)	2.5 (0.5)	3.3 (0.2)	2.0 (1.0)	2.0 (0.6)	7.5 (1.8)	0.1 (0.0)
12-May	1.8 (0.0)	8.3 (0.3)	15.8 (0.6)	11.2 (0.5)	2.1 (0.1)	4.4 (0.0)	1.9 (0.2)	2.4 (0.2)	8.2 (0.4)	0.4 (0.1)
16-May	1.8 (0.1)	8.6 (0.3)	17.7 (0.2)	10.5 (0.3)	2.4 (0.1)	4.7 (0.0)	1.8 (0.1)	2.1 (0.0)	6.1 (0.3)	0.1 (0.1)
19-May	2.5 (0.2)	9.3 (0.3)	18.1 (0.3)	10.3 (0.2)	2.4 (0.2)	5.0 (0.0)	1.7 (0.2)	1.9 (0.1)	5.5 (0.6)	0.1 (0.0)
23-May	2.4 (0.6)	7.0 (3.1)	18.8 (2.8)	12.0 (1.5)	3.0 (1.1)	1.5 (0.1)	2.3 (0.0)	2.4 (0.2)	6.1 (0.2)	0.3 (0.2)
26-May	2.2 (0.3)	6.4 (3.2)	16.7 (3.2)	13.2 (3.2)	2.2 (0.8)	4.9 (0.1)	2.3 (0.4)	2.0 (0.6)	5.9 (1.4)	0.4 (0.2)
30-May	2.9 (0.8)	9.3 (0.5)	20.2 (3.3)	9.2 (0.4)	3.2 (0.9)	4.0 (0.3)	1.2 (1.3)	1.7 (1.1)	8.4 (3.8)	0.5 (0.2)
06-Jun	2.3 (0.6)	10.1 (0.6)	12.4 (4.1)	8.8 (3.3)	1.9 (0.8)	4.0 (0.1)	1.6 (0.1)	1.9 (0.2)	5.3 (1.2)	0.3 (0.0)
14-Jun	2.5 (0.5)	10.0 (0.4)	16.3 (1.9)	11.3 (0.3)	2.6 (0.3)	1.5 (0.0)	1.0 (0.3)	2.4 (0.4)	6.2 (0.9)	0.3 (0.0)
27-Jun	2.9 (0.3)	10.4 (0.2)	14.0 (0.7)	10.4 (3.0)	2.2 (0.6)	2.2 (0.0)	0.8 (0.5)	2.2 (1.0)	8.7 (0.6)	0.0 (0.0)
11-Jul	3.9 (3.3)	9.3 (0.9)	13.5 (2.4)	9.6 (1.4)	1.6 (0.8)	3.2 (0.1)	1.7 (0.4)	2.3 (0.2)	6.5 (0.4)	0.4 (0.0)
27-Jul	1.9 (0.4)	5.8 (3.3)	12.6 (3.2)	10.0 (0.9)	3.0 (0.4)	3.9 (0.2)	2.2 (0.3)	2.6 (0.1)	7.2 (0.5)	0.6 (0.1)
16-Aug	2.2 (0.5)	8.3 (0.6)	13.8 (0.9)	9.4 (2.2)	1.6 (0.1)	3.0 (0.1)	1.7 (0.9)	2.9 (0.3)	7.4 (0.2)	0.3 (0.2)
30-Aug	1.8 (0.1)	8.5 (0.3)	15.2 (2.6)	8.0 (1.0)	1.8 (0.5)	4.0 (0.0)	2.4 (0.4)	2.9 (0.4)	7.3 (0.2)	0.2 (0.0)
05-Sep	1.6 (0.3)	7.9 (0.9)	17.0 (4.2)	7.9 (1.3)	2.2 (1.1)	2.7 (0.1)	2.4 (0.5)	2.9 (0.5)	7.8 (1.3)	0.4 (0.3)
03-Oct	3.0 (2.5)	7.1 (2.7)	13.0 (1.6)	5.5 (1.3)	1.9 (1.3)	2.6 (0.2)	2.1 (1.1)	2.5 (1.6)	7.4 (3.0)	0.3 (0.3)
24-Oct	3.0 (2.2)	6.8 (2.8)	11.1 (3.2)	6.3 (2.7)	2.6 (1.3)	1.9 (0.4)	2.0 (1.1)	2.5 (1.7)	7.5 (3.3)	0.1 (0.1)

Table 3 (continued)

18:4(n-3)	20:4(n-6)	20:5(n-3)	22:6(n-3)	SAFA	MUFA	PUFA	DMA	(n-3)	(n-6)
2.7 (0.1)	3.9 (0.4)	16.2 (0.1)	14.9 (1.2)	24.9 (0.6)	8.6 (1.0)	47.3 (2.5)	10.3 (3.8)	38.5 (2.6)	6.4 (1.0)
2.7 (0.1)	3.7 (0.6)	16.0 (0.4)	15.7 (1.8)	25.0 (0.6)	8.8 (1.0)	48.1 (2.7)	12.1 (4.5)	39.7 (3.2)	6.1 (1.5)
3.0 (0.4)	3.6 (0.5)	14.1 (1.0)	16.9 (0.7)	27.6 (2.0)	8.8 (0.3)	49.5 (1.4)	11.7 (1.3)	40.2 (1.8)	6.5 (0.8)
3.8 (0.7)	3.3 (0.8)	16.0 (1.4)	13.2 (1.6)	25.7 (1.7)	9.7 (1.0)	47.3 (2.8)	9.9 (1.7)	38.8 (2.4)	5.9 (1.4)
5.0 (0.3)	3.0 (0.2)	17.9 (0.7)	11.4 (0.3)	26.7 (0.7)	11.1 (0.8)	47.5 (1.7)	6.4 (0.3)	39.9 (0.8)	5.4 (0.9)
3.8 (0.8)	3.8 (0.7)	17.5 (1.1)	12.4 (1.3)	25.6 (1.5)	10.7 (0.5)	47.7 (3.3)	9.6 (0.2)	39.1 (3.3)	6.1 (0.9)
3.4 (0.2)	5.0 (0.5)	18.7 (1.5)	10.8 (1.5)	23.7 (3.6)	12.7 (0.8)	48.2 (4.4)	8.4 (0.7)	38.4 (3.8)	7.6 (0.8)
2.6 (0.3)	4.5 (0.5)	16.0 (2.0)	10.2 (0.7)	29.1 (2.4)	14.9 (0.9)	42.9 (3.6)	7.9 (1.2)	33.5 (2.5)	7.1 (1.2)
3.1 (0.4)	4.1 (0.3)	18.1 (1.3)	11.5 (0.6)	25.3 (3.0)	14.5 (1.2)	46.6 (2.2)	7.4 (0.2)	37.7 (2.3)	6.7 (0.8)
2.0 (0.1)	3.6 (0.2)	13.0 (0.3)	9.0 (0.5)	31.7 (0.3)	14.6 (0.8)	36.2 (0.5)	7.1 (0.8)	27.9 (0.3)	5.7 (0.4)
2.2 (0.1)	3.6 (0.8)	13.6 (1.4)	11.9 (1.1)	31.2 (1.7)	13.3 (0.5)	40.8 (3.9)	7.5 (1.6)	31.8 (2.9)	5.8 (1.2)
2.8 (0.2)	3.3 (0.4)	11.6 (0.6)	11.1 (0.6)	32.3 (0.6)	13.3 (0.7)	38.4 (0.8)	7.0 (0.3)	29.9 (0.4)	5.6 (0.6)
3.6 (0.8)	3.0 (0.3)	13.5 (2.5)	12.3 (2.0)	30.6 (2.8)	12.4 (1.9)	41.9 (5.9)	7.8 (0.2)	34.2 (5.7)	5.4 (0.6)
3.2 (0.2)	3.3 (0.3)	13.1 (1.5)	11.6 (1.9)	30.6 (1.3)	12.1 (1.8)	40.9 (4.5)	7.6 (0.6)	32.8 (3.6)	5.6 (0.7)
1.9 (0.4)	6.7 (0.6)	15.3 (1.4)	10.6 (0.8)	27.5 (0.9)	11.0 (0.6)	47.5 (1.7)	6.1 (0.6)	32.1 (2.6)	9.4 (1.2)
2.1 (0.5)	6.2 (0.5)	16.7 (1.3)	9.8 (1.1)	29.2 (1.3)	12.3 (0.9)	44.1 (0.5)	7.9 (1.1)	32.9 (0.8)	8.7 (0.8)
3.6 (0.3)	4.8 (0.1)	16.6 (0.8)	10.7 (0.7)	26.0 (2.3)	12.7 (1.1)	47.1 (2.6)	7.7 (0.5)	36.2 (1.7)	7.6 (0.4)
2.9 (0.6)	4.9 (0.6)	17.0 (0.7)	11.7 (0.2)	29.3 (0.7)	11.9 (0.9)	46.3 (1.1)	6.3 (0.9)	36.1 (1.7)	7.7 (1.8)
4.0 (1.0)	4.3 (0.3)	17.5 (1.6)	12.7 (0.5)	28.7 (0.3)	11.0 (0.8)	49.4 (2.2)	5.8 (1.2)	40.0 (2.9)	7.0 (1.2)
3.8 (0.4)	3.8 (0.1)	15.4 (1.5)	16.0 (0.5)	25.9 (1.0)	9.2 (0.9)	53.0 (1.4)	6.9 (0.2)	40.3 (2.0)	6.3 (0.4)
3.9 (0.5)	3.9 (0.4)	15.4 (1.0)	15.1 (0.9)	26.3 (2.5)	9.6 (0.9)	52.6 (1.8)	6.8 (0.4)	39.7 (1.1)	6.5 (0.4)
4.2 (0.3)	4.4 (0.5)	16.5 (0.2)	13.8 (0.4)	28.5 (1.3)	10.8 (0.3)	50.1 (0.5)	7.1 (0.9)	40.3 (0.6)	7.1 (0.4)
4.7 (0.4)	4.8 (0.9)	17.5 (1.6)	13.8 (0.5)	27.4 (3.1)	11.6 (1.8)	51.7 (2.1)	5.6 (1.4)	41.2 (0.5)	7.6 (1.0)
4.4 (0.7)	5.3 (0.5)	17.1 (1.9)	14.1 (0.9)	26.3 (2.3)	11.3 (2.2)	51.8 (2.1)	6.1 (1.8)	40.7 (1.0)	8.2 (0.5)

Table 4 (continued)

20:4(n-6)	20:4(n-3)	20:5(n-3)	22:6(n-3)	SAFA	MUFA	PUFA	DMA	(n-3)	(n-6)
3.3 (0.0)	1.0 (0.5)	23.0 (0.0)	6.8 (0.0)	26.5 (0.0)	18.4 (0.6)	51.1 (0.0)	1.5 (0.0)	41.7 (0.2)	6.8 (0.1)
2.4 (0.0)	1.1 (1.3)	21.5 (0.0)	6.6 (0.0)	27.1 (0.0)	18.1 (0.3)	50.4 (0.1)	1.4 (0.0)	41.9 (0.3)	6.0 (0.2)
1.5 (1.1)	1.2 (0.2)	21.6 (0.8)	6.6 (0.2)	27.0 (1.5)	15.7 (1.0)	53.1 (2.1)	2.2 (0.4)	44.9 (2.3)	5.2 (0.8)
2.5 (0.3)	1.3 (0.2)	21.8 (1.3)	6.6 (0.4)	28.1 (0.9)	17.2 (1.2)	51.5 (0.9)	0.7 (0.8)	42.9 (1.3)	5.9 (0.7)
2.8 (0.4)	1.0 (0.4)	23.6 (3.2)	6.2 (0.6)	26.3 (4.0)	17.5 (2.7)	53.4 (6.2)	0.2 (0.1)	44.7 (5.1)	6.4 (0.6)
2.6 (1.1)	1.3 (0.4)	24.6 (0.7)	6.6 (0.9)	25.0 (2.6)	18.2 (1.5)	54.4 (4.0)	0.1 (0.1)	46.2 (5.0)	6.4 (0.6)
3.2 (0.4)	0.7 (0.2)	25.8 (5.5)	6.4 (1.6)	26.5 (3.8)	16.8 (4.3)	54.2 (7.6)	0.5 (0.8)	45.3 (8.2)	6.2 (0.5)
2.4 (0.7)	1.0 (0.4)	19.9 (3.2)	6.7 (2.2)	29.5 (4.3)	19.7 (5.7)	48.3 (1.8)	0.7 (0.8)	39.5 (1.5)	5.9 (1.0)
2.2 (0.2)	1.2 (0.0)	22.5 (1.3)	4.9 (0.5)	28.0 (0.9)	20.7 (0.7)	48.0 (1.6)	0.2 (0.0)	41.4 (1.5)	5.4 (0.1)
2.8 (0.1)	1.0 (0.1)	20.1 (0.1)	5.0 (0.5)	31.3 (0.1)	21.3 (0.5)	44.6 (0.5)	0.2 (0.0)	36.2 (0.5)	5.9 (0.0)
2.6 (0.6)	0.9 (0.1)	17.8 (0.4)	5.7 (0.3)	32.7 (0.0)	21.7 (0.6)	42.3 (0.8)	0.2 (0.1)	34.0 (0.5)	5.7 (0.9)
2.8 (1.0)	0.9 (0.3)	18.5 (4.3)	5.9 (1.1)	31.1 (5.5)	21.7 (1.9)	44.0 (6.4)	0.1 (0.1)	35.8 (5.0)	6.7 (1.1)
2.4 (0.8)	1.1 (0.2)	19.1 (7.0)	7.2 (2.5)	27.1 (7.1)	23.5 (3.0)	46.1 (10.0)	0.1 (0.1)	37.8 (9.6)	6.3 (1.6)
2.5 (1.0)	0.7 (0.2)	13.4 (0.4)	6.6 (2.2)	35.2 (4.2)	21.2 (2.3)	39.5 (5.0)	0.4 (0.3)	32.5 (5.0)	4.8 (0.0)
4.9 (0.9)	0.7 (0.2)	25.6 (2.7)	5.6 (1.7)	26.8 (4.0)	18.1 (5.3)	52.0 (4.5)	0.1 (0.0)	41.6 (4.9)	8.2 (1.0)
3.8 (0.5)	0.9 (0.1)	22.7 (1.2)	5.4 (0.6)	30.3 (2.1)	17.7 (0.7)	48.5 (1.1)	0.2 (0.0)	39.7 (2.1)	6.0 (0.8)
3.4 (0.4)	0.7 (0.1)	23.0 (0.5)	6.8 (0.7)	27.3 (0.8)	18.0 (1.7)	50.8 (2.3)	0.2 (0.1)	43.4 (1.6)	5.5 (0.5)
3.7 (0.6)	1.0 (0.1)	24.3 (2.1)	6.8 (0.6)	25.7 (3.0)	17.4 (2.2)	51.6 (2.6)	0.5 (0.5)	42.6 (1.9)	6.9 (0.3)
3.5 (0.6)	0.9 (0.1)	22.3 (2.2)	7.7 (0.3)	22.9 (6.3)	20.8 (2.0)	52.8 (4.1)	0.3 (0.1)	43.3 (2.7)	7.4 (1.1)
2.8 (1.0)	1.2 (0.1)	24.0 (1.2)	7.4 (2.3)	25.9 (0.7)	18.0 (1.6)	53.5 (2.6)	0.1 (0.1)	44.9 (0.6)	6.0 (1.6)
3.1 (0.2)	1.0 (0.0)	21.9 (1.2)	7.3 (1.3)	27.9 (3.2)	17.3 (0.3)	52.3 (3.7)	0.2 (0.0)	42.4 (2.8)	6.7 (0.6)
3.0 (0.3)	1.1 (0.2)	21.9 (3.0)	7.5 (1.8)	28.6 (4.6)	15.9 (2.2)	53.2 (7.2)	0.2 (0.0)	43.7 (6.6)	6.7 (0.7)
4.4 (1.7)	0.8 (0.5)	22.9 (3.5)	11.0 (3.8)	26.1 (3.5)	12.3 (3.2)	58.0 (3.6)	0.3 (0.2)	47.3 (4.5)	7.9 (0.7)
4.4 (1.3)	0.7 (0.5)	22.3 (4.0)	10.5 (3.4)	23.6 (5.2)	14.3 (6.0)	56.0 (4.6)	2.8 (3.7)	46.0 (5.5)	7.9 (0.3)

Table 5. Sterol composition of the digestive gland of *Pecten maximus* (expressed as sterol percentage of total) in the Bay of Brest in 2011. NOR: norcholesterol; CHO: cholesterol; BRA: brassicasterol; DES: desmosterol; CAM: campesterol; 24M: 24 methylene; STI: stigmasterol; BSI: β-sitosterol; FUS: fucosterol; OTH: other sterols; TOT: total sterol concentration (in µmol g^{-1}). Standard deviations are in brackets

| Date (dd/mo)	NOR	CHO	BRA	DES	CAM	24M	STI	BSI	FUC	OTH	TOT
21/03	4.0 (0.4)	29.6 (2.1)	15.8 (1.1)	4.6 (0.3)	2.5 (0.2)	12.4 (1.0)	3.3 (0.6)	6.9 (0.4)	1.7 (0.2)	19.2 (1.3)	46.77 (3.74)
04/04	4.3 (0.2)	29.8 (1.9)	13.6 (3.3)	4.0 (1.3)	2.3 (0.5)	11.6 (0.4)	3.0 (1.0)	5.9 (1.4)	1.5 (0.4)	23.9 (0.8)	50.85 (10.28)
11/04	4.1 (0.5)	28.1 (3.8)	14.7 (2.3)	3.7 (0.3)	2.5 (0.3)	13.3 (1.5)	2.9 (0.3)	5.9 (0.8)	1.7 (0.3)	23.5 (1.3)	35.92 (10.31)
18/04	4.3 (0.2)	29.1 (2.2)	15.4 (1.0)	2.9 (0.2)	2.1 (0.1)	14.4 (0.8)	2.9 (0.4)	6.1 (0.6)	1.6 (0.2)	21.8 (0.6)	55.65 (5.89)
26/04	3.6 (0.5)	25.4 (0.4)	12.1 (0.7)	2.2 (0.8)	1.9 (0.1)	15.7 (0.7)	2.1 (0.3)	5.3 (0.8)	1.9 (0.2)	29.8 (0.4)	58.81 (1.80)
28/04	2.7 (0.1)	24.3 (1.8)	10.7 (0.4)	2.8 (0.6)	1.7 (0.1)	18.5 (2.2)	2.5 (0.3)	5.6 (1.1)	1.6 (0.2)	30.1 (0.7)	53.73 (6.10)
02/05	3.3 (0.4)	23.9 (2.8)	9.9 (0.9)	2.2 (0.2)	1.9 (0.1)	21.7 (3.8)	2.5 (0.4)	5.0 (0.9)	1.3 (0.0)	28.5 (0.4)	50.75 (9.01)
09/05	3.6 (0.3)	24.5 (0.4)	10.0 (0.9)	1.8 (0.2)	1.7 (0.2)	21.6 (3.7)	2.5 (0.5)	5.0 (0.2)	1.3 (0.1)	27.9 (0.6)	49.75 (3.02)
12/05	3.2 (0.1)	23.6 (1.4)	10.4 (0.6)	2.9 (0.3)	1.8 (0.3)	21.9 (1.1)	3.0 (0.5)	6.4 (0.3)	1.3 (0.5)	25.4 (0.3)	47.28 (3.71)
16/05	3.4 (0.3)	24.4 (1.4)	10.2 (0.1)	2.9 (0.7)	1.7 (0.5)	16.5 (1.2)	3.1 (0.3)	6.2 (0.6)	1.4 (0.1)	30.1 (0.4)	44.42 (3.44)
19/05	0.0 (0.0)	26.4 (0.6)	12.6 (1.1)	3.9 (0.5)	2.3 (0.1)	20.4 (1.0)	4.1 (0.7)	7.3 (0.8)	2.0 (0.4)	20.9 (1.2)	41.18 (6.64)
23/05	3.7 (0.2)	24.4 (1.9)	12.6 (1.1)	2.9 (0.4)	2.2 (0.1)	15.4 (0.9)	3.3 (0.2)	6.2 (0.6)	1.7 (0.3)	27.6 (1.6)	48.88 (7.28)
26/05	3.6 (0.1)	22.6 (1.7)	13.5 (2.3)	2.7 (0.5)	2.3 (0.2)	12.9 (2.0)	3.4 (0.7)	6.3 (1.0)	2.0 (0.3)	30.7 (0.7)	52.73 (8.72)
30/05	3.0 (0.4)	21.7 (2.4)	12.1 (1.7)	3.2 (0.6)	2.0 (0.2)	12.6 (0.9)	3.0 (1.0)	5.7 (1.4)	1.7 (0.4)	34.9 (1.1)	73.73 (9.28)
06/06	3.4 (0.2)	23.8 (1.9)	10.5 (0.7)	2.2 (0.1)	2.4 (0.4)	24.0 (2.9)	2.5 (0.3)	4.8 (0.6)	1.1 (0.0)	26.5 (0.5)	183.08 (4.59)
14/06	2.6 (0.1)	21.4 (0.6)	12.9 (1.2)	2.8 (0.4)	3.2 (0.4)	17.9 (1.4)	2.0 (0.4)	4.9 (0.8)	1.2 (0.2)	31.1 (0.4)	66.02 (5.00)
27/06	3.3 (0.1)	23.4 (1.3)	12.4 (0.9)	2.4 (0.1)	2.2 (0.2)	13.9 (0.9)	2.7 (0.2)	4.7 (0.1)	1.4 (0.1)	33.6 (0.8)	62.34 (4.91)
11/07	2.5 (0.3)	22.4 (1.3)	11.1 (0.3)	3.5 (0.7)	1.9 (0.2)	16.0 (0.6)	2.4 (0.3)	4.9 (0.3)	0.9 (0.5)	34.3 (1.2)	63.79 (2.78)
27/07	2.4 (0.1)	22.4 (2.6)	11.3 (0.8)	2.8 (1.2)	2.3 (0.2)	12.9 (1.3)	2.6 (0.4)	4.9 (0.5)	1.8 (0.5)	37.4 (1.7)	54.35 (9.16)
16/08	2.8 (0.6)	22.7 (4.7)	11.4 (2.4)	2.3 (1.1)	2.3 (0.1)	9.8 (0.5)	2.7 (0.2)	6.4 (2.2)	2.0 (0.1)	42.4 (1.5)	42.89 (4.49)
30/08	2.4 (0.3)	19.7 (0.6)	9.6 (0.0)	2.7 (0.3)	2.1 (0.1)	9.3 (0.1)	2.6 (0.3)	4.5 (0.3)	1.6 (0.4)	45.2 (0.5)	91.12 (0.88)
05/09	2.9 (0.2)	21.1 (2.7)	10.3 (1.7)	1.5 (0.9)	2.2 (0.4)	10.2 (1.4)	2.5 (0.7)	3.2 (1.5)	1.5 (0.4)	44.7 (0.8)	98.50 (6.42)
03/10	1.9 (0.3)	22.0 (0.4)	8.7 (2.1)	1.3 (0.9)	1.8 (0.3)	22.0 (5.6)	2.3 (0.5)	2.6 (1.7)	1.3 (0.2)	36.2 (1.4)	65.43 (3.47)
24/10	3.0 (0.4)	20.5 (0.8)	10.6 (1.8)	2.0 (0.6)	2.2 (0.3)	14.8 (5.2)	2.1 (0.1)	4.4 (0.6)	1.6 (0.2)	39.5 (0.5)	105.31 (8.02)
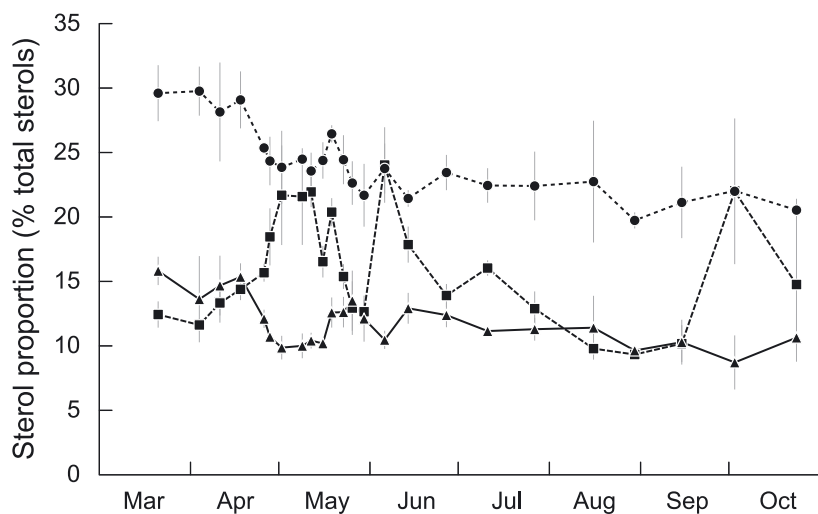
Fig. 7. Proportion of cholesterol (dotted line, black dots), 24 methylene cholesterol (dashed line, black squares) and brassicasterol (solid line, black triangles) in the digestive gland of *Pecten maximus* (expressed as sterol percentage of total) in the Bay of Brest in 2011. Standard deviations are indicated by vertical grey lines

activity of female *Crassostrea corteziensis*. They concluded that these indicators were relevant when trying to infer energy reserve dynamics, because they are essential membrane components provided by diet and food availability.

There was a clear relationship between pigment concentration and composition in the water and the phytoplankton cell microscopic observations (Fig. 8). The variation of the fucoxanthin proportion corresponded well to each diatom bloom in early May, early June and late September (Fig. 2). Indeed, this pigment has often been reported to highlight the presence of diatoms (Jeffrey 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015), which contain the most im-
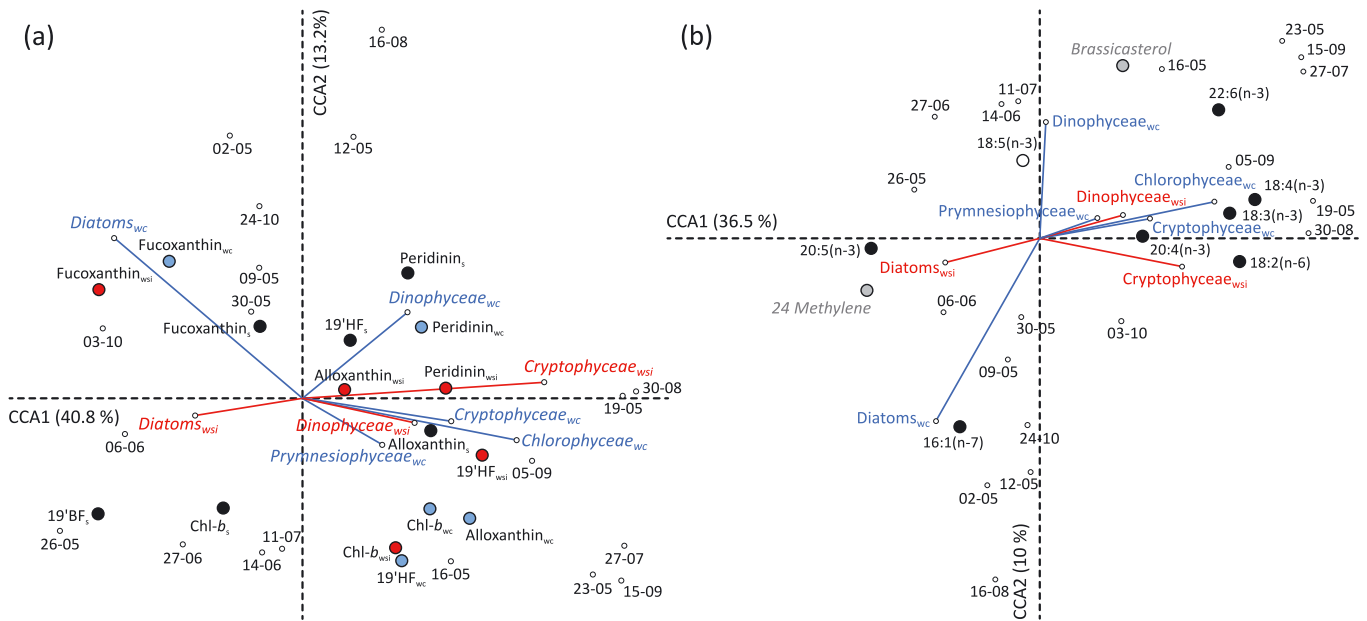
Fig. 8. Triplots from the canonical correspondence analysis performed on pigment and lipid data. Plots include phytoplankton counts in the water column (wc; blue vectors) and the water–sediment interface (wsi; red vectors) and (a) proportions of pigments in the water column (blue dots), the water–sediment interface (red dots) and the stomach contents of *Pecten maximus* (s; black dots) and (b) proportions of fatty acids from the polar fraction (black dots), the neutral fraction (white dot) and sterols (grey dots) in the digestive gland of *P. maximus*

portant proportions of this pigment (Lampert 2001). Likewise, the highly variable dynamics of Dinophyceae blooms were very well reproduced by the proportion of peridinin in the water. Alloxanthin proportions recorded in both water compartments precisely matched the identification of Cryptophyceae, which is also in accordance with previous observations (Jeffrey 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015). The Prymnesiophyceae algae class has been reported to especially contain 19′HF (Jeffrey 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015), which in the present study seemed to be well correlated with the development of these microalgae. However, cell identification did not fit well with measurements of chl *b*, a pigment usually linked to Chlorophyceae (Jeffrey 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015). While the proportion of chl *b* in the filtered water was rather high during the study (up to 15% in the water column and 25% at the bottom, especially during summer), only one bloom of Chlorophyceae was registered in late May. This pigment also constitutes a major component of green macroalgae, which are often observed in the Bay of Brest (Ménesguen et al. 2006). The high concentrations of this pigment in our filtrates could be attributed to discarded pieces of macroalgae in the water (Lampert 2001). The micro-

algae *Lepidodinium chlorophorum*, which bloomed between mid-July and mid-August, is also the only known Dinophyceae to contain chl *b* (Zapata et al. 2012), which is likely to explain the presence of this pigment in the water at this time.

Lipid proportions in the DG fluctuated less than pigment concentrations in the digestive tract content. The incorporation of lipids in the tissues of the organism is a regulated process, smoothed by homeostatic processes and selective incorporation mechanisms (Soudant et al. 1996). On the other hand, pigments are not edible compounds to be assimilated by the scallops (Shuman & Lorenzen 1975). Most of them are egested within faeces in a more or less degraded form. Considering the properties of these 2 biochemical compounds, the strong changes observed in both FAs from the PLs and sterols were important and indicated critical modifications of the cell membrane of DG tissue, notwithstanding the rather strong homeostasis that would be expected for these structural compounds. Our results indicated stronger variations of FAs from the polar fraction compared to those of the neutral fraction in the DG. In this organ, reserve lipids (i.e. NLs) remain relatively unmodified by the diet (Nérot et al. 2015). Measurement of FA composition in other tissues such as the muscle usually focuses on reserve lipids, as dietary FAs are more selectively incorporated into the muscle due to its longer turnover rate (Nérot et al. 2015). Finally, the difference in turnover

rate between pigments and lipids in organisms is an advantage which helps overcome the change in sampling frequency (twice a week in April and May, once every 2 wk for the rest of the study), which might impact the temporal variability of data.

Feeding ecology

Importance of Dinophyceae in the diet of *Pecten maximus*

This study highlights the complexity of the diet of *P. maximus*, both temporally and in terms of composition. During monitoring, the proportion of DHA, representative of Dinophyceae (Parrish et al. 1995, Dalsgaard et al. 2003, Parrish 2013), in the PLs of the DG showed rather important variations. Another FA usually found in some Dinophyceae species is 18:5(n-3) (Dijkman & Kromkamp 2006), which displayed variations that were very similar to the dynamics of peridinin and DHA. The level of peridinin, a specific pigment of the Dinophyceae (Jeffrey, 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015), in the digestive tract of *P. maximus* averaged 18% in the stomach, indicating a significant ingestion of this microalgae class. This is rather surprising as the proportion of peridinin in the water did not exceed 13%. Loret et al. (2000) observed similar results with the oyster *Pinctada margaritifera*, which actively selected Cryptophyceae despite their relatively low presence in the water. Dinophyceae did not dominate phytoplankton composition in the water, yet biomarkers of this phylum were high in the scallop and showed clear covariation between scallop tissues and the environment (Fig. 8). This suggests that *P. maximus* could be actively selecting Dinophyceae out of the available microalgae mix. DHA and EPA are 2 essential FAs for bivalves (Soudant et al. 1996, 1998b), and DHA is largely present in Dinophyceae and also found in some Prymnesiophyceae (Parrish et al. 1995, Volkman et al. 1998, Dalsgaard et al. 2003, Mansour et al. 2005, Parrish 2013). Foraging on Dinophyceae, even when they are not dominant in the water, could be a way for scallops to get this essential component into their diet. However, these findings conflict with the fact that many Dinophyceae species (not observed during this study) are known for their toxicity. Several studies have reported the negative impacts of blooming events of such microalgae on the physiology of *P. maximus* (Erard-LeDenn et al. 1990, Lorrain et al. 2000, Chauvaud et al. 2001). Not all species of Dinophyceae produce toxins, but they have long been con-

sidered as more of a threat than an essential food source for the great scallop (Smolowitz & Shumway 1997, Lorrain et al. 2000, Chauvaud et al. 2001, Bougrier et al. 2003). *P. maximus* might be able to select and sort ingested food on a qualitative basis. Suggestions have been made in this way to explain the active selection of food particles in suspension-feeding bivalves, involving ctenidia retention, labial palps sorting and physico-chemical interactions with algal cell surface (Shumway et al. 1985, Ward & Shumway 2004, Espinosa et al. 2010, Rosa et al. 2013).

Moreover, the great scallop seemed to be able to switch from one source to another depending on the season. Indeed, despite the predominant proportion of diatoms as a food source for scallops, there is an alternation in the stomach content of fucoxanthin, found mainly in diatoms, and peridinin, found exclusively in Dinophyceae (Fig. 4). These shifts are also observed in the FA dynamics (Fig. 6), the proportion of 16:1(n-7) and EPA, found in high amounts in diatoms, alternating with the proportion of DHA and 18:5(n-3), representative of Dinophyceae (Dalsgaard et al. 2003). The ratio between EPA and DHA is a common indicator of a diatom- or flagellate-based diet (St. John & Lund 1996, Auel et al. 2002). The variations of this index are in complete accordance with the evolution of the other biomarkers. Further evidence of this alternation is shown in the evolution of 24 methylene cholesterol and brassicasterol in the DG tissues (Fig. 7), which have also been reported to be major sterols of diatoms and Dinophyceae respectively (Volkman 1986).

While Dinophyceae in the water did not seem to negatively impact ingestion, diatom concentration on the other hand might have stopped feeding activity altogether at some point. During the most important bloom in the water column in early May, proportions of fucoxanthin rapidly increased in the scallops' digestive glands before dropping on 2 May and increasing back to the previous high level 4 d after. During this bloom, the dominant species were the diatoms *Chaetoceros* sp., *Dactiliosolen fragilissimus*, *Chaetoceros debilis* and *Cerataulina pelagica*. A temporary stop in diatom ingestion while cell concentrations are at their maximum in the water could result from an overload of sedimenting microalgae. This hypothesis was suggested by Lorrain et al. (2000), who claimed that overly elevated food particles of high quality surrounding the scallop may have a negative effect on filtration and growth. Flocculation is well established as a dominant process in the transport of nutrients to the benthos (Alldredge & Gotschalk 1989, Cranford et al. 2005), and is particu-

larly significant in supplying food to species such as *P. maximus* that can reside on the continental shelf at depths of up to approximately 220 m (Nérot et al. 2012). However, the aggregation of blooming microalgae may have caused the scallop to close its valves to prevent/respond to gill clogging. Moreover, these blooming events might lead to oxygen depletion at the bottom, to which the scallop reacts by closing its valves (Taylor et al. 1985, Lorrain et al. 2000).

Diet switches

Suspension-feeding bivalves are known to feed mainly on microalgae, filtrated from the surrounding seawater (Le Pennec et al. 2003, MacDonald et al. 2006). The 'bottom-up' relationship between the great scallop and its food implies a feeding plasticity in response to environmental availability. First of all, feeding activity seemed to correlate with phytoplankton dynamics at the water–sediment interface at the beginning of spring, which goes along with the hypothesized ability of *P. maximus* to resuspend microphytobenthos and sedimented microalgae by clapping its valves (Lorrain et al. 2000, Chauvaud et al. 2001, MacDonald et al. 2006). For the rest of the study period, however, the diet of the scallop more closely followed the seston dynamics of the water column, which is also in compliance with a well described benthic–pelagic coupling (Chauvaud et al. 2000, Cranford et al. 2005). More specifically, scallops seemed to feed on Dinophyceae, Prymnesiophyceae, Cryptophyceae and Chrysophyceae, given the proportions of peridinin, 19′HF, alloxanthin and 19′BF in their stomachs until the first large bloom in the water column in early May. This is also supported by the evolution of DHA, found in high proportion in the Dinophyceae (Fig. 8), 18:4(n-3), a common FA in Prymnesiophyceae and 20:4(n-3), rarely found in other microalgae classes than the Cryptophyceae (Volkman et al. 1998, Dalsgaard et al. 2003), even though the latter can result from the elongation of 18:4(n-3). Then, during spring and the renewal of primary production in the water, *P. maximus* seemed to feed mainly on diatoms and Dinophyceae, whose specific pigments respectively account for more than 90% of the total pigments found in the stomach contents. At the end of May, the proportion of Prymnesiophyceae in the scallops' diet briefly increased, as shown by the ingestion of 19′HF-rich microalgae (Fig. 2) and the accumulation of 18:4(n-3) (Fig. 6). Later, the level of chl *b* in the gut of the scallop, which remained under 2% before the last spring

bloom in early June, increased up to 15% during the first part of summer, indicating an ingestion of green microalgae or green macroalgae debris (Lampert 2001). The FA 18:2(n-6) and 18:3(n-3) in PLs, usually used as trophic signatures of green macroalgae or Chlorophyceae (Jamieson & Reid 1972, Parrish et al. 1995, Zhukova & Aizdaicher 1995, Volkman et al. 1998, Dalsgaard et al. 2003, Parrish 2013), showed a fairly smooth increase from June until October. However, this could also reflect an ingestion of Cryptophyceae or Prymnesiophyceae (observed in the seawater at this period), which can also present significant amounts of these FAs (Viso & Marty 1993, Soudant et al. 1996, Renaud et al. 2002). Finally, at the end of the monitoring period (late September/ early October), sharp increases of 24 methylene cholesterol, fucoxanthin and EPA evidenced a diatom-based diet.

Zeaxanthin was measured at levels varying between 5 and 10% in the water, while its contribution to digestive tract contents remained close to zero, except in the first stomach sample and 2 rectum samples in August (Table 2). High levels of zeaxanthin have been reported in cyanobacteria (Jeffrey 1974, 1997, Mackey et al. 1996, Lampert 2001, Wright & Jeffrey 2006, Sanz et al. 2015), the size of which is under 2 μm, and in other microorganisms (Grossman et al. 1995). The gill retention capacity of most bivalves is not fine enough to capture such tiny particles (Møhlenberg & Riisgård 1978, MacDonald et al. 2006, Strohmeier et al. 2012). Nevertheless, several studies have shown evidence that free bacterioplankton, typically ranging in size from 0.3 to 1 μm, can be ingested and assimilated by Pectinidae and other bivalves when bound in aggregates (McHenery & Birkbeck 1985, Prieur et al. 1990, Alber & Valiela 1995, MacDonald et al. 2006, Ward & Kach 2009). This could explain the occurrence of zeaxanthin in relatively high amounts (3%) in a few samples. Similarly, the dynamics of *iso*17:0 FA, a specific marker of marine bacteria (Vestal & White 1989, Kharlamenko et al. 2001, 2008), in PLs of scallop tissues indicates that bacteria might be part of the diet of *P. maximus*. At certain times of the year, a large proportion (>70%) of natural particulates of varying size and quality can form aggregates mixed with high molecular weight substances (flocs, marine snow; Alldredge et al. 1993, Crocker & Passow 1995). More investigations are required to determine if the ingestion of microorganisms could be a way for the scallops to meet energetic requirements when no other trophic sources are available, especially during the bleak season (Quéguiner & Tréguer 1984).

Ingestion, digestion and assimilation

Some pigments detected in the water did not appear in the same proportions in the scallops' digestive tract. Several experimental studies have pointed out the capacity of *P. maximus* to select different algal species on the basis of their size, nutritional value, physiological state or lipid content (Shumway et al. 1985, Soudant et al. 1998b, Ward & Shumway 2004). Recently, interactions between some proteins (lectins) of the mucus that covers oysters' gills and carbohydrate present on the surface of microalgae cells have been evidenced (Espinosa et al. 2010) and may contribute to microalgal species selection. Rosa et al. (2013) also highlighted non-specific interactions involving surface-charge and wettability of particles in the particle discrimination process.

Beyond the matter of ingestion stands the question of the digestion and assimilation of the ingested products. Some pigments found in the stomach were found in the rectum in similar proportions, possibly meaning that the algae they belong to were not digested (Hawkins et al. 1986, Louda et al. 2008). This is the case for fucoxanthin during the month of May and at the end of July or for peridinin in late May and in mid-July to mid-August. Chl *b* also seemed to go through the digestive tract rather unaltered, as elevated amounts were found in the rectum, which could reflect a weak affinity to this trophic source. The microalgal cell wall, varying in size, structure and composition among species, might affect the digestibility of phytoplankton.

Pheophorbide-*a* and pheophytin-*a* are 2 pigments resulting from the degradation of chl *a* (Hawkins et al. 1986). Significant amounts (up to 36% of pheophorbide-*a* and 25% of pheophytin-*a*) were found in the stomach contents, suggesting that one of the first steps of food processing occurs in this compartment (Fig. 5). In *P. maximus*, digestion is both extra- and intracellular and occurs at several sites in the digestive tract (Beninger & Le Pennec 2006). The first step of degradation occurs in the stomach with both a mechanical and chemical action of the crystalline style that breaks and releases enzymes attacking the external layer of the microalgae (sometimes a hard cell wall like the frustule of diatoms). Pigments contained in microalgae undergo different levels of degradation through the enzymatic activity of digestion (Bayne et al. 1987, Pastoureaud et al. 1995). Degradation peaks observed after periods of low ingestion could result from the degradation of the food remains in the stomach and the DG. Indeed, scallops have to cope with periods of low food availability over the winter or between phytoplankton blooms. The degradation of the last food particles in the stomach could provide them with nutrients while awaiting a renewal of food supply. Another possible explanation could be the passive deterioration (not due to active digestion) of the remaining products of digestion inside the stomach.

CONCLUSIONS

This in-depth investigation of the seasonal feeding ecology of *P. maximus* shows the species' ability to switch from one trophic source to another. Some food sources seem to be neglected by the great scallop (green algae), in favor of diatoms and dinoflagellates, supporting the hypothesis of food preference (Møhlenberg & Riisgård 1978, Shumway et al. 1985, Ward & Shumway 2004, MacDonald et al. 2006, Ward & Kach 2009, Espinosa et al. 2010, Rosa et al. 2013).

Relating *in situ* physiological performance to food quantity and quality is not an easy task (Chauvaud et al. 1998, 2001, Lorrain et al. 2000, Strohmeier et al. 2009). Variations in growth performance and survival of scallops rely on numerous environmental factors. An improved description of the dynamics and complexity of energy intake helps with the interpretation of these mechanisms and could be useful in understanding the consequences of ever-changing environments.

LITERATURE CITED

Alber M, Valiela I (1995) Organic aggregates in detrital food webs: incorporation by bay scallops *Argopecten irradians*. Mar Ecol Prog Ser 121:117–124

Alldredge AL, Gotschalk CC (1989) Direct observations of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. Deep Sea Res I 36:159–171

Alldredge AL, Passow U, Logan BE (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. Deep Sea Res I 40:1131–1140

Auel H, Harjes M, da Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. Polar Biol 25: 374–383

Aya FA, Kudo I (2017) Nitrogen stable isotopes reveal age-dependent dietary shift in the Japanese scallop *Mizuhopecten yessoensis*. Isotopes Environ Health Stud 53:80–90

Bachok Z, Mfilinge PL, Tsuchiya M (2003) The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. J Exp Mar Biol Ecol 292: 187–197

Bachok Z, Meziane T, Mfilinge PL, Tsuchiya M (2009) Fatty acid markers as an indicator for temporal changes in food sources of the bivalve *Quidnipagus palatum*. Aquat Ecosyst Health Manage 12:390–400

Bayne BL, Hawkins AJS, Navarro E (1987) Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. J Exp Mar Biol Ecol 111:1–22

Beninger PG, Le Pennec M (2006) Structure and function in scallops. In: Shumway SE, Parsons GJ (eds) Scallops: biology, ecology and aquaculture. Developments in aquaculture and fisheries science, Vol 35. Academic Press, London, p 123–228

Bode A, Alvarez-Ossorio MT, Varela M (2006) Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Mar Ecol Prog Ser 318:89–102

Bougrier S, Lassus P, Bardouil M, Masselin P, Truquet P (2003) Paralytic shellfish poison accumulation yields and feeding time activity in the Pacific oyster (*Crassostrea gigas*) and king scallop (*Pecten maximus*). Aquat Living Resour 16:347–352

Chatterjee A, Klein C, Naegelen A, Claquin P and others (2013) Comparative dynamics of pelagic and benthic micro-algae in a coastal ecosystem. Estuar Coast Shelf Sci 133:67–77

Chauvaud L, Thouzeau G, Paulet YM (1998) Effects of environmental factors on the daily growth rate of *Pecten maximus* juveniles in the Bay of Brest (France). J Exp Mar Biol Ecol 227:83–111

Chauvaud L, Jean F, Ragueneau O, Thouzeau G (2000) Long-term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. Mar Ecol Prog Ser 200: 35–48

Chauvaud L, Donval A, Thouzeau G, Paulet YM, Nézan E (2001) Variations in food intake of *Pecten maximus* (L.) from the Bay of Brest (France): influence of environmental factors and phytoplankton species composition. C R Acad Sci III 324:743–755

Claustre H, Ras J (2009) The LOV method. In: Hooker SB, Van Heukelem L, Thomas CS, Claustre H and others (eds) The third seaWiFS HPLC analysis round-robin experiment (SeaHARRE-3). NASA/TM-2009-215849, NASA Center for AeroSpace Information, Hanover, MD, p 80–83

Cranford PJ, Armsworthy SL, Mikkelsen OA, Milligan TG (2005) Food acquisition responses of the suspension-feeding bivalve *Placopecten magellanicus* to the floccu-

lation and settlement of a phytoplankton bloom. J Exp Mar Biol Ecol 326:128–143

Crocker KM, Passow U (1995) Differential aggregation of diatoms. Mar Ecol Prog Ser 117:249–257

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Dijkman NA, Kromkamp JC (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. Mar Ecol Prog Ser 324:113–125

Erard-Le Denn E, Morlaix M, Dao JC (1990) Effects of *Gyrodinium* cf. *aureolum* on *Pecten maximus* (post larvae, juveniles and adults). In: Granéli E, Sundström EL, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, NY, p 132–136

Espinosa PE, Perrigault M, Ward JE, Shumway SE, Allam B (2010) Microalgal cell surface carbohydrates as recognition sites for particle sorting in suspension-feeding bivalves. Biol Bull (Woods Hole) 218:75–86

Grossman AR, Bhaya D, Apt KE, Kehoe DM (1995) Light-harvesting complexes in oxygenic photosynthesis: diversity, control, and evolution. Annu Rev Genet 29:231–288

Hawkins AJS, Bayne BL, Mantoura RFC, Llewellyn CA, Navarro E (1986) Chlorophyll degradation and absorption throughout the digestive system of the blue mussel *Mytilus edulis* L. J Exp Mar Biol Ecol 96:213–223

Hurtado MA, Racotta I, Arcos F, Morales-Bojórquez E, Moal J, Soudant P, Palacios E (2012) Seasonal variations of biochemical, pigment, fatty acid, and sterol compositions in female *Crassostrea corteziensis* oysters in relation to the reproductive cycle. Comp Biochem Physiol B Biochem Mol Biol 163:172–183

Iverson SJ, Field C, Don Bowen W, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecol Monogr 74:211–235

Jamieson GR, Reid EH (1972) The component fatty acids of some marine algal lipids. Phytochemistry 11:1423–1432

Jeffrey SW (1974) Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar Biol 26: 101–110

Jeffrey SW (1997) Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO, Paris

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Mar Ecol Prog Ser 220:103–117

Kharlamenko VI, Kiyashko SI, Rodkina SA, Imbs AB (2008) Determination of food sources of marine invertebrates from a subtidal sand community using analyses of fatty acids and stable isotopes. Russ J Mar Biol 34:101–109

Lampert L (2001) Dynamique saisonnière et variabilité pigmentaire des populations phytoplanctoniques dans l'Atlantique Nord (Golfe de Gascogne). Seasonal dynamics and pigmentary variability of phytoplanktonic populations in the Northern Atlantic (Bay of Biscay). PhD thesis, University of Brest

Lavaud R, Flye-Sainte-Marie J, Jean F, Emmery A, Strand Ø, Kooijman SALM (2014) Feeding and energetics of the great scallop, *Pecten maximus*, through a DEB model. J Sea Res 94:5–18

Le Pennec M, Paugam A, Le Pennec G (2003) The pelagic life of the pectinid *Pecten maximus*—a review. ICES J Mar Sci 60:211–233

Loret P, Pastoureaud A, Bacher B, Delesallel B (2000) Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis. Mar Ecol Prog Ser 199:55–67

Lorrain A, Paulet YM, Chauvaud L, Savoye N, Nézan E, Guérin L (2000) Growth anomalies in *Pecten maximus* from coastal waters (Bay of Brest, France): relationship with diatom blooms. J Mar Biol Assoc UK 80:667–673

Lorrain A, Paulet YM, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar Biol Ecol 275:47–61

Louda JW, Neto RR, Magalhaes ARM, Schneider VF (2008) Pigment alterations in the brown mussel *Perna perna.* Comp Biochem Physiol B Biochem Mol Biol 150:385–394

MacDonald BA, Bricelj MV, Shumway SE (2006) Physiology: energy acquisition and utilisation. In: Shumway SE, Parsons GJ (eds) Scallops: biology, ecology and aquaculture. Developments in aquaculture and fisheries science, Vol 35. Academic Press, London, p 417–492

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144: 265–283

Mansour MP, Frampton DMF, Nichols PD, Volkman JK, Blackburn SI (2005) Lipid and fatty acid yield of nine stationary-phase microalgae: applications and unusual C_{24}-C_{28} polyunsaturated fatty acids. J Appl Phycol 17: 287–300

Marín Leal JC, Dubois S, Orvain F, Galois R and others (2008) Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and modelling as tools to estimate the trophic ecology of cultivated oysters in two contrasting environments. Mar Biol 153:673–688

Mason J (1957) The age and growth of the scallop, *Pecten maximus* (L.), in Manx waters. J Mar Biol Assoc UK 36: 473–492

McHenery JG, Birkbeck TH (1985) Uptake and processing of cultured microorganisms by bivalves. J Exp Mar Biol Ecol 90:145–163

Ménesguen A, Cugier P, Leblond I (2006) A new numerical technique for tracking chemical species in a multi-source, coastal ecosystem, applied to nitrogen causing *Ulva* blooms in the Bay of Brest (France). Limnol Oceanogr 51:591–601

Møhlenberg F, Riisgård HU (1978) Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17:239–246

Napolitano GE, Ackman RG, Silva-Serra MA (1993) Incorporation of dietary sterols by the sea scallop *Placopecten magellanicus* (Gmelin) fed on microalgae. Mar Biol 117: 647–654

Nérot C, Lorrain A, Grall J, Gillikin DP, Munaron JM, Le Bris H, Paulet YM (2012) Stable isotope variations in benthic filter feeders across a large depth gradient on the continental shelf. Estuar Coast Shelf Sci 96:228–235

Nérot C, Meziane T, Schaal G, Grall J, Lorrain A, Paulet YM, Kraffe E (2015) Spatial changes in fatty acids signatures of the great scallop *Pecten maximus* across the Bay of Biscay continental shelf. Cont Shelf Res 109:1–9

Oksanen J, Blanchet FG, Friendly M, Kindt R and others (2017) vegan: community ecology package. R package version 2.4-3

Parrish CC (2013) Lipids in marine ecosystems. ISRN Oceanogr 2013:604045

Parrish CC, McKenzie CH, MacDonald BA, Hatfield EA (1995) Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. Mar Ecol Prog Ser 129:151–164

Pastoureaud A, Heral M, Prou J, Razet D, Russu P (1995) Particle selection in the oyster *Crassostrea gigas* (Thunberg) studied by pigment HPLC analysis under natural food conditions. Aquaculture 19:79–88

Prieur D, Mevel G, Nicolas JL, Plusquellec A, Vigneulle M (1990) Interactions between bivalve molluscs and bacteria in the marine environment. Oceanogr Mar Biol Annu Rev 28:277–352

Quéguiner B, Tréguer P (1984) Studies on the phytoplankton in the Bay of Brest (Western Europe). Seasonal variations in composition, biomass and production in relation to hydrological and chemical features (1981–1982). Bot Mar 27:449–459

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Renaud SM, Thinh LV, Lambrinidis G, Parry DL (2002) Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211:195–214

Roditi HA, Fisher NS, Sañudo Wilhelmy SA (2000) Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature 407:78–80

Rosa M, Ward JE, Shumway SE, Wikfors GH, Espinosa PE, Allam B (2013) Effects of particle surface properties on feeding selectivity in the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. J Exp Mar Biol Ecol 446:320–327

Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation of $\delta^{13}C$ and $\delta^{15}N$ in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. Limnol Oceanogr 49:408–414

Sanz N, García-Blanco A, Gavalás-Olea A, Loures P, Garrido JL (2015) Phytoplankton pigment biomarkers: HPLC separation using a pentafluorophenyloctadecyl silica column. Methods Ecol Evol 6:1199–1209

Shuman FR, Lorenzen CJ (1975) Quantitative degradation of chlorophyll by a marine herbivore. Limnol Oceanogr 20: 580–586

Shumway SE, Cucci TL, Newell RC, Yentsch CM (1985) Particle selection, ingestion, and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91:77–92

Shumway SE, Selvin R, Schick DF (1987) Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. J Shellfish Res 6: 89–95

Smolowitz R, Shumway SE (1997) Possible cytotoxic effects of the dinoflagellate, *Gyrodinium aureolum*, on juvenile bivalve molluscs. Aquacult Int 5:291–300

Soudant P, Marty Y, Moal J, Samain JF (1995) Separation of major polar lipids in *Pecten maximus* by high-performance liquid chromatography and subsequent determination of their fatty acids using gas chromatography. J Chromatogr B Biomed Sci Appl 673:15–26

Soudant P, Marty Y, Moal J, Robert R, Quéré C, Le Coz JR, Samain JF (1996) Effect of food fatty acid and sterol qual-

ity on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143:361−378

Soudant P, Le Coz JR, Marty Y, Moal J, Robert R, Samain JF (1998a) Incorporation of microalgae sterols by scallop *Pecten maximus* (L.) larvae. Comp Biochem Physiol A Mol Integr Physiol 119:451−457

Soudant P, Marty Y, Moal J, Masski H, Samain JF (1998b) Fatty acid composition of polar lipid classes during larval development of scallop *Pecten maximus* (L.). Comp Biochem Physiol A Mol Integr Physiol 121:279−288

St. John M, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131: 75−85

Strohmeier T, Strand Ø, Cranford P (2009) Clearance rates of the great scallop (*Pecten maximus*) and blue mussel (*Mytilus edulis*) at low natural seston concentrations. Mar Biol 156:1781−1795

Strohmeier T, Strand Ø, Alunno-Bruscia M, Duinker A, Cranford PJ (2012) Variability in particle retention efficiency by the mussel *Mytilus edulis.* J Exp Mar Biol Ecol 412:96−102

Taylor FJ, Taylor NJ, Walsby JR (1985) A bloom of the planktonic diatom, *Cerataulina pelagica*, off the coast of northeastern New Zealand in 1983, and its contribution to an associated mortality of fish and benthic fauna. Int Rev Gesamten Hydrobiol Hydrograph 70:773−795

Vestal JR, White DC (1989) Lipid analysis in microbial ecology. Bioscience 39:535−541

Viso AC, Marty JC (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521−1533

Volkman JK (1986) A review of sterol markers for marine and terrigenous organic matter. Org Geochem 9:83−99

Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F (1998) Microalgal biomarkers: a review of recent research developments. Org Geochem 29:1163−1179

Ward JE, Kach DJ (2009) Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Mar Environ Res 68:137−142

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83−130

Wright SW, Jeffrey SW (2006) Pigment markers for phytoplankton production. In: Volkman JK (ed) Marine organic matter: biomarkers, isotopes and DNA. Springer, Berlin, p 71−104

Zapata M, Fraga S, Rodríguez F, Garrido JL (2012) Pigment-based chloroplast types in dinoflagellates. Mar Ecol Prog Ser 465:33−52

Zhukova NV, Aizdaicher NA (1995) Fatty acid composition of 15 species of marine microalgae. Phytochemistry 39: 351−356