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Nutrient storage and utilization in relation to reproductive condition of the New Zealand scallop *Pecten novaezelandiae*

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ABSTRACT: The New Zealand native scallop *Pecten novaezelandiae* has high economic value from wild catch and has aquaculture potential. We investigated scallop nutrient (carbohydrates, proteins, lipids) storage and utilization in relation to a reproductive cycle at 2 sites (Hauraki Gulf, Auckland, New Zealand) over 1 yr. Water samples were collected for seston and chlorophyll *a* analyses, and sediment samples were taken to evaluate the potential for re-suspended nutrients as food sources for scallops. Isotope (carbon and nitrogen) and proximate analyses were conducted for gonad, adductor muscle and digestive gland, seston (1.2–5 and >5 µm) and sediment samples. Isotope analyses revealed different signatures in suspended sediment and scallop tissues, indicating that re-suspended nutrients are unlikely to contribute to their diet. However, seston (particularly small fractions) signatures were closely related to scallop tissues, suggesting a main food source. Scallops from both sites exhibited similar reproductive cycles and utilization of nutrients. Gametogenesis started in winter (increased gonad index and energy content) and used carbohydrates stored in adductor muscles. Spawning events in spring (October–November) and summer (January–March) were associated with a sharp drop in visual gonad index (VGI), gonad index, lipid and energy contents. The energy demand required during spawning was supported by digestive gland protein. Gonad re-maturation was indicated by increases in VGI, gonad index, lipid and energy contents post spring spawning, and gonad recovery was supported by lipids from digestive glands. Results show that *P. novaezelandiae* utilizes energy reserves from both adductor muscle and digestive gland to cover the full costs of gametogenesis.

KEY WORDS: Pectinidae · Biochemical composition · Nutrient utilization · Proximate analysis · Reproduction

1. INTRODUCTION

Most marine bivalves have well-defined annual reproductive cycles that take into account environmental conditions, such as water temperature and food availability. The ultimate purpose of these cycles is to produce gametes during optimal periods to ensure successful fertilization and larval development. Usually, gonad maturation and spawning take place once or twice a year in spring and summer (Mathieu & Lubet 1993). Since gonad maturation and/or gametogenesis represent periods of particularly high energy demands, the cost of gamete synthesis must be met by high food supplies and/or stored reserves. Two reproductive strategies commonly used by bivalves are conservative and opportunistic strategies, which are based on the relationship between gonad development and the accumulation and utilization of nutrients (Bayne 1976). In conservative species, gametogenesis takes place using previously acquired reserves, while for opportunistic species gametogenesis occurs when food availability is high. As described for many scallop species, energy is stored during periods of high

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food supplies, and subsequently these reserves are mobilized for use during times of food shortage, decreased feeding rates and/or high energy demands (Sastry 1968, Zandee et al. 1980, Martínez 1991, Barber & Blake 2006). Storage of energy in bivalves, such as scallops, undergoes various biochemical processes that convert assimilated nutrients into storage compounds that can be re-mobilized when needed.

Seasonal changes in body tissue weight (condition index) and biochemical composition provide information regarding the ways in which nutritional components (i.e. carbohydrates, lipids, proteins) are used in gamete production. In general, carbohydrates are used as primary energy sources in bivalves, and are important for gamete development and maintenance of adult condition during periods of nutritive stress (Bayne 1976, Barber & Blake 1981, Berthelin et al. 2000). Indeed, a direct relationship has been established between gonad index and somatic carbohydrate content in many bivalve species (Beninger & Lucas 1984, Pérez-Camacho et al. 2003). Lipids also represent an important component in energy reserves during periods of nutritional deficiency (Farias & Uriarte 2001) and are accumulated within oocytes during development (Holland 1978). Proteins constitute the largest fraction in the composition of oocytes and other soft tissues within bivalves, and somatic protein loss during winter has been observed in numerous bivalve studies (Bayne 1976, Beninger & Lucas 1984, Epp et al. 1988). In addition to direct use of nutritional components for metabolic demands, inter-conversion of these components can take place, such as stored carbohydrates to lipids (lipogenesis) to support gonad development (Gabbott 1975).

Due to the high commercial value of scallops, considerable research has been generated with regards to reproductive physiology (Saout et al. 1999, Racotta et al. 2003, Guerra et al. 2012), broodstock conditioning (Martínez et al. 1992, Pazos et al. 1996, Pernet et al. 2003) and energy storage and utilization (Epp et al. 1988, Pazos et al. 1997, Lodeiros et al. 2001) of a number of species. Based on such studies, it has been well established that pectinids filter food particles from their surrounding environment. Phytoplankton and detrital material may be consumed from the water column (seston) and/or from re-suspended material from the seabed. In addition, scallops are known to have the ability to discriminate microalgae from other particles and reject particles of poor nutrition as pseudofaeces (Nicholson 1978, Cranford & Gordon 1992). However, nutritional preferences, storage of energy and timing of utilization for reproductive activity seem to vary greatly among scallop species and populations (Giese et al. 1967, Bayne 1976, Sastry 1979). While it is generally accepted that stored nutrients are mobilized from somatic tissues (adductor muscle and digestive gland) to support yolk synthesis for gonadal maturation (Barber & Blake 2006), there are some notable differences regarding the type of nutrients utilized in this process. For example, maturation of gametes is fuelled by carbohydrate and protein reserves stored in the adductor muscle in *Chlamys opercularis* (Taylor & Venn 1979), *Pecten maximus* (Faveris & Lubet 1991) and *Argopecten purpuratus* (Martínez 1991). However, in *Argopecten irradians irradians*, gametogenesis occurs mainly at the expense of adductor muscle protein and lipid reserves (Epp et al. 1988). Furthermore, *Lyropecten* (*Nodipecten*) *nodosus* utilizes carbohydrates and proteins from the digestive gland for reproduction (Lodeiros et al. 2001). Thus, it is clear that more specific research is needed to identify the nutritional requirements and biochemical processes involved in reproductive allocation and development for each species in question.

The New Zealand native scallop *Pecten novaezelandiae* is a species that has been subjected to extensive harvesting pressure (commercial and recreational fishing) and has experienced significant population declines in the last 20 yr (Marsden & Bull 2006). Dramatic scallop biomass declines recorded in 2000 have put this fishery at risk (Williams 2005). Knowledge of the ecology of *P. novaezelandiae* is limited and restricted to grey literature, mainly unpublished government reports and graduate theses (Bull 1976, Nicholson 1978, Morrison 1999, Nesbit 1999, Lyon 2002, Williams 2005). A few studies have hinted at the importance of environmental factors (e.g. sedimentation, freshwater runoff, habitat complexity) and food availability to *P. novaezelandiae* growth, reproductive condition, and survivability (Morrison 1999, Morrison & Cryer 2003, Talman et al. 2004). However, these studies have not clearly identified the role of different environmental parameters and food supplies on scallop nutrition and reproductive condition. Thus, the aims of this study were to investigate the source (sediment and water column) of nutrients consumed by wild scallop (i.e. *P. novaezelandiae*) populations, and the storage and utilization of nutrients (carbohydrates, proteins and lipids) within scallop tissues (gonad, adductor muscle, digestive gland) during the reproductive cycle.

2. MATERIALS AND METHODS

2.1. Study sites

Two study sites were selected in the Southern Tawharanui Peninsula, Hauraki Gulf, New Zealand (Fig. 1). The subtidal sites were ~800 m apart along the peninsula and approximately ~100 m away from land. Initial surveys of the general area indicated that these sites contained extensive scallop populations that were subjected to minimal fishing pressure. The first site (36°23′13″S, 174°46′44″E) was located at 5–8 m water depth and composed mostly of sand with sparse seagrass beds. The second site (36°23′ 20″S, 174°47′09″E) was a muddy-sand habitat without benthic vegetation, also located at 5–8 m depth.

2.2. Sample collections

A total of 7 field sampling events were carried out at approximately bi-monthly intervals over 1 yr to collect sediment, water and scallop samples from each of the 2 sites. The sampling period extended across a reproductive season from May 2012 to March 2013. Bottom water temperatures were recorded at the same time as scallops were collected.

2.2.1. Sediment samples

Two replicate sediment samples were collected during each sampling event at slack tide to minimize collection variability. The samples were collected by SCUBA divers with a core sampler (130 mm diameter, 3 cm depth). Each sample was double bagged and transported to the laboratory in a cold box. Once at the laboratory, the sediment samples were centrifuged to remove excess water and freeze-dried until further analysis. Particulate organic matter (POM) quantification of each sediment sample was conducted by combustion at 490°C. The loss on ignition (LOI) was then calculated according to:

$$LOI_{490} = [(DW_{freeze\ dried} - DW_{490}) / DW_{freeze\ dried}] \times 100 \quad (1)$$

where DW is the dry weight. One-off sediment grain-size analyses were conducted at the start of the study to characterize the habitats within each site. Sediment grain-size characteristics are presented as median particle diameter (MPD) and classified according to Gray & Elliott (2009).

2.2.2. Water samples

To quantify seston concentrations in the water column, 2 replicate water samples of 20 l each were collected from both surface (0.5 m below the water surface) and bottom (0.1 m above the seabed) waters at each site during each sampling event. The water samples were screened through a 250 µm mesh net to remove large particles. Water was transported to the laboratory in dark containers cooled with cold packs. Once at the laboratory, the samples were kept at 4°C and processed within 24 h. First, the samples were filtered through 5 µm polypropylene membranes to collect the large seston fraction, and then through 1.2 µm (GF/C) precombusted glass fibre filters to obtain the small seston fraction. The filters containing seston were then washed twice with 5 ml of 0.9% ammonium formate and freeze-dried for further biochemical analysis. These seston size fractions were selected following Cranford et al. (1998), who suggested that scallops consume mostly particles larger than 5 µm, and other authors (MacDonald & Thompson 1985, Pazos et al. 1997, Lorrain et al. 2002), who reported the consumption of smaller-sized fractions. In addition to these potential food particle size classes, chlorophyll *a* (chl *a*) concentrations were determined using 1–3 l aliquots from each replicate water sample. These sub-samples were concentrated on Whatman® GF/C filter discs, and the chl *a*

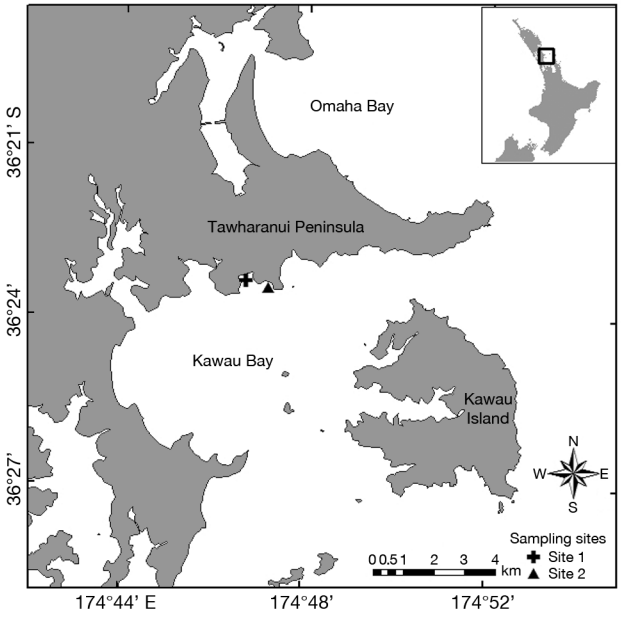

Fig. 1. Study sites in southern Tawharanui Peninsula, Hauraki Gulf, New Zealand

was extracted with 90% methanol (24 h at 4°C). The absorbance of each sample was measured at 652, 665 and 750 nm (Porra 2002). Chl *a* concentrations were calculated according to:

$$\text{Chl } a \text{ (µg ml}^{-1}) = 16.29A^{665-750} - 8.54A^{652-750} \quad (2)$$

where A is the absorbance.

2.2.3. Scallop samples

In total, 13–19 scallops (>80 mm shell height) were collected by SCUBA divers at each site during each sampling event. Scallops were transported in a cool bin without water and dissected within 6 h of collection. Each scallop was cleaned thoroughly, and shell heights and widths were measured to the nearest 0.01 mm with Vernier callipers. The adductor muscle, digestive gland and gonad were then removed from each individual and weighed separately. Tissues were then freeze-dried and weighed. Condition indices of gonad (GI), adductor muscle (AMI) and digestive gland (DGI) were determined according to Barber & Blake (1981):

$$\text{Condition index} = (DW_{tissue} / DW_{total\ tissue}) \times 100 \quad (3)$$

2.3. Reproductive condition

Visual gonad index (VGI) was determined for all animals just prior to dissections. Visual grades 1–8 were assigned to each gonad, based on their appearance and following the classification by Williams & Babcock (2004). These grades included spent (grade 1), partially spawned (grades 2 and 3), active (grades 4–6) and ripe (grades 7 and 8), and were based on morphological appearance of the gonad to the naked eye. For gonads that were >3 g wet weight, tissue samples of approximately 15 × 10 × 5 mm were taken from the centre of each gonad at the interface of the testis and ovary, and stored separately in 10% buffered formalin for histological analysis. For sampling events when all gonads were too small (i.e. all gonads <3 g), the entire gonad from 5–10 individuals were used for histology, and separate animals from the same sampling event were used for biochemical analyses (see below).

Histological sections of gonad tissue were observed to identify scallop reproductive stages. Fresh gonad tissues were fixed in 10% buffered formalin for a minimum of 48 h. Tissues were dehydrated in A-graded ethanol series, cleared in xylene and embedded in paraffin wax. Blocks were sectioned to 5 µm thickness, mounted on slides and stained with haematoxylin and eosin. Each histological section was observed to determine the state of follicle and gamete development, and to assign a developmental stage (I–V according to Bull 1976). The diameter of 30 randomly selected nucleated oocytes was measured per scallop to provide quantitative data on the reproductive condition of each individual.

2.4. Nutritional condition

Biochemical composition of 3 main tissues (adductor muscle, digestive gland and gonad) was determined for each scallop from each of the sampling sites and sampling events. Biochemical analyses included total carbohydrates, total protein, total lipids and fatty acid profiles. The fatty acid profiles of female and male gonad samples from October and November 2012 and January 2013 were analyzed separately when enough material was available. In addition, sufficient material was only available from the >5 µm seston samples to conduct fatty acid profile analyses. Stable isotope (^{13}C and ^{15}N) signatures were obtained for scallop tissues, seston and sediment, as discussed below.

2.4.1. Biochemical composition

Carbohydrates were determined using the Anthrone method according to Hedge & Hofreiter (1962) and reported as glucose equivalents. Carbohydrates were hydrolyzed into simple sugars using dilute hydrochloric acid (2.5 N), then mixed with 4 parts of anthrone solution. Samples were then incubated for 8 min at 100°C, and the optical density of the green coloured solution was read at 630 nm. Protein was determined using a commercial kit (The Pierce BCA Assay; Thermo Scientific) and reported as bovine serum albumin (BSA) equivalents. Samples were digested with 0.5 N NaOH for 30 min at 56°C for this analysis. The supernatant was then incubated with BCA solution for 2 h at 37°C. The optical density of the solution was read at 562 nm. Total lipid contents were measured according to Bligh & Dyer (1959) and Marsh & Weinstein (1966), and are reported as tripalmitin equivalents. Lipids were extracted in a 2:1 chloroform:methanol mixture. Extracted lipids were then dried under a flow of nitrogen and the amount of total

lipid was determined by charring with concentrated sulphuric acid for 15 min at 200°C. The optical density of the solution was read at 375 nm.

2.4.2. Energy content

Energy conversion factors were used to obtain energy contents (Saout et al. 1999). These factors were 17.2, 23.9 and 33 kJ g^{-1} for carbohydrates, proteins and lipids, respectively. The energy content of each tissue type was calculated for individual scallops in order to determine the energy contribution of each organ to the animal's energetics over the reproductive cycle.

2.4.3. Fatty acid profiles

Fatty acids were prepared by a gas chromatographic one-step methylation extraction method, which was adopted from a method by de La Cruz Garcia et al. (2000). An aliquot (10–20 mg) of homogenized freeze-dried sample was added to 0.49 ml of toluene, 0.75 ml freshly prepared 5% methanolic HCl (0.75 ml) and 10 µl of 2 g l^{-1} tridecanoic acid (internal standard). Tubes with the sample solutions were closed under nitrogen to avoid lipid oxidation and heated at 70°C in a water bath for 2 h. Then, 1 ml of 6% aqueous K_2CO_3 and 0.5 ml of toluene were added and centrifuged at 1100 × *g* for 5 min. The organic phase on the top layer, which included fatty acid methyl esters (FAMEs), was transferred into a beaker while the water was precipitated by anhydrous Na_2SO_4.

The separation and quantification of FAMEs were conducted with a 2010 Shimadzu GC-2010, which was integrated with a split–splitless injector and flame ionisation detector (FID). The individual FAMEs were separated and identified by a Zebron ZB-Wax capillary column (30 m × 0.25 mm × 0.25 µm) from Phemomenex. The temperature of the oven was 140–245°C. The temperature was increased at 5°C min^{-1} to 245°C and held for 15 min. One cycle took a total of 50 min. The gas carrier was nitrogen with a flow rate of 60 ml min^{-1} and the flow of hydrogen was 40 ml min^{-1}. The average linear velocity was set to 20 cm s^{-1}, with a head pressure of 8.7 psi (1 psi = 6894.76 Pa). The temperature of the detector was 250°C and the volume of injection was 1 µl.

FAMEs were identified by comparing the retention times with a standard (Supelco 37 Component FAME Mix, 18919-1AMP) and FAMEs from vegetable oils, fish oils and New Zealand green-lipped mussels *Perna canaliculus*. The response factor of the internal standard was used for quantification.

2.4.4. Stable isotopes

Two samples of sediment, seston (from the 2 size classes) and isolated scallop tissues (adductor muscle, digestive gland and gonad), all from each sampling site and event, were used for stable isotope analyses. Isotope analyses were processed by the Waikato Stable Isotope Unit, The University of Waikato, Hamilton, New Zealand. Isotopic analyses were carried out on a fully automated Europa Scientific 20/20 isotope analyzer. Samples were combusted and the resulting gases were separated by gas chromatography, and then analyzed by continuous flow-mass spectrometry. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to:

$$\delta^{13}C_{sample} \text{ or } \delta^{15}N_{sample} = (R_{sample}/R_{standard} - 1) \times 1000 \quad (4)$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. $\delta^{13}C$ was measured to a precision of ±0.5%. All samples were referenced to pre-calibrated C_4 sucrose, which was cross-referenced to the PeeDee Belemnite standard. $\delta^{15}N$ was measured to a precision of ±1%. All samples were referenced to a urea standard that is traceable to atmospheric nitrogen

2.5. Statistical analyses

A 2-way ANOVA was used to identify differences in scallop reproductive and nutrition condition between months and sites, performed using the SPSS statistics package. Data were not transformed since the parametric requirements were met. POM of sediment, surface seston concentration, bottom seston concentration and surface and bottom chl *a* were used as dependent variables for environmental parameters. VGI, gonad developmental stage and oocyte diameter were used as dependent variables for reproductive condition, while condition index, carbohydrates, protein and lipid content were used as dependent variable for nutrition condition. Energy content within individual organs (i.e. gonad, adductor muscle and digestive gland) were used as dependent variables for ANOVA tests on energy levels.

Multidimensional scaling (MDS) was used to identify patterns in fatty acid composition among different scallop tissues throughout the sampling period, and centroids by tissue type and by sampling month were plotted. SIMPER analysis was used to identify the fatty acids that primarily provided the discrimination between groups (i.e. tissue types). PRIMER was used to perform the MDS and SIMPER analyses on untransformed fatty acid data (organ-specific fatty acid compositions in animals always consist of arrangements of the same fatty acids present in different proportions [Napolitano et al. 1997], hence data were not transformed to avoid giving more weight to fatty acids present in small quantities), with a nonparametric Bray-Curtis similarity matrix, following the methods of Parrish et al. (2015).

3. RESULTS

3.1. Sediment samples

The sediment data from the 2 sites revealed slight differences in sediment composition. Site 1 had medium to fine sand of 1.3 φ medium particle diameter (MPD), while Site 2 had fine sand of 2 φ MPD.

Analysis of POM in sediment samples collected throughout the year revealed significant differences among the 2 sites and between months (Table 1). While the POM values at Site 1 were relatively stable between 4.1 ± 0.3 and 5.8 ± 0.5% DW over the year with a small peak in November, Site 2 had a wide range of values from 3.2 ± 0.3 to 6.8 ± 0.4% DW with a large peak in October.

3.2. Water samples

Water temperature was the same at both sites, with high values in summer and early autumn (maximum: 22°C in January 2013) and low in winter (minimum: 12°C in August 2012).

Analysis of water samples indicated that seston abundances were relatively similar between sites, with no significant differences (Table 1) found throughout the sampling period (Fig. 2). Surface water samples from both sites had consistently low seston concentrations (<5 mg l^{-1}) for both size classes (1.2–5 and >5 µm), except for a peak in concentration of about 13 mg l^{-1} for the 1.2–5 µm particle fraction in October 2013. For bottom water samples, seston concentrations varied greatly, from 3–22 and 0.7–16 mg l^{-1} for small (1.2–5 µm) and large (>5 µm) size classes, respectively, at Site 1, while seston concentrations at Site 2 ranged from 3–24 and 2.2–27 mg l^{-1} for small and large size classes, respectively. The temporal pattern of seston concentrations at the bottom revealed a major peak at both sites in August 2012. Surface water samples from the 2 sites were not significantly different (Table 1), and had consistently low chl *a* concentrations (<2 µg l^{-1}), except for a peak in concentration of about 6 µg l^{-1} at Site 2 in August 2012. Bottom chl *a* concentrations were significantly different between the 2 sites (Table 1), with a major peak between July and November at Site 2, and a smaller peak in October at Site 1. There was no agreement between the patterns in bottom chl *a* concentration and seston abundance at Site 1. At Site 2, chl *a* concentrations overlapped well with seston abundances for both size classes.

3.3. Reproductive condition

3.3.1. Visual gonad index

VGI analyses showed seasonal variations for scallops at both sites over the sampling period (Fig. 3), and VGI were not significantly different between

Table 1. Statistical analysis of particulate organic matter (POM) of sediment, surface seston concentration, bottom seston concentration and surface and bottom chl *a* for Sites 1 and 2

Source	df	*F*	p		
Sediments					
Site	1	6.58	**0.02**		
Month	6	5.60	**0.00**		
Site × Month	6	10.39	**0.00**		
Error	14				
		1.2–5 µm		>5 µm	
		F	p	*F*	p
Surface seston					
Site	1	1.86	0.19	0.12	0.74
Month	6	1.94	0.14	0.55	0.76
Site × month	6	0.05	0.69	0.73	0.64
Error	14				
Bottom seston					
Site	1	1.06	0.32	0.01	0.95
Month	6	4.04	0.02	2.26	0.10
Site × month	6	0.49	0.81	0.48	0.81
Error	14				
Chlorophyll *a*					
Site	1	1.06	0.32	32.66	**0.00**
Month	6	2.01	0.13	19.35	**0.00**
Site × month	6	0.86	0.55	11.50	**0.00**
Error	14				

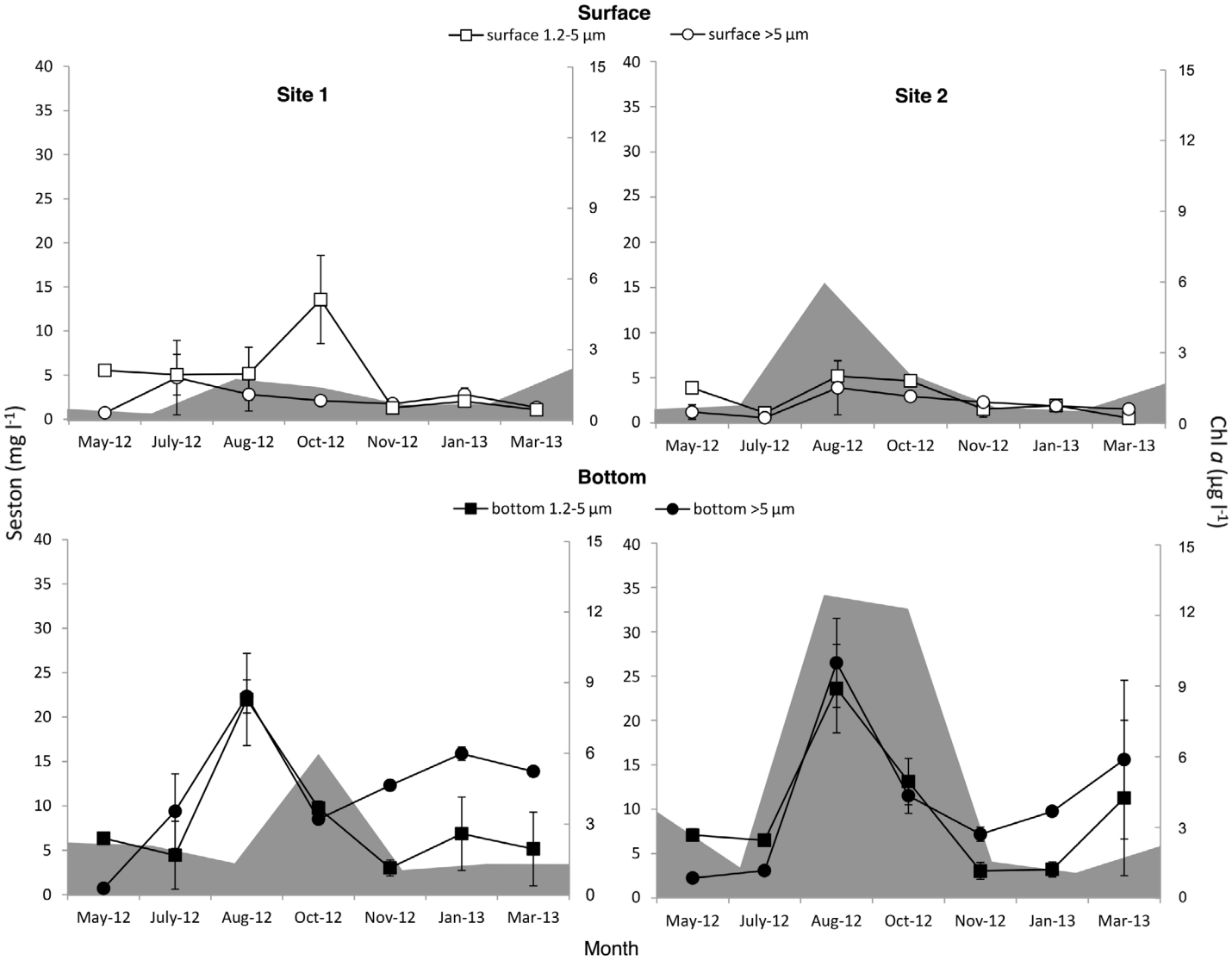

Fig. 2. Mean (±SE) surface and bottom seston abundances (2 size classes: 1.2–5 and >5 µm) at the 2 study sites between May 2012 and March 2013. Grey shading: chl *a* concentration

Sites 1 and 2 (Table 2). Immature/spent gonads (grade 1) were most frequent in March 2013 followed by May 2012 at both sites, while partially spawned gonads (grade 2) were most frequent in May 2012 for Site 1 and July 2012 for Site 2. Active gonads (grades 3–5) were recorded most often in August and November 2012 at both sites, while ripe gonads (grades 6–8) were dominant in October 2012 and January 2013 at both sites.

3.3.2. Histology

Analyses of histological sections of scallop gonads indicated that all 5 developmental stages used in the gonadal development classification by Bull (1976) could be observed over the sampling period (Figs. 3 & 4). Gonadal development was not significantly different between the 2 sites (Table 2). Spent gonads (stage I) were most frequent in May 2012 at Site 1 and March 2013 at Site 2, while gonads in the early development stage (stage II) were most frequent in May 2012 at both sites. Gonads within the active developmental stage (stage III) were recorded in high percentages in July, August and November 2012 and January 2013 at both sites, while ripe gonads (stage IV) were dominant between August 2012 and January 2013 at Site 1, and August 2012 to March 2013 (except November 2012) at Site 2. Partly spent gonads (stage V) were

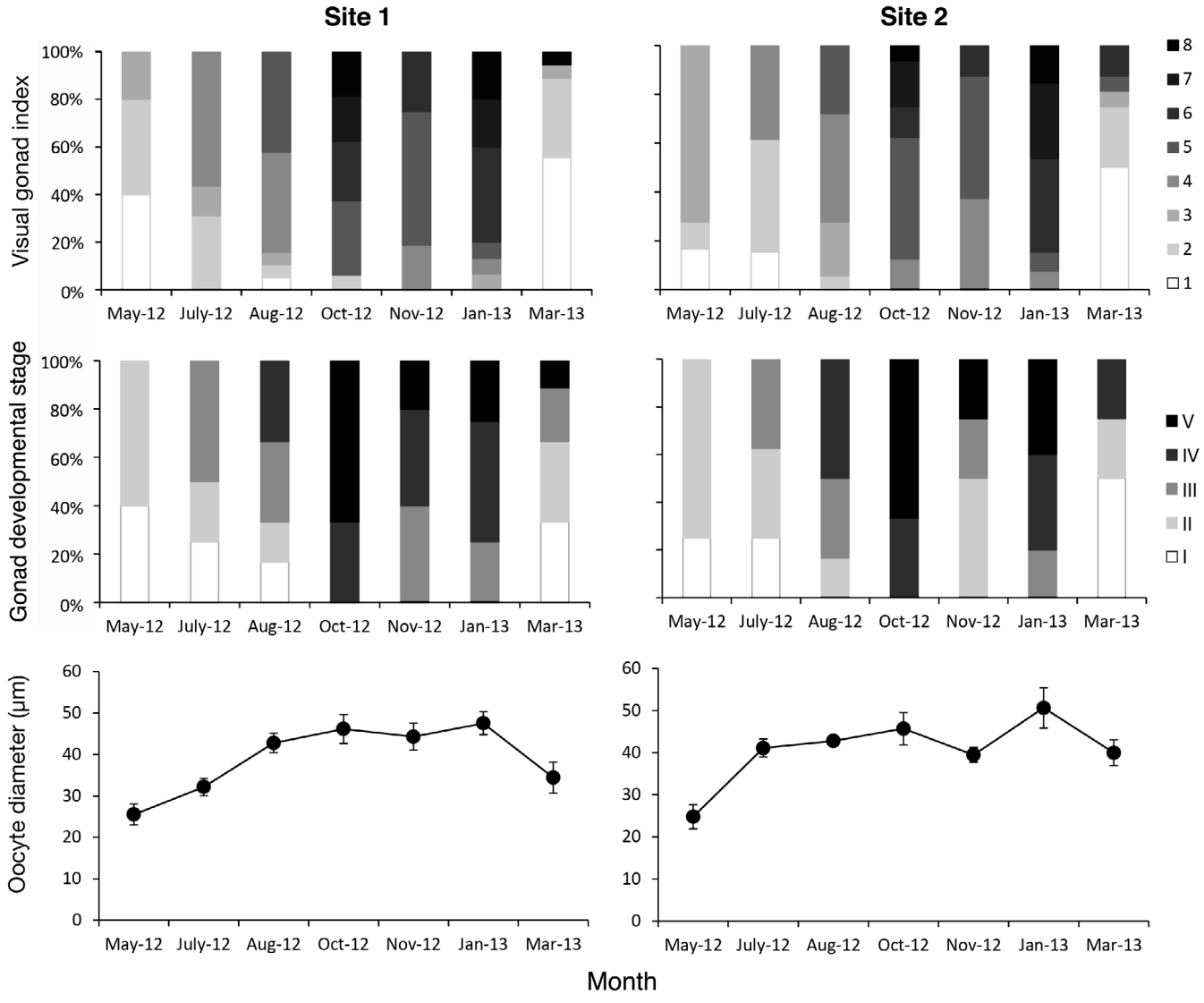

Fig. 3. Frequency (%) of visual gonad index classes (1–8) and gonad developmental stages (I–V), and mean (±SE) oocyte diameter (µm) of *Pecten novaezelandiae* at Sites 1 and 2 from May 2012 to March 2013

recorded most frequently between October 2012 and January 2013 at both sites. Oocyte diameters measured from photomicrographs of gonadal sections showed similar overall patterns at both sites, with 2 maxima in oocyte diameter in October 2012 and January 2013, followed by respective declines due to spawning (Fig. 3). Oocyte diameters ranged from 25–48 and 25–51 µm for Sites 1 and 2, respectively, over the entire sampling period. The seasonal variation of oocyte diameters overlapped well with the VGI data.

Scallop GIs did not differ significantly between the 2 sites (Table 2, Fig. 5). At both sites, notable increases in GI were observed from May 2012 and reached the highest values in October 2012, at 13.7% for Site 1 and 15.1% for Site 2. GI values dropped in November 2012, and reached a second peak at 12.7% (Site 1) and 13.6% (Site 2) in January 2013. The seasonal variation of GI values was found to be similar to that of VGI values and oocyte diameters over the sampling period.

3.3.3. Biochemical analyses

Biochemical compositions of gonad tissues are presented in Fig. 5. Analyses of carbohydrates, proteins and lipids within gonads resulted in relatively similar trends for scallops at both sites, but some noteworthy differences were observed. Gonad protein and lipid

levels were significantly different between the 2 sites (Table 2). The fluctuation in carbohydrate contents within gonad samples was low, but there was a notable association with gonad maturation, with a dramatic increase in October 2012 to a maximum of 50 ± 7 mg g^{-1} at Site 1, and 70 ± 10 mg g^{-1} at Site 2 (Fig. 5). At Site 1, protein content in gonads reached 2 peak levels in October 2012 and January 2013, each followed by a dramatic decline in the next sampling month. Gonad protein levels of scallops from Site 1 dropped from 355 ± 13 to 261 ± 13 mg g^{-1} between October and November, while protein levels dropped from 305 ± 11 to 195 ± 14 mg g^{-1} between January and March. On the other hand, gonad protein levels of scallops from Site 2 did not reach a maximum until January 2013 (322 ± 13 mg g^{-1}), and a minimum level was recorded in July 2012 (201 ± 13 mg g^{-1}). The changes in lipid contents in gonads were similar to those of carbohydrates and gonad maturation. Lipid contents increased gradually from May 2012 to October 2012, when the first peak was reached (Site 1: 137 ± 8 mg g^{-1}; Site 2: 116 ± 5 mg g^{-1}). Subsequent declines coincided with spawning events. Lipid levels reached a second peak in January (Site 1: 153 ± 9 mg g^{-1}; Site 2: 111 ± 8 mg g^{-1}), followed by a dramatic decline and second spawning in March 2013.

Table 2. Statistical analyses of reproductive condition (visual gonad index [VGI], gonad developmental stage, oocyte diameter), nutrition condition (condition index, carbohydrate, protein and lipid contents) and energy level of *Pecten novaezelandiae* from Sites 1 and 2

Source	df	VGI					
		F	p				
Reproductive condition							
Site	1	0.01	0.94				
Month	6	64.67	**0.00**				
Site × month	6	1.49	0.18				
Error	208						
		Stage		Oocyte diameter			
		F	p	*F*	p		
Histology							
Site	1	1.88	0.17	0.01	0.94		
Month	6	11.95	**0.00**	16.87	**0.00**		
Site × month	6	1.48	0.21	0.96	0.46		
Error	56						
		Gonad		Adductor muscle		Digestive gland	
		F	p	*F*	p	*F*	p
Condition index							
Site	1	2.67	0.10	0.17	0.68	1.34	0.25
Month	6	51.26	**0.00**	19.06	**0.00**	4.87	**0.00**
Site × month	6	1.20	0.31	1.53	0.17	3.31	**0.00**
Error	208						
Carbohydrates							
Site	1	1.35	0.25	0.29	0.59	0.01	0.94
Month	6	29.35	**0.00**	18.73	**0.00**	19.59	0.00
Site × month	6	5.80	**0.00**	1.56	0.16	2.77	**0.01**
Error	208						
Protein							
Site	1	13.68	**0.00**	5.90	**0.02**	3.64	0.06
Month	6	14.48	**0.00**	15.58	**0.00**	28.79	**0.00**
Site × month	6	9.46	**0.00**	4.70	**0.00**	2.33	**0.03**
Error	208						
Lipids							
Site	1	21.50	**0.00**	41.52	**0.00**	1.18	0.28
Month	6	179.54	**0.00**	44.19	**0.00**	8.69	**0.00**
Site × month	6	27.68	**0.00**	5.14	**0.00**	6.34	**0.00**
Error	208						
Energy							
Site	1	2.97	0.09	12.14	**0.00**	0.36	0.55
Month	6	42.50	**0.00**	5.70	**0.00**	18.35	**0.00**
Site × month	6	0.88	0.51	4.32	**0.00**	2.77	**0.01**
Error	208						

3.4. Nutritional condition

3.4.1. Adductor muscle

Adductor muscles had the largest condition indices, ranging from 44–53 and 4–54% for Sites 1 and 2, respectively. Scallop AMIs were generally negatively correlated with GIs over the annual cycle. The AMI values were generally higher in winter (July and August 2012) compared to summer for both sites. The maximum value was reached in July 2012 and the minimum in October 2012 for both sites (Fig. 5). AMI was not significantly different between the 2 sites (Table 2).

Analysis of adductor muscle tissues revealed similar overall patterns in biochemistry for scallops from both sites over the sampling period; however, some differences were observed, and muscular protein and lipid contents were significantly different between sites (Table 2, Fig. 5). The fluctuations of carbohydrate contents within scallop muscles from both sites were similar, with a clear maximum in November of 134 ± 10 and 112 ± 12 mg g^{-1} for animals from Sites 1 and 2, respectively. Conversely, protein content in

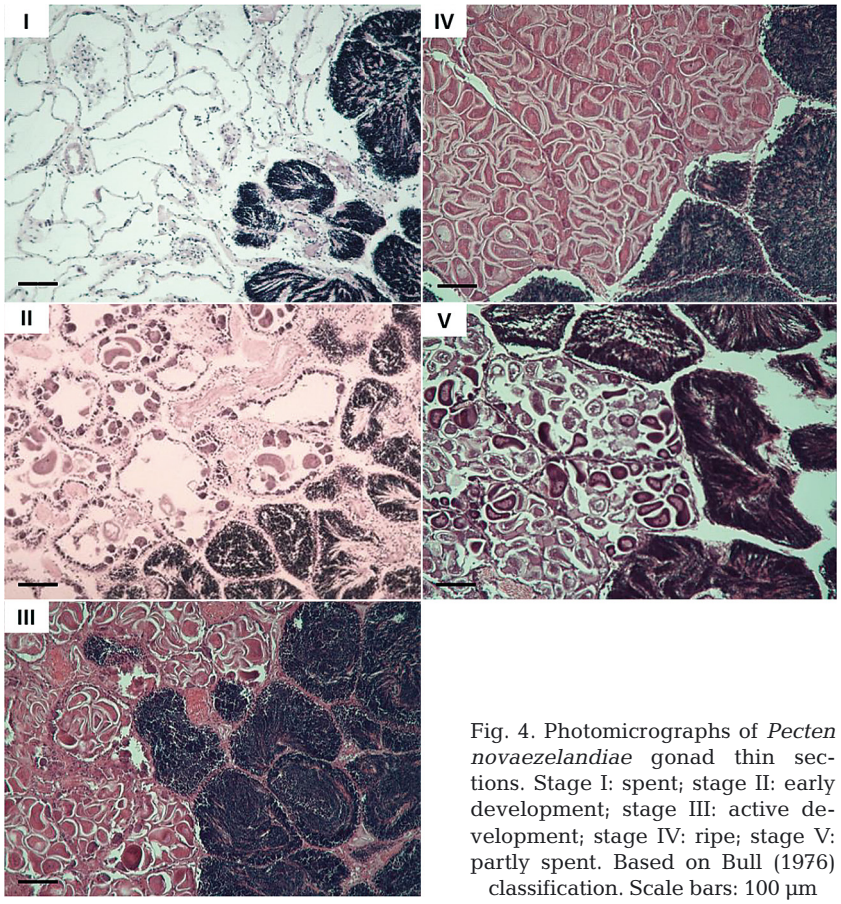

Fig. 4. Photomicrographs of *Pecten novaezelandiae* gonad thin sections. Stage I: spent; stage II: early development; stage III: active development; stage IV: ripe; stage V: partly spent. Based on Bull (1976) classification. Scale bars: 100 µm

the adductor muscle of scallops from both sites was lowest in November. Protein values ranged from 110 ± 3 mg g^{-1} in November to 234 ± 12 mg g^{-1} in January for Site 1 and from 125 ± 9 mg g^{-1} in November to 257 ± 4 mg g^{-1} in July at Site 2. The drop in protein content in the adductor muscle tissues in November coincided well with the drop in scallop condition index in October, suggesting a lag time of about 1 mo between these physiological parameters (Fig. 5). Lipid levels in adductor muscle tissues were low (26 ± 1.6 to 55 ± 3.1 mg g^{-1} and 38 ± 1.4 to 81 ± 4.3 mg g^{-1} for Sites 1 and 2, respectively) throughout the year with a slight increase to 55 ± 3.1 mg g^{-1} in October at Site 1 and to 81 ± 4.3 mg g^{-1} in November at Site 2 (Fig. 5). This slight increase in muscular lipid content appears to be associated with post-spawning condition.

3.4.2. Digestive gland

Analysis of digestive gland tissues revealed no significant difference between the 2 sites over the sampling period (Table 2). DGI of scallops from both sites did not fluctuate much over the seasonal cycle (Fig. 5). The variation in DGI was from 7.3–9.1 and 7.6–8.7 % for Sites 1 and 2, respectively.

The biochemical analyses of digestive gland revealed no significant difference between the 2 sites (Table 2). Carbohydrate contents within digestive glands were relatively similar for scallops from both sites, and relatively constant between 56 ± 8 to 116 ± 10 mg g^{-1} at Site 1 and 54 ± 9 to 129 ± 15 mg g^{-1} at Site 2 throughout the year, except for a small peak in November of 112 ± 20 and 129 ± 15 mg g^{-1} for Sites 1 and 2, respectively (Fig. 5). Protein contents in digestive glands were similar for animals from both sites (Fig. 5). Protein values followed a constant increase, from the lowest values in May (291 ± 9 mg g^{-1} at Site 1; 358 ± 12 mg g^{-1} at Site 2) to the highest values in October (535 ± 19 mg g^{-1} at Site 1; 546 ± 17 mg g^{-1} at Site 2). Then, there was a small decrease in protein content to about 450 mg g^{-1} that was maintained in scallops at both sites until March 2013. Lipid contents in digestive glands were relatively constant throughout the year, ranging from 105 ± 5 to 221 ± 15 mg g^{-1} for scallops at Site 1 and from 106 ± 4 to 179 ± 11 mg g^{-1} for scallops at Site 2 (Fig. 5). While a distinct November peak in lipid content was observed in digestive glands of scallops at Site 1, no distinct peak was evident for individuals at Site 2.

3.4.3. Energy content

The temporal pattern of average energy content within gonads, muscles and digestive glands showed relatively similar trends for scallops from Sites 1 and 2 throughout the year (Fig. 5), but statistical analyses revealed that the adductor muscle energy was significantly different between the 2 sites (Table 2). For scallops from both sites, the tissue with the highest energy content was the adductor muscle (Site 1: 4.7–15.8 kJ; Site2: 7.4–19.5 kJ), followed by the digestive gland (Site 1: 2.8–8.7 kJ; Site 2: 4.8–7.8 kJ) and gonads (Site 1: 1.1–10 kJ; Site 2: 1.9–8.7 kJ). The pattern in gonadal energy content was consistent with that of the GI, which was lowest in May 2012

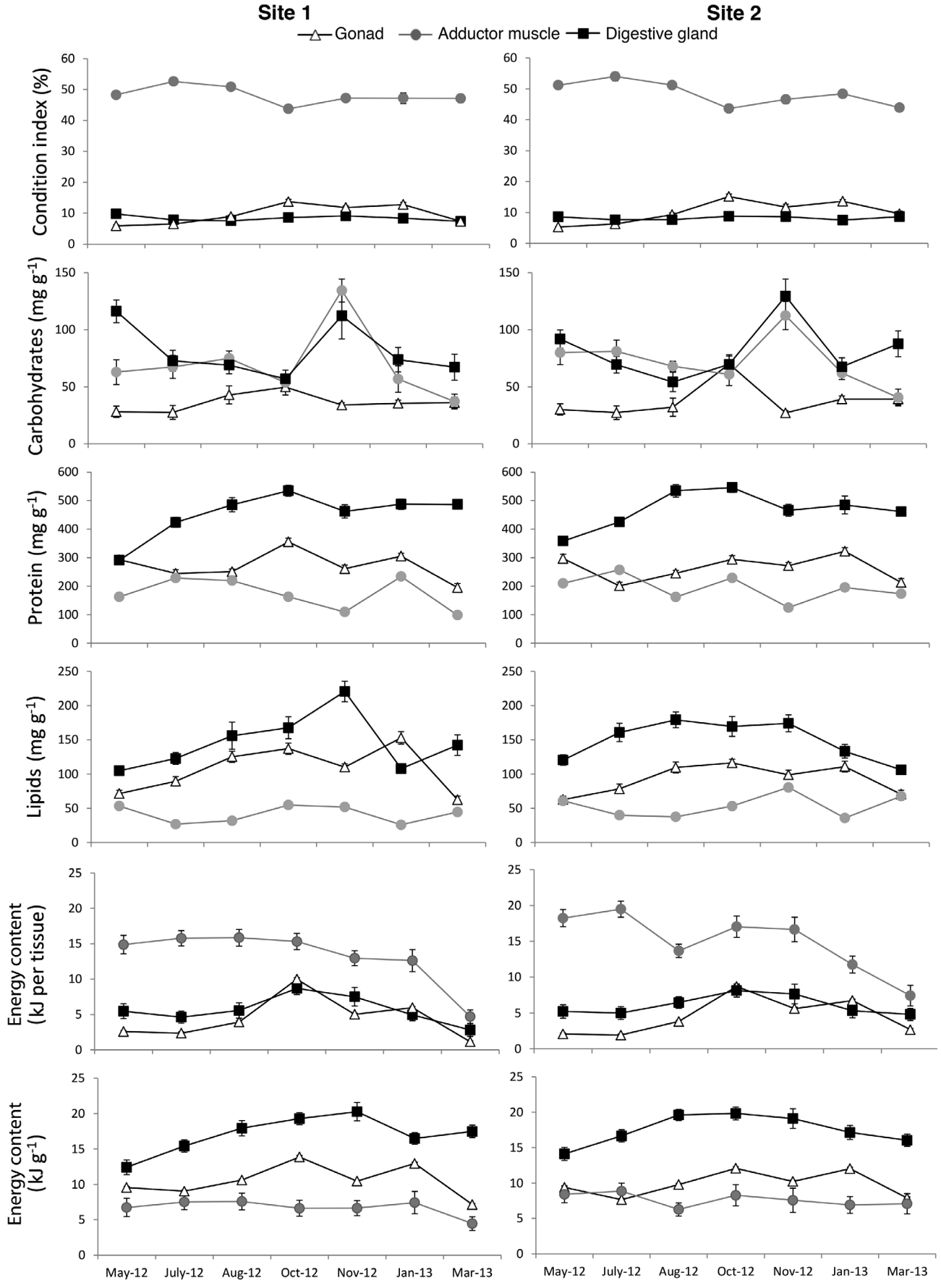

Fig. 5. Mean (±SE) condition indices, biochemical composition (carbohydrates, proteins and lipids) and energy content for gonad, muscle and digestive glands of *Pecten novaezelandiae* from Sites 1 and 2 from May 2012 to March 2013

and highest in October 2012 and January 2013. The energy content in scallop adductor muscles was lowest in March 2013 (after the January spawning), while the energy content in digestive glands was low in the colder season, at approximately 5 kJ for both sites, but contribution to the overall energy pool increased during spawning and/or summer (up to 9 kJ).

The highest values in calorific content per gram were found in digestive glands due to protein accumulation. Thus, digestive glands exhibited a maximum energy of 20.3 kJ g^{-1} (Site 1) and 19.8 kJ g^{-1} (Site 2) in November and August 2012, respectively, and a minimum of 12.4 kJ g^{-1} (Site 1) and 14.1 kJ g^{-1} (Site 2) in May 2012. In the adductor muscle, there was no clear variation in caloric content, which ranged between 4.5–7.6 and 6.9–8.9 kJ g^{-1} for Sites 1 and 2, respectively. The seasonal variation of gonad energy content was found to be similar to that of gonadal maturation (i.e. GI, VGI values, oocyte diameter), with a maximum energy content of 13.7 kJ g^{-1} (Site 1) and 12.1 kJ g^{-1} (Site 2) in October 2012, and minimum of 7.2 kJ g^{-1} in March 2013 for Site 1 and 7.7 kJ g^{-1} in July 2012 for Site 2.

3.5. Fatty acid profiles

MDS plots showed clear clusters of fatty acids from seston and scallop tissues (gonad, adductor muscle and digestive gland) for each site. The gonad samples clustered between adductor muscle samples, while adductor muscle samples clustered tightly and separated well from the more dispersed group of digestive gland samples (Fig. 6). These spatial patterns were quantified by SIMPER, having similarities of >82% for all groups, with seston, female gonad, male gonad, adductor muscle and digestive gland having similarities of 85, 85, 88, 92 and 83%, respectively, for Site 1, and 82, 92, 93, 95 and 82%, respectively, for Site 2 (Table 3). As identified by SIMPER, C16:0, C16:1 and C20:2 were the main contributors for the seston grouping, while C16:0, C20:5n3 and C22:6n3 were the top contributors for clustering of all 3 types of scallop tissues (gonad, adductor muscle and digestive gland), with the addition of C18:0 for digestive gland.

3.6. Stable isotopes

Clear separation and clustering in $\delta^{15}N$ and $\delta^{13}C$ signatures were observed for all scallop tissues, sediment and seston samples from both sites over the sampling period (Fig. 7). Scallop tissues (gonad, adductor muscle and digestive gland) had tight and separate clusters with $\delta^{13}C$ signatures between −18.1 to −20.4, −17.7 to −18 and −19.4 to −22‰ for each tissue, respectively, and $\delta^{15}N$ signatures between 8.5–9.9, 9.6–10.3 and 8.0–9.3‰ for each tissue, respectively, at both sites. Sediment samples also separated well from seston and scallop samples with a narrow range of $\delta^{13}C$ signatures between −9.3 and −11.6 ‰, and a wide spread of $\delta^{15}N$ values from 4.7–9.3‰ (sites combined). $\delta^{13}C$ for seston samples within the >5 µm size class clustered between −18.1 and −24.1‰, while seston samples within the 1.2–5 µm size class were slightly more enriched between −20.7 and −26.3‰ for both sites. $\delta^{15}N$ values for the >5 µm seston fraction ranged from 5.3–6.4‰, while those of the 1.2–5 µm size fraction ranged from 5.1–7.1‰ for both sites.

Temporal differences were found in $\delta^{13}C$ and $\delta^{15}N$ isotopes among tissues (Fig. 8). In samples from both sites, $\delta^{13}C$ values for adductor muscle varied little over time. Greater and comparable temporal variations were found for gonad and digestive gland tissues, with a general increase in $\delta^{13}C$ values from August 2012 to January 2013. Nitrogen values for gonad tissues exhibited a temporal shift in relation to somatic tissues. Gonads and adductor muscles revealed similar signatures in winter (May–October 2012); gonad signatures became closely related to those of digestive glands during November 2012.

4. DISCUSSION

4.1. Environmental parameters

The 2 sampled sites had similar sandy grain size composition, and higher seston abundances and chl *a* concentrations in the bottom water column compared to the surface. There were also comparable temporal patterns of low nutrient composition in the water column in winter followed by a spring bloom. However, the presence of seagrasses at Site 1 provided a distinctive 3-dimensional protective structure with potentially higher nutritional content for scallops compared to Site 2, which was composed of a flat sandy substrate. These physical site characteristics support the higher variation of POM values, seston levels and chl *a* concentrations at Site 2 compared to Site 1 throughout the sampling period. Although chl *a* was the only significantly different environmental parameter between the 2 sites, the year-long monitoring showed a more stable environment at the seagrass site (Site 1) than the sandy site (Site 2).

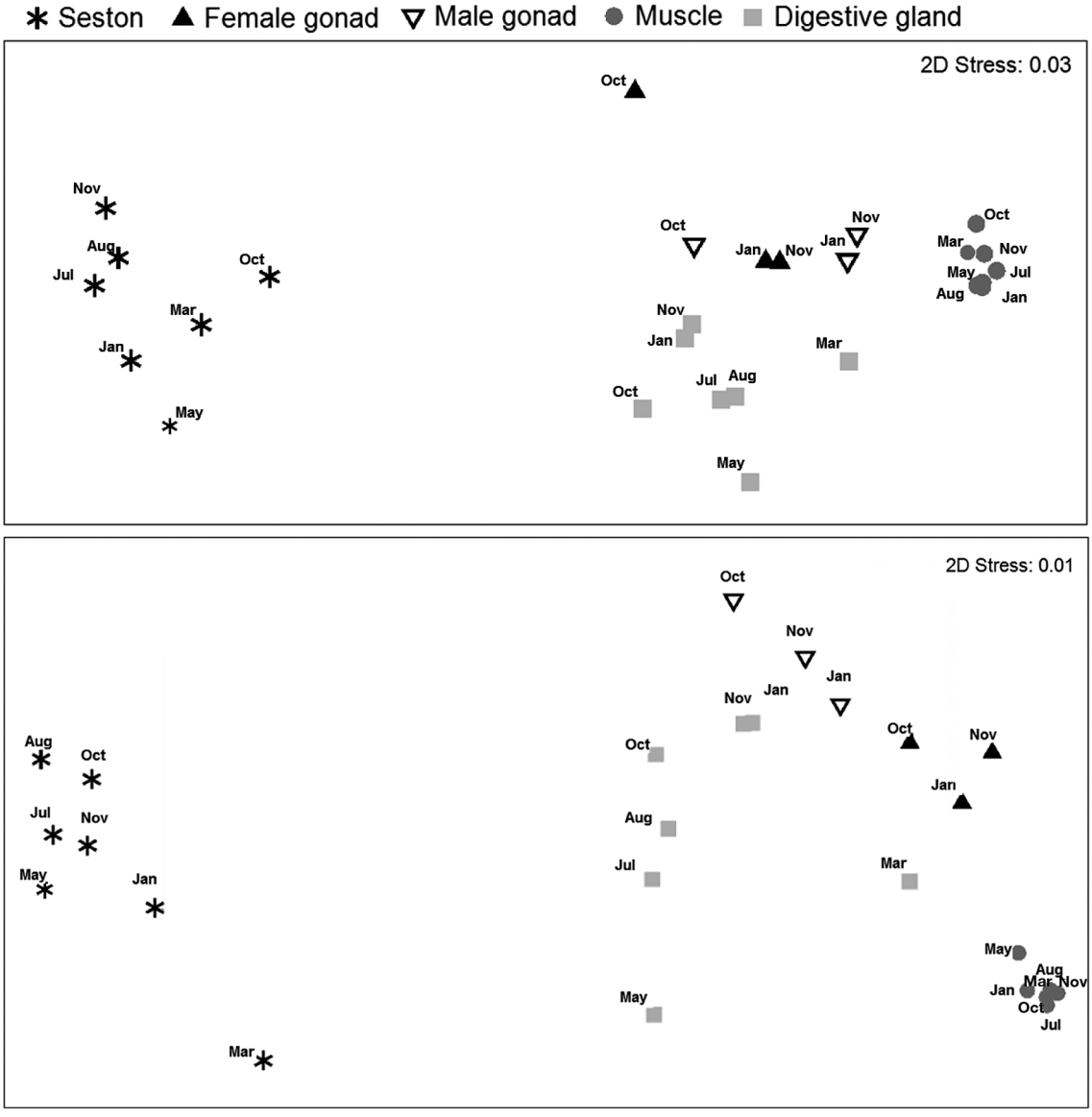

Fig. 6. Multidimensional scaling plots of fatty acid proportions (%) of >5 µm seston and *Pecten novaezelandiae* tissues (female gonad, male gonad, adductor muscle, digestive gland) for Sites 1 and 2 from May 2012 to March 2013. Resemblance matrix was created using Bray-Curtis similarity coefficients

The seston and chl *a* levels at the 2 sites fall within the range of values previously recorded in the Hauraki Gulf, and share the same seasonal cycle previously reported of minimum concentrations in winter followed by a spring bloom (Booth & Sondergaard 1989, Rhodes et al. 1993, Chang et al. 2003). Seston and chl *a* concentrations were consistently high in August and October 2012, indicating that phytoplankton was most abundant in spring in both surface and bottom waters, although bottom waters always had higher concentrations. The overall lower chl *a* concentrations in bottom waters at Site 1 likely indicate limited benthic diatom growth on the seabed due to shading from the seagrass communities (Hemminga & Duarte 2000). Indeed, yellow–green biofilms (commonly containing diatoms) were observed on the seabed at Site 2, but were absent at Site 1. This material may be re-suspended by strong water currents and/or during storm events, and provides a potentially important food supply for scallops. While the 2 sites may provide slightly different food types to scallops, it is difficult to associate these differences with specific patterns in nutritional and reproductive condition, especially when scallops are a mobile spe-

Table 3. Average similarities (SIMPER) by fatty acid proportions (%) of >5 µm seston and *Pecten novaezelandiae* tissues (female gonad, male gonad, adductor muscle, digestive gland) for Sites 1 and 2, from May 2012–March 2013. Similarity, the cut-off is shown at 80%

Site 1		Site 2	
Fatty acids	Cum.%	Fatty acids	Cum.%
Seston			
Average similarity:	84.59	Average similarity:	81.39
C16:0	41.61	C16:0	44.98
C16:1	68.01	C16:1	73.65
C20:2	77.24	C20:2	84.03
C20:4n6	86.21		
Female gonad			
Average similarity:	85.28	Average similarity:	92.07
C16:0	30.26	C20:5n3	25.3
C20:5n3	56.69	C16:0	50.25
C22:6n3	69.79	C22:6n3	68.45
C16:1	78.39	C16:1	76.86
C18:0	83.39	C18:0	84.28
Male gonad			
Average similarity:	88.31	Average similarity:	92.78
C16:0	25.52	C20:5n3	24.96
C20:5n3	50.99	C22:6n3	49.19
C22:6n3	72.13	C16:0	71.48
C18:0	81.57	C18:0	81.78
Adductor muscle			
Average similarity:	92.44	Average similarity:	94.84
C22:6n3	38.46	C22:6n3	40.22
C16:0	57.71	C16:0	59.8
C20:5n3	76.67	C20:5n3	78.11
C18:0	87.2	C18:0	88.26
Digestive gland			
Average similarity:	83.05	Average similarity:	81.81
C16:0	30.18	C16:0	30.52
C18:0	45.48	C18:0	45.98
C20:5n3	60.09	C22:6n3	60.77
C22:6n3	73.5	C20:5n3	74.61
C16:1	80.51	C16:1	80.54

cies that may use seagrass beds for other reasons, such as a protective environment against predators.

The $\delta^{13}C$ and $\delta^{15}N$ signatures for seston, sediment and scallop samples indicated that the small seston fraction (1.2–5 µm) is likely to be the main source of food for *Pecten novaezelandiae* (especially at Site 2), while the large seston fraction (>5 µm) contributed to a lesser extent. As can be seen in Fig. 7, sediment samples had considerably greater carbon values than those of scallops. Contrary to the present study, Cranford & Grant (1990) reported that the optimal retention efficiency of *Placopecten magellanicus* included food particles of 5–40 µm. However, Ward & Shumway (2004) reviewed particle selection in suspension-feeding bivalves and concluded that retention efficiency is species-specific, especially for particles <6 µm. These species-specific differences have been suggested to be related to the morphology of feeding parts (Ward & Shumway 2004) and food availability (Zhang et al. 2010). While *P. magellanicus* (Cranford & Grant 1990) and *Monia squama* (Jørgensen et al. 1984) primarily consume seston particles that are >5 µm, high retention efficiency of 2–5 µm seston has been shown for *Mytilus edulis* (Vahl 1972), *Argopecten irradians* (Palmer & Williams 1980), and *Petricola pholadiformis* (Jørgensen et al. 1984). In addition, pectinids have the ability to control the quantity (Cahalan et al. 1989) and quality (organic versus inorganic) of particles consumed (Brillant & MacDonald 2002). Nicholson (1978) documented that selectivity in *P. novaezelandiae* is achieved by using their lips and palps to control the ingestion of seston <5 µm, and these seston contributed as much as 50% of the total diet in this species.

It is difficult to classify wild seston into phytoplankton groups based on size difference, due to the seasonal variation in phytoplankton combination (flagellates and cryptophytes dominate in spring and winter, whereas diatoms are abundant in summer) (Bănaru et al. 2014), and the variation in cell size among populations (i.e. cell size decreases when population size increases) (Cassie 1959). In the present study, isotopic analyses revealed different carbon signatures between the 2 seston fractions collected, with large seston being more enriched (–20.9 ± 0.9‰ at Site 1; –19.5 ± 0.4‰ at Site 2) than small seston (–23.2 ± 0.9‰ at Site 1; –22.8 ± 1‰ at Site 2). Typically, suspended organic matter (mainly detrital material) has a more depleted ^{13}C than phytoplankton (Harmelin-Vivien et al. 2008). Nevertheless, small phytoplankton might also be reflected by lower carbon values. Few studies have reported that larger microphytoplankton particles are markedly ^{13}C-enriched compared to small cells (<6 µm) (Rau et al. 1990, Rolff 2000). Although carbon isotope signatures of diatoms were more enriched than other phytoplankton when pure net plankton samples were analyzed by Fry & Wainright (1991), the authors also reported an insignificant correlation between carbon signatures and taxonomic composition of phytoplankton when samples were classified by dominant phytoplankton (Wainright & Fry 1994). Hence, isotopic signature cannot clearly determine wild seston composition. Therefore, in the present study, the isotope signatures and environment parameters indicate that *P. novaezelandiae* consumes primarily detritus and

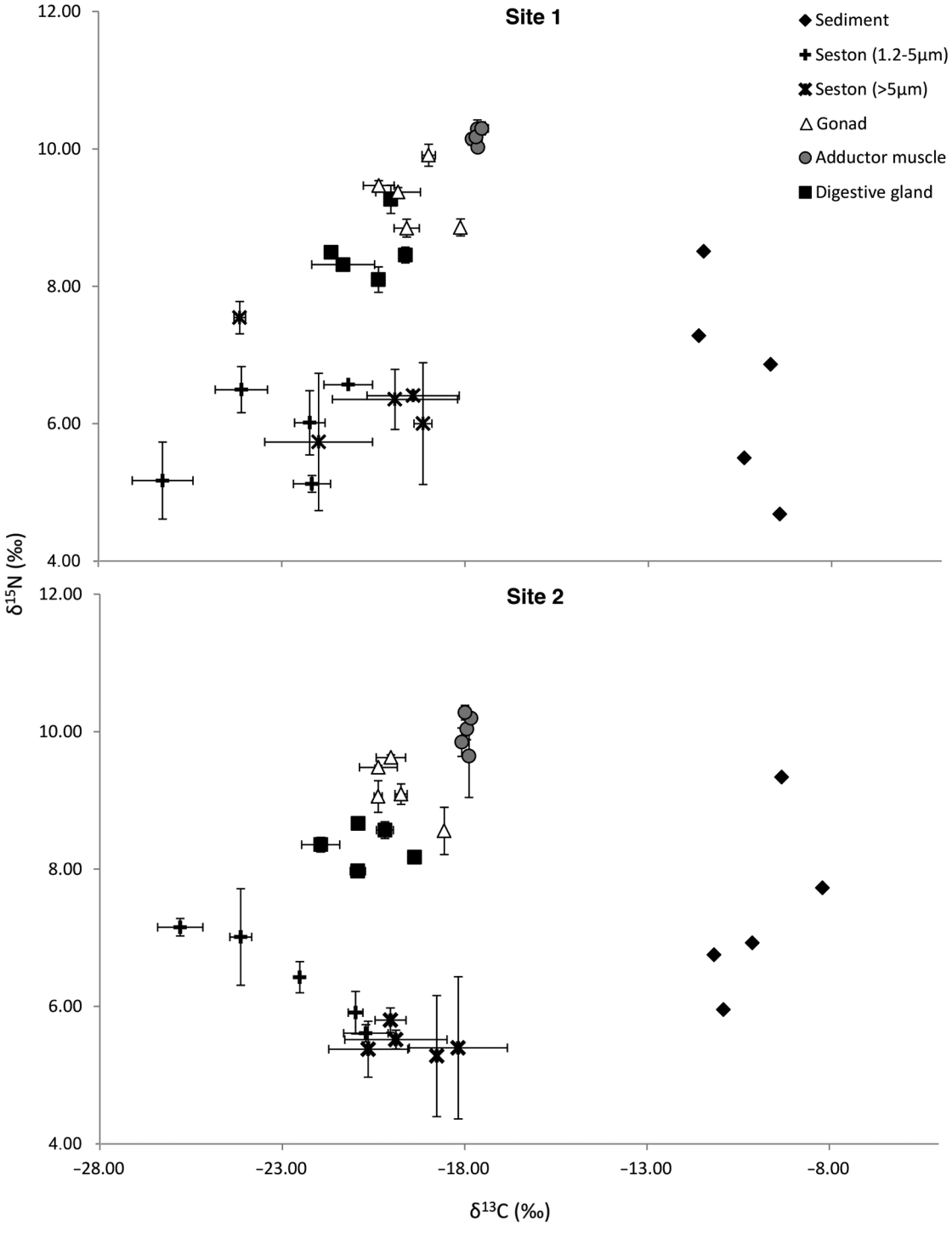

Fig. 7. Stable isotope ($\delta^{15}N$ vs. $\delta^{13}C$) signatures of seston (1.2–5 and >5 µm size classes), sediment and *Pecten novaezelandiae* tissues (gonad, adductor muscle and digestive gland). Values for seston and scallop tissues are means (±SE) of 2 replicates; only 1 replicate was analyzed for sediment samples

small phytoplankton with larger phytoplankton as a secondary food source at the study sites. Indeed, detrital material has been shown to be consumed by *P. novaezelandiae* (Nicholson 1978) and *P. magellanicus* (Cranford & Grant 1990), while phytoplankton has been reported to be a food item of *P. maximus* (Chauvaud et al. 2000) and *Zygochlamys patagonica* (Botto et al. 2006).

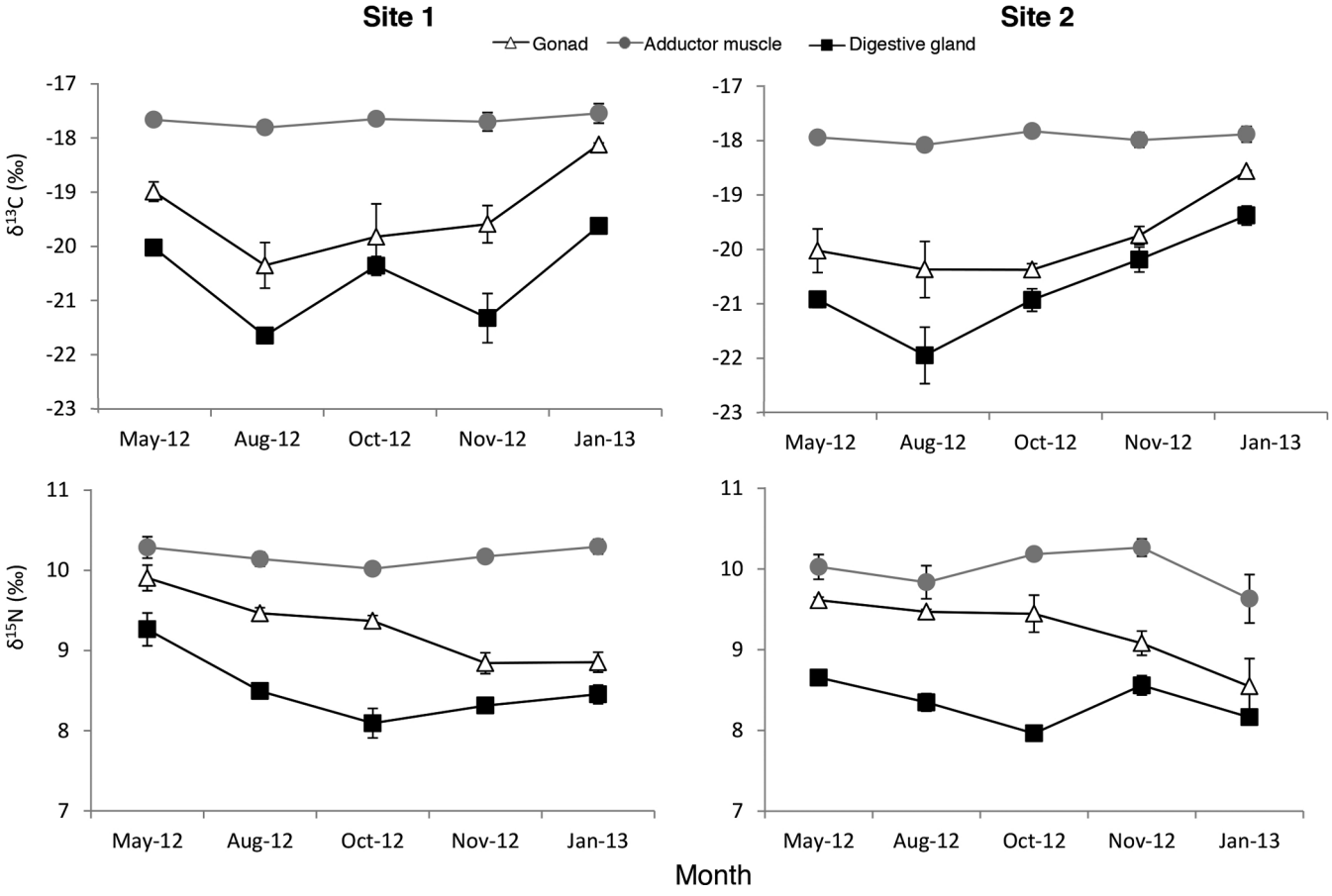

Fig. 8. Mean (±SE) temporal isotopic variation in gonad, adductor muscle and digestive gland of *Pecten novaezelandiae*

4.2. Reproduction

Despite the potentially different nutritional environments found at the 2 sampling sites, the gametogenic cycle of *P. novaezelandiae* did not differ between sites. The reproductive cycle was characterized by 2 major spawning periods, reflected by a sharp decrease in VGI, oocyte diameter, GI and lipid content. Spawning events occurred in November 2012 (referred to as 'spring spawning') when the temperature was about 18°C; a second spawning occurred in March 2013 (referred to as 'summer spawning') when the temperature reached 22°C. Nicholson (1978) and Morrison (1999) recorded a similar spawning pattern for *P. novaezelandiae* in the Hauraki Gulf. Nicholson (1978) surveyed populations in Takatu and Ti Point in the Hauraki Gulf (~1.5 km east and ~8.5 km northeast of our sampling sites, respectively) to investigate the reproductive cycle of *P. novaezelandiae.* He measured GI and macroscopic visual classification between 1976 and 1977, and concluded that *P. novaezelandiae* was limited to one spring spawning event with a further potential spawning in February and March, should environmental conditions prove suitable. Fourteen years later, Morrison (1999) collected reproductive data again from Takatu, south of Ti Point (~7 km northeast of our sampling sites) and Motuketekete Island (~9.5 km southeast of our sampling sites) and confirmed a clear biannual spawning pattern for *P. novaezelandiae* in the Hauraki Gulf. Nicholson (1978), Morrison (1999) and the present study recorded 2 spawning events per year, which indicates that spawning of *P. novaezelandiae* is not necessarily predictable. Williams (2005) recorded 3 spawning events between 2000 and 2001 near Plembles Island (~4.5 km southeast of our sampling sites), with the addition of partially spawned individuals recorded throughout the year. He concluded that the spawning timing of this species is variable among populations, and the largest spawning events tend to occur mostly in December (referred to as 'spring spawning' in this study). Such spawning variations may be a result of size structure variations among populations and/or environmental

conditions, such as temperature and food availability. Indeed, Sastry (1968) found that temperature was a strong determinant on the rate of gamete maturation, whereas fecundity and size of gonads were primarily determined by food availability. Experiments using *Pecten fumatus* (Heasman et al. 1996) and *P. novaezelandiae* (Nesbit 1999) provide further evidence that gonad size is strongly affected by diet quantity, whereas gonad condition varies more with temperature. Although laboratory experiments have shown that low temperatures (12°C) may slow down the rate of gametogenesis (Nesbit 1999), spawning has been recorded in the Hauraki Gulf during winter when the surface water temperature was 14°C (bottom water temperatures would have been lower) (Williams 2005). Thus, it is possible to conclude that gametogenesis may be slowed down but not prevented by winter temperatures at the study site. In addition, food availability may be a primary factor responsible for variations in spawning timing and number of spawning events.

In the present study, the biochemical composition of gonad samples generally supported the VGI and histological data; carbohydrates, proteins and especially lipids showing an increase in nutrition levels with gonad maturation. Energy content in gonads started to increase in August 2012, indicating the beginning of gametogenesis, and reached a maximum in October, followed by a dramatic drop in November, representing spring spawning. Carbohydrates, proteins and lipids have been shown to increase in gonads during gametogenesis, leading to spawning in other pectinid species (Racotta et al. 1998, Saout et al. 1999, Barber & Blake 2006). For example, higher carbohydrate contents were found to be associated with gonad development during broodstock conditioning of *Argopecten purpuratus* (Martínez et al. 1992) and *A. ventricosus* (Racotta et al. 1998), and it has been suggested that the carbohydrates accumulated in the gonads of molluscs can be used directly as energy for spawning (Racotta et al. 1998, Ruiz-Verdugo et al. 2001). Protein is the fundamental component of all tissues, as it provides structural material for the cell. Accumulation of proteins in gonads during maturation is commonly reported in scallop species in association with gonad growth (Barber & Blake 1981, Epp et al. 1988, Couturier & Newkirk 1991, Pazos et al. 1996, Racotta et al. 1998, Martínez et al. 2000, Ruiz-Verdugo et al. 2001). Also, accumulation of lipids in gonads during gametogenesis is an important and well-documented process (Vassallo 1973, Couturier & Newkirk 1991, Martínez 1991, Pazos et al. 1997, Barber & Blake 2006), and is essential for equipping eggs with the resources needed to fuel their energetic embryo and larval development processes (Holland 1978). Indeed, Devauchelle & Mingant (1991) demonstrated that the hatching success rate of *P. maximus* was highly dependent on egg lipid reserves. In the present study, spat were not collected to assess the hatching success rate of *P. novaezelandiae* in the sampling area. However, the polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are essential for bivalve larval survival, and these were the main fatty acids in the gonad samples tested. In addition, lipid contents in gonads decreased dramatically during the 2 spawning events, indicating that lipids were accumulated in the developing eggs, which were then released to the ocean.

4.3. Nutrition

The high energy requirements of gametogenesis usually require storage of nutrients where they can be easily accessed when needed (Barber & Blake 2006). Only 3 main tissues (gonad, adductor muscle and digestive gland) were analyzed in this study, and it is important to note that other tissues (mainly mantle and gills) may also play some role in energy allocation during gametogenesis. The energy contribution of these additional tissues is currently unclear. Indeed, some researchers suggest that the mantle may supply energy during bivalve reproduction (e.g. Barber & Blake 1981, Epp et al. 1988), while others suggest its role in gametogenesis is negligible (Vite-Garciá & Saucedo 2008). In addition, gills may have a limited energy contribution for respiration and feeding (Lodeiros & Himmelman 1994). In the present study, energy gains in scallop gonads were more than the losses of energy recorded in the adductor muscle and/or digestive gland, indicating that energy demands for gametogenesis in *P. novaezelandiae* were either supported by (1) both ingested food and nutrient reserves in adductor muscle and digestive gland or (2) by nutrient reserves from adductor muscle, digestive gland and other tissues that were not analyzed in this study. Nevertheless, the biochemical data revealed that energy reserves used during gametogenesis were mainly obtained from carbohydrates and proteins in the adductor muscle and lipids in the digestive gland.

In pectinids, it has been documented that the large adductor muscle provides energy for reproduction, but storage cells have not yet been described (Mathieu & Lubet 1993). In the present study, the energy

level in the adductor muscle progressively decreased from July 2012 to March 2013, with a more dramatic decrease post spring spawning. This indicates that energy from adductor muscles is likely to fuel gametogenesis and satisfy the high energy demand during spawning periods. In addition, carbohydrate levels decreased during August to October and November to March in adductor muscles, indicating that these carbohydrates are likely to be used for oogenic development and gonad maturation. Once in the gonads, carbohydrates could be converted into lipids and stored in the ripening gametes, as reported for many marine bivalves (Gabbott 1975, Berthelin et al. 2000). The role of carbohydrates (mainly glycogen) is predominant in most bivalves in terms of nutrient storage to support gametogenesis (Barber & Blake 1981, Martínez et al. 2000, Lodeiros et al. 2001). In contrast, proteins are the major structural components in cells and have a high contribution to total energy levels in the adductor muscle. The decrease in protein in the adductor muscle from July (when food availability and temperature are lowest) through to spring spawning in November indicates that protein is likely used to meet the maintenance requirements during winter and the high energy demands of spawning. Indeed, the breakdown of adductor muscle protein to provide maintenance energy has been recorded in *A. irradians concentricus* (Barber & Blake 1981), *A. irradians irradians* (Epp et al. 1988) and *Euvola zizac* (Brokordt et al. 2000), as well as other bivalve species such as *Tapes philippinarum* (Beninger & Lucas 1984) and *Mytilus edulis* (Bayne et al. 1982).

The digestive gland is known to play an important role in regulating the distribution of assimilated nutrients in body tissues (Vassallo 1973). Depending on seasonal food supplies and reproductive state, the digestive gland can also serve as a storage organ (Thompson et al. 1974). In the present study, the fatty acid profiles support the notion that the digestive gland was involved in the initial assimilation and subsequent transfer of nutrients from the ingested food (seston) to the gonad during the sampling period. Indeed, the MDS plots of fatty acid signatures show a closer association between digestive gland and seston samples than gonad or adductor muscle samples and seston. This likely indicates that fatty acids originating from seston were first incorporated into the digestive gland and then re-allocated to the gonad, while the adductor muscle had no or little involvement in storage of fatty acids from seston. Caers et al. (2003) also reported dietary lipids being stored in the digestive gland and subsequently transferred to the developing female gonad in *A. purpuratus*. In addition, adductor muscle tissues are unlikely to store large quantities of lipids, and lipid components within this organ are normally used for cellular structure (Napolitano et al. 1997).

In the present study, energy levels in digestive gland samples continuously increased from winter to spring at both sampling sites, and energy utilization started from January (post spring spawning) onwards, suggesting that the energy from the digestive gland was being used for gonad recovery. Consistently low carbohydrate levels in the digestive gland were recorded over the course of the study, but an inverse relationship between carbohydrates in the digestive gland and gonad maturation was observed. This relationship may indicate the use of digestive gland carbohydrates for gametogenesis. However, previous studies have found no evidence for the use of digestive gland carbohydrates for reproductive purposes in pectinids (Barber & Blake 1981, Pazos et al. 1997, Racotta et al. 2003). Comparatively high protein content was observed in digestive gland tissues, with an increasing trend during gametogenesis peaking in October (prior to spring spawning), followed by a slight drop during spring spawning. This pattern suggests that digestive gland proteins are not required for gametogenesis, but accumulate in digestive gland tissues and are used during gonad spawning. Lipid content within digestive gland tissues increased with increasing gonad maturation in scallops prior to spring spawning, but appeared to be depleted by January. This pattern suggests that digestive gland lipids are not required to prepare for spring spawning, but stored lipids contribute to gonad re-development after this spawning event. Although the digestive gland lipids appeared to fluctuate more in samples from Site 1 than Site 2, the general pattern at both sites was similar over the recorded period. Lipid reserves in digestive glands have been reported to be important energy reserves for gametogenesis in *A. irradians concentricus* (Barber & Blake 1981), *P. maximus* (Comely 1974, Pazos et al. 1997, Saout et al. 1999) and *P. magellanicus* (Robinson et al. 1981).

Energy reserves and utilization in *P. novaezelandiae* and their relationship to the reproductive cycle represent complex physiological interactions between gonad, adductor muscle and digestive gland. Often, gamete production needs to compete for energy resources with somatic growth and/or maintenance expenses (Barber & Blake 1985). For the most part, the utilization and storage of energy can be identified through investigation of biochemical

and/or metabolic processes within and among different tissues. For example, Lorrain et al. (2002) suggested that $\delta^{15}N$ signatures can be used to indicate metabolic fluxes (energy transfer) among tissues in the scallop *P. maximus* (i.e. variations in $\delta^{15}N$ are consistent with energy transfers). As can be seen in Fig. 8, nitrogen signatures in gonad samples experienced temporal changes in relation to somatic tissue in the present study: gonad samples shared a similar signature to adductor muscle samples during winter, then became comparable to digestive gland signatures during and after spring spawning (November). This pattern suggests that energy was transferred from adductor muscle to gonads to support the energy demand for gametogenesis during winter, and digestive gland energy was allocated to gonads from November for spawning and gonad recovery.

Overall, the nutritional condition of *P. novaezelandiae* revealed by $\delta^{15}N$ signatures and biochemical analyses of tissues suggests that this scallop species exhibits temporal changes in energy transfer. These are indicative of the use of energy (mostly carbohydrates) from the adductor muscle to fuel gametogenesis (gonad maturation) during winter, and a shift to energy derived from the digestive gland for use during spring spawning (mostly protein) and gonad recovery (mostly lipids) during summer (Fig. 9).

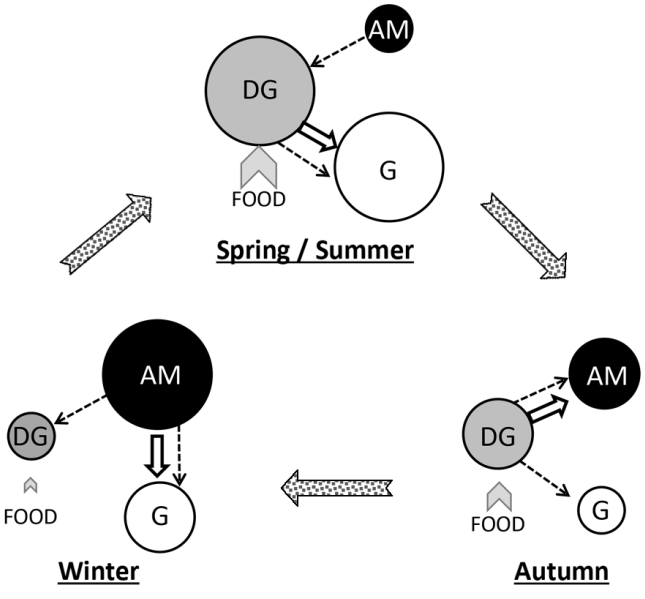

Fig. 9. Simplified scheme of energy distribution in *Pecten novaezelandiae*. Circle size corresponds to relative energy level in gonad (G), adductor muscle (AM) and digestive gland (DG). Stippled arrows: seasonal cycle. Thick and dashed arrows: primary and secondary/maintenance energy transfer, respectively, among gonad, adductor muscle and digestive gland

4.4. Summary

The findings of this study show that energy allocation between gonad and somatic tissues in *P. novaezelandiae* exhibits a temporal (seasonal) trend. When food availability is low in winter, adductor muscle energy reserves are utilized to initiate gametogenesis. In spring/summer when food resources are abundant, assimilated food is directly transferred from the digestive gland to the gonads for spawning and gonad recovery. During autumn, when food availability decreases, assimilated food within the digestive gland is used for maintenance expenses, and excess energy is transferred to the adductor muscle for storage. Thus, *P. novaezelandiae* requires both long-term energy reserves from the adductor muscle and energy mobilized from ingested food through the digestive gland to achieve its reproductive cycle. Carbohydrates in the adductor muscle provide energy to initiate gametogenesis in winter, protein from the digestive gland provides the threshold levels of energy for spawning in spring/summer and lipids from the digest gland provide energy for gonad recovery after spring spawning.

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