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Diet and performance in the scallop, *Argopecten purpuratus*: force production during escape responses and mitochondrial oxidative capacities

Helga Guderley[1,2,a], Katherina Brokordt[2], Hernán M. Pérez Cortés[1,2], Yanic Marty[3] and Edouard Kraffe[3]

[1] Département de biologie, Université Laval, Québec, P.Q. Canada G1K 7P4, Canada
[2] CEAZA (Centro de Estudios Avanzados en Zonas Áridas), Facultad de Ciencias de Mar, Universidad Católica del Norte, Coquimbo, Chile
[3] Laboratoire des Lipides marins, UMR CNRS 6521, Université de Bretagne occidentale, CS 93837, 29238 Brest Cedex 3, France

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Abstract – We examined whether escape response performance and mitochondrial capacities could reveal differences created by feeding scallops, *Argopecten purpuratus*, mono-specific algal diets composed of either *Chaetoceros calcitrans* or *Isochrysis galbana* (variety *T. iso*) hereafter *T. iso*. Before and after feeding scallops with these diets, we assessed force production in vivo to evaluate escape response performance (initial and repeat). We measured oxidative capacities of mitochondria isolated from the adductor muscle and from the male and female portions of the gonad. Initial escape response performance was reduced more by the *C. calcitrans* diet than by the *T. iso* diet. Repeat escape responses, which require aerobic recuperation, were reduced by both treatments. The oxidative capacity of mitochondria isolated from muscle and female gonad was markedly lower in scallops fed *C. calcitrans* than in those fed *T. iso*. Flux through complex I–IV and through complex IV was also lower in mitochondria from muscle of scallops fed *C. calcitrans* than in those fed *T. iso*. Muscle aerobic capacity, assessed by citrate synthase activity, was lower in scallops fed *C. calcitrans* than in those fed *T. iso*. Despite the marked differences in fatty acid (FA) composition of the algal diets, the FA composition of mitochondrial phospholipids differed little between scallops fed *C. calcitrans* and those fed *T. iso*. Both escape response behaviour and mitochondrial capacities changed with feeding mono-specific diets. The simplicity of measurements of scallop escape responses suggests this as a practical means of evaluating the status of scallops for the two monoalgal diets tested.

Key words: Escape response / Contractile properties / Mitochondrial capacities / Adductor muscle / Gonad / Fatty acid / Algal diet / Pectinidae

1 Introduction

With the decline of wild stocks of most traditional marine food sources, aquaculture is of increasing importance. Marine bivalves are particularly interesting for sustainable aquaculture as they filter environmental phytoplankton and do not require costly feedstuffs. Nonetheless, broodstock conditioning and hatchery rearing of progeny are often required to obtain sufficient numbers of spat for culture. Cultured phytoplankton is generally used during conditioning of bivalves (Martínez et al. 2000; Farías and Uriate 2006).

Trial and error has shown that not all microalgal species lead to good gonadal production and gamete performance in bivalves (Enright et al. 1986). Algal shape, size, toxicity, digestibility and biochemical composition affect their nutritional value (Webb and Chu 1983; Brown et al. 1997). The microalgae used in mariculture differ in their contents of protein, carbohydrate and lipid (Brown 1991). While the amino-acid compositions of microalgal proteins are similar, the sugar composition of their polysaccharides can differ considerably (Whyte 1987). The contents of vitamins, for example riboflavin, a key component of mitochondrial flavoproteins, or ascorbate, a major antioxidant, vary markedly among phytoplankton species and between growth phases within a species (Brown and Miller 1992; Brown et al. 1997; Brown 2002). Deficiencies in vitamin intake could reduce metabolic capacities of bivalves. Among lipids, the polyunsaturated fatty acids (PUFA) with 20 and 22 carbons and more than 3 double bonds are sparingly, or not at all, synthesised by bivalves and must be obtained from the diet (De Moreno et al. 1976; Chu and Webb 1984; Soudant et al. 1998). Species of phytoplankton frequently used for scallop culture differ considerably in their fatty acid and 20-22 PUFA composition (Chu and Webb 1984; Volkman et al. 1989). As the 20- or 22-carbon PUFA, notably 22:6*n*-3, 20:5*n*-3 and 20:4*n*-6, are of particular importance in membranes, compositional differences of

[a] Corresponding author: Helga.guderley@bio.ulaval.ca

dietary phytoplankton could modify membrane FA composition and change membrane functions (Delaporte et al. 2003). Given the compositional variety of phytoplankton species, the dietary needs of bivalves are best met by mixtures of phytoplankton (notably diatoms + flagellates) (Farías and Uriarte 2006).

When the desired endpoint of bivalve conditioning is high gamete quality and offspring survival, establishing optimal diets becomes a lengthy and complex process (Martínez et al. 2000). The composition of ovocytes and spermatozoids in scallops reflects that of the food on which the broodstock are conditioned (Soudant et al. 1996), indicating that dietary quality can influence tissue composition and may modify function. We reasoned that simple bioassays that evaluate physiological performance could facilitate the establishment of optimal diets during hatchery conditioning. For scallops, two fairly easily characterised levels of performance, which could be affected by diet, are the escape response and the oxidative capacities of isolated mitochondria.

The escape response of scallops is a dramatic behaviour requiring the coordinated response of the neuromuscular system (Wilkens 1991). During escapes, rapid contractions of the phasic adductor muscle expel water from the cavity between the valves. As escapes occur in response to the most dangerous predators (Legault and Himmelman 1993; Himmelman et al. 2009), their contribution to overall fitness would seem considerable. Escape response performance is sensitive to the physiological status of scallops. On the short term, cold stress, air exposure, handling stress and previous swimming activity can reduce the intensity of escape responses (Brokordt et al. 2000a,b; Lafrance et al. 2002; Guderley et al. 2008a). On the longer term, reproductive investment and seasonal changes can decrease performance, particularly during a second response to the predator (Brokordt et al. 2000a,b; Brokordt et al. 2006; Kraffe et al. 2008; Guderley et al. 2008a). The sensitivity of the scallop escape responses to physiological status suggests that food quality could modify this performance.

Mitochondrial capacities could be modified by biochemical differences between dietary phytoplankton or by endocrine regulation in response to nutritional status (Fuller and Randle 1984). Dietary composition could modify the FA composition of mitochondrial phospholipids and change mitochondrial performance, as shown for rainbow trout (Guderley et al. 2008b). The specific interactions between membrane phospholipids and membrane proteins (Philipps et al. 2009), such as between cardiolipins and certain complexes of the mitochondrial respiratory chain (Zhang et al. 2002), suggest that flux through the respiratory chain may be particularly sensitive to dietary modifications. If escape response performance or mitochondrial properties prove sensitive to dietary quality, assays at these levels could help optimise diets for broodstock conditioning.

To examine these questions, we characterised the escape response performance and recuperation of scallops, *Argopecten purpuratus*, before and after feeding with monospecific algal diets (*I. galbana* variety *T. iso* and *Chaetoceros calcitrans*, respectively). Force production during escape responses was used to quantify phasic and tonic contractile force and number, as well as the rate and timing of phasic and tonic contractions (Fleury et al. 2005). Repeat performance was used to assess recuperation from exhaustive escapes. In parallel, we compared oxidative capacities and flux through the respiratory chain complexes of mitochondria isolated from the phasic adductor muscle, male and female gonad of scallops fed these mono-specific algal diets. We also examined the impact of these diets upon tissue aerobic capacity, by measuring the activity of the mitochondrial enzyme, citrate synthase, in muscle, male and female gonad.

2 Materials and methods

2.1 Animals and holding conditions

Adult *Argopecten purpuratus* (72–84 mm shell height) were obtained from scallop growers at Tongoy Bay (30° 16′S, 71° 35′W) and transferred to the Centro de Cultivos de la Universidad Católica del Norte, Coquimbo Chile. Scallops (60) of intermediate gonadal maturity (gonads that were not as brightly coloured as when fully mature) were used. Scallops were held at ambient water temperatures (~19 °C) in flow through tanks supplied with natural, partly filtered (50 μm) seawater for approximately one week before initial characterisation of the force production during escape responses (see below). After these force recordings, the scallops were marked and returned to the tanks for one week before beginning dietary conditioning.

Dietary conditioning occurred in two tanks per diet. Scallops were haphazardly assigned to a dietary treatment. Fourteen scallops were placed in each tank (4 tanks of 200 L each); each tank held 8 marked scallops for which we had taken escape response data and 6 additional scallops. Scallops were fed either *Isochrysis galbana* (variety *T. iso*) or *Chaetoceros calcitrans*. Algae in exponential growth phase were obtained from the culture facility at a concentration of 5–6 $\times$ 10^6 cells ml^{-1}. The scallops were supplied with algae to provide approximately 6% the animals' dry mass per day, by continuous dripping, during 6–8 h per day. The tanks were continuously aerated during feeding, but flow through the tanks was stopped during feeding. Once algal feeding was finished, circulation of the partly filtered seawater was started. We completely changed the water in the tanks every second day. Dietary conditioning started in late January 2007 and continued until final escape response tests in early April 2007 (approximately 8 weeks). This duration was chosen to insure assimilation of the diets but to prevent marked growth or physiological change. In late March 2007, when the mitochondrial characterisation was done, feeding had lasted 7–8 weeks.

2.2 Force recordings during escape responses

For these tests, scallops were gently removed from the holding tank and carried in buckets full of seawater to the test apparatus. Each scallop was tested for 10 min, given 15 min of recuperation in seawater and then tested again for 10 min. This provided information on the escape response capacities as well as on the capacity to recuperate after exhausting exercise. Temperature during force recordings was ~19 °C. The same

starfish was used for the two escape tests carried out for a scallop on a given day. A given starfish was used at most for tests with 4 scallops. The water in the test basin was changed between scallops. Behavioral results used only scallops for which an initial (pre-treatment) and a final (post-feeding) escape response tests were measured.

Force production during escape responses was measured according to Guderley et al. (2008a). A force gauge (Quantrol by Mecmesin) assessed force production through the sensor placed under the upper valve of the scallop. The bottom valve was attached to the bottom of the test basin by a clamp maintained in position with a suction cup. The valves were separated by the distance the scallops separated their valves during routine ventilation (1 cm) using the test stand (Mecmesin). The scallop's upper valve was free to move above the sensor. The scallops were stimulated to contract by contact with an arm of their starfish predator, *Myenaster gelatinosus*. The operator gently and constantly stroked the scallop's mantle with the starfish, even during tonic contractions. Scallops were marked and returned to the holding tanks after the force measurements either to start their feeding treatment (initial tests) or to recuperate from the final test before dissection and measurement of anatomic properties.

Data output from the force gauge was transmitted to a PC through a RS232 cable. Dataplot software (Mecmesin) was used to acquire the force data before their transfer to MS-Excel for analysis.

2.3 Mitochondrial preparation

Preliminary experiments validating isolation and assay methods used scallops that had recently arrived from Tongoy Bay and were based on two to three preparations (each using tissues pooled from 3 scallops). Mitochondria were isolated from muscle, male and female gonad (separated by dissection) following Guderley et al. (1995). The extraction medium contained 480 mM sucrose, 30 mM Hepes, 230 mM KCl, 3 mM EDTA (ethylene dinitrilotetraacetic acid), 6 mM EGTA (ethyleneglycol tetra-acetic acid), 5 mM $MgCl_2$, 0.1% bovine serum albumin (BSA), pH 7.0. Tissues were chopped and then homogenised (1:10 (m/v) for muscle and 1:6 for the gonadal portions) in the extraction buffer using three passes of a motorised Potter-Elvehjem tissue grinder. We increased the dilution of the muscle extracts because, if they were too concentrated, the supernatants became gelatinous making it impossible to obtain mitochondrial pellets. An initial centrifugation (900 × g) for 10 min was followed by a centrifugation of the first supernatant at 10 000 × g for 10 min. This "mitochondrial" pellet was re-centrifuged in the reaction medium (480 mM sucrose, 70 mM Hepes, 158 mM KCl, 10 mM KH_2PO_4, 50 mM taurine, 50 mM β-alanine, pH 7.0, 0.5% BSA) at 10 000 × g for 10 min. The final pellet was resuspended in a minimal volume of reaction medium. Coupled mitochondria could be isolated from the male and female gonad using this procedure, with the only change in methodology being that the levels of BSA were increased to 0.5% for the female gonad. For scallops fed the mono-specific diets, we characterised 5 mitochondrial preparations from each tissue, using material pooled from 3 scallops. It was necessary to pool the scallops to obtain sufficient mitochondrial preparation for the desired assays, reducing the number of scallops for which final escape response data could be obtained.

2.4 Mitochondrial oxidative capacities and flux through electron transport chain complexes

For mitochondria from muscle, male and female gonads, 1 mM pyruvate (with sparking levels of malate, 0.5 mM) was oxidised at equal or higher rates than 24 mM succinate or 30 mM glutamate (data not shown). Thus, comparisons of maximal mitochondrial capacities used malate + pyruvate. Maximal rates (State 3) of oxygen uptake were obtained after stimulation with 0.5 mM ADP whereas state 4 rates were obtained after this ADP was depleted.

We estimated flux through the electron transport system (ETS) complexes following the approach outlined by Muleme et al. (2006), whereby maximal flux through the complexes is measured using specific substrates and inhibitors. Flux through complexes I–IV was assessed at 1 mM pyruvate and 0.5 mM malate in the presence of 1 mM ADP, with oxygen uptake remaining after inhibition by rotenone (2 μg ml^{-1}) being subtracted from the initial rate. After inhibition with rotenone and antimycin (5 μg ml^{-1}; dissolved in ethanol), ascorbate (8 mM) and N,N,N,N,-tetramethyl-p-phenylenediamine (TMPD; 0.8 mM dissolved in ethanol) were added to measure flux through complex IV. The autooxidation of ascorbate and TMPD was subtracted from flux estimates for complex IV.

Mitochondrial oxygen uptake was evaluated using optodes (PreSens) and the Microx program provided for their control. The implantable optodes were placed in plastic sheaths and inserted into 1 cc plastic syringes that in turn were placed in the Plexiglas holder for YSI polarographic electrodes. YSI water-jacketed chambers and stirring bars were used for oxygen uptake measurements. The minimal volume for use with this setup was 2.5 ml. Manual calibration of the optodes used sodium sulphite to eliminate oxygen in the reaction medium and set 100% saturation at the saturation obtained after stirring the reaction medium equilibrated at the experimental temperature (19 °C) for 5 min. Oxygen contents were calculated using the atmospheric pressure at the start of measurements, the experimental temperature and the solubility coefficients provided by Graham (1987) for dextran-containing media. Data were transferred from the ASCII files generated by the Microx program to MS-Excel to generate graphs from which rates were determined.

For protein analysis, samples of the mitochondrial preparation (50 μl) were centrifuged (13 000 × g for 10 min) to sediment the mitochondria and were re-suspended in reaction buffer without BSA and centrifuged two additional times. The pellet was frozen (–20 °C) in 50 μl distilled water for subsequent analysis of protein contents following Lowry et al. (1951). Approximately 50 μl mitochondrial preparation was frozen for subsequent analysis of lipid composition.

2.5 Citrate synthase activity

Samples of phasic adductor muscle, female and male gonads were homogenised on ice, using a homogenizer (Scilent cruncher M, Heidolph Instrument GMBH & Co. KG, Germany) in ten volumes of 50 mM imidazole-HCl, 2 mM EDTA-Na_2, 5 mM EGTA, 1 mM dithiothreitol, 0.1% Triton X-100, pH 7.2. The homogenates were centrifuged at 600 × *g* at 4 °C for 15 min.

Assay temperature was controlled at 16 °C with a circulating refrigerating water bath (Haake D8). Citrate synthase (EC 4.1.3.7) activity was measured using a UV/Vis spectrophotometer (Varian Cary 50Bio) to follow the absorbance changes due to the transfer of sulphydryl groups from CoASH to 5,5′dithio-bis(2-nitro)benzoic acid (DTNB) at 412 nm. The molar extinction coefficient for DTNB was 13.6 L mol^{-1} cm^{-1}. Assays were run in duplicate and the specific activities were expressed in international units (μmol of substrate converted to product min^{-1}) g^{-1} wet mass. Conditions for citrate synthase assay were (all concentrations in mM): TRIS-HCl 75, oxaloacetate 0.3 (omitted for the control), DTNB 0.1, acetyl CoA 0.2, pH 8.0.

2.6 Fatty acid composition of mitochondrial phospholipids

The membrane lipids of mitochondrial suspensions, prepared as indicated above, were extracted by resuspending mitochondrial pellets in 4 ml of a chloroform/methanol mixture (2:1, vol/vol). To ensure a complete lipid extraction, vials were sonicated 10 min at 4 °C. The final extract was stored at –80 °C under nitrogen after 0.01% w/v butylated hydroxytoluene (BHT, antioxidant) was added. Algal suspensions were centrifuged and the pellet was extracted using the same method.

An aliquot of the lipid extracts was evaporated to dryness and lipids were recovered with three washings of 500 μl of $CHCl_3$/MeOH (98/2, v/v) and deposited at the top of a silica gel micro-column (30 × 5 mm i.d., packed with Kieselgel 60 70–230 mesh previously heated at 450 °C and deactivated with 6 wt% H_2O) (Marty et al. 1992). Neutral lipids were eluted with 10 ml of $CHCl_3$/MeOH (98/2 v/v). The polar lipid fraction was recovered with 20 ml of MeOH and stored at –20 °C before FA composition analysis by gas chromatography.

The polar lipid fraction was used for the direct determination of the total mitochondrial glycerophospholipid FA composition after transesterification (MeOH/boron trifluoride, 10 min at 100 °C). Fatty acid methyl esters (FAME) obtained were identified and quantified by gas chromatography using C23:0 as an internal standard. FAME were analysed in a Varian CP 8400 gas chromatograph equipped with an on-column injector and a flame ionisation detector. FA were identified by comparing their retention times using both polar (CPWAX 52 CB – 30 m × 0.25 mm, 0.25 μm film thickness) and non-polar (CP-Sil 8 CB – 30 m × 0.25 mm, 0.25 μm film thickness) capillary columns by means of a standard mixture containing 37 FAME (SUPELCO/Sigma-Aldrich, St-Quentin Fallavier, France), and other known standard mixtures from pectinids (Kraffe et al. 2010).

2.7 Biochemicals

All products were obtained from Sigma Chemicals (SIGAL, Chile). BHT and boron trifluoride (14% by weight in methanol) were obtained from Sigma-Aldrich. Silica gel 60 (63–230 μm mesh) was purchased from Merck.

2.8 Statistics

Mitochondrial rates were determined using the curve fitting functions in MS-Excel. Mitochondrial capacities were compared between treatment groups using *t*-tests. The change in escape response behaviour due to the feeding treatment was examined using paired *t*-tests, comparing the pre- and post-feeding behaviours. The post-feeding escape response behaviour of the dietary groups, the mitochondrial capacities and the FA composition of mitochondrial phospholipids were compared with *t*-tests. Percentage data for FA composition were transformed (arcsin of the square root) before *t*-tests, but are presented in table as untransformed percentage values.

3 Results

3.1 Morphological characteristics of scallops

The scallops fed the two mono-specific diets during 7–8 weeks, did not differ in their shell size (73 mm height, 77 mm width, 25 mm depth) or in the wet mass of soft tissues ($p > 0.05$). This was true both for the scallops used for mitochondrial characterisation ($n = 15$ for each diet) and for those used to compare initial and final escape response performance ($n = 13$ fed *T. iso*; and $n = 11$ fed *C. calcitrans*). Nonetheless, the mass of the female gonad was slightly greater in scallops fed *T. iso* than in those fed *C. calcitrans* (1.97 ± 0.79 vs. 1.56 ± 0.32, $p = 0.07$). No mortalities occurred during the dietary conditioning. One scallop in the *C. calcitrans* group had an infection by *Polydora* which markedly reduced muscle mass, its data were removed from the analysis.

3.2 Escape response performance

The force traces indicated the number and force of phasic and tonic contractions, the minimum interval between phasic contractions, the proportion of time spent in tonic contractions and the duration of responsiveness. When scallops have a strong escape response, they show many phasic contractions, high contractile force, a small minimum interval between phasic contractions, many tonic contractions (as these occur between phasic contractions), a low % time spent in tonic contractions and a long response time. Weakened scallops make few phasic contractions, start their tonic contractions early during the escape tests and as they have a high % time spent in tonic contractions, make few tonic contractions. Fatigued and weakened scallops may fail to respond to the predator. The initial and repeat escape response performance of each scallop fed a given diet (post-feeding) was compared to its performance shortly after arrival from Tongoy Bay (pre-treatment).

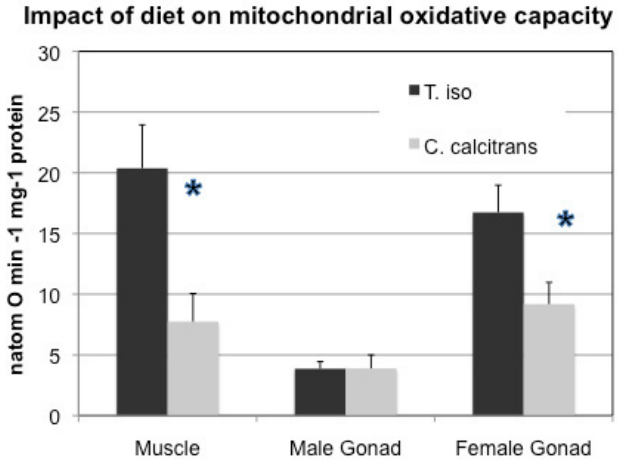

Fig. 1. Maximal (state 3) rates of pyruvate oxidation by mitochondria isolated from muscle, male and female gonad of *Argopecten purpuratus* after 7–8 weeks of feeding with *Isochrysis galbana* (*T. iso*) or with *Chaetoceros calcitrans*. Values are shown as X ± SE (n = 5 pools of tissues from 3 animals). Mitochondria from muscle and female gonad of scallops fed *T. iso* had higher capacities than those from scallops fed *C. calcitrans* ($p < 0.05$).

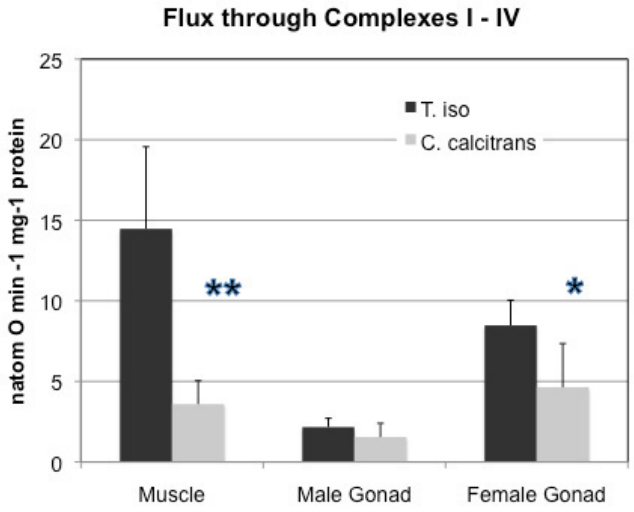

Fig. 2. Impact of diet on flux from complex I to complex IV in mitochondria isolated from muscle, male and female gonad of *Argopecten purpuratus* after 7–8 weeks of feeding with *Isochrysis galbana* (*T. iso*) or with *Chaetoceros calcitrans*. Mitochondria from muscle and female gonad of scallops fed *T. iso* had higher rates of flux than those from scallops fed *C. calcitrans* ($p < 0.01$ and 0.05 respectively).

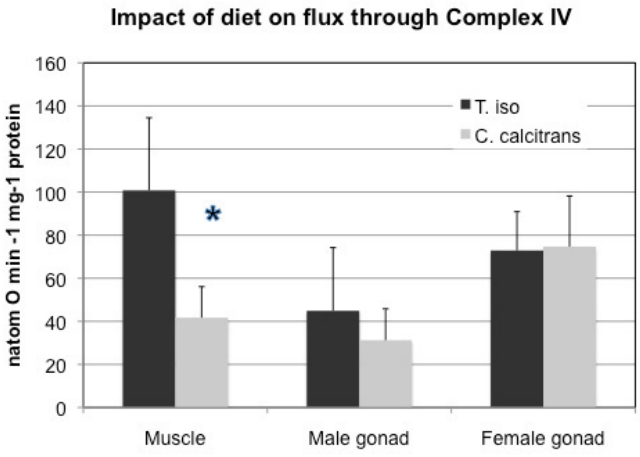

Fig. 3. Flux through complex IV in mitochondria isolated from muscle, male and female gonad of *Argopecten purpuratus* after 7–8 weeks of feeding with *Isochrysis galbana* (*T. iso*) or with *Chaetoceros calcitrans*. Mitochondria from muscle of scallops fed *T. iso* had higher capacities than those from scallops fed *C. calcitrans* ($p < 0.05$).

Feeding *A. purpuratus* mono-specific algal diets only slightly modified their initial escape responses (Table 1). The behavioural changes differed with diet. Scallops fed *C. calcitrans* decreased the number of phasic contractions in their initial attempts to escape their predator by 25%, increased the overall duration of tonic contractions and shortened the response time (Table 1). Feeding scallops *T. iso* increased the use of tonic contractions in the first 60 s of stimulation as well as the overall duration of tonic contractions. Neither diet changed phasic or tonic force production. Repeat performance was markedly weakened by feeding scallops with mono-specific algal diets. Of the scallops fed *C. calcitrans*, only 64% (7/11) responded with more than one clap during repeat tests whereas before the dietary treatment, all had responded during repeat tests. Of the scallops fed *T. iso*, 85% (11/13) responded with more than one clap in the repeat tests. For both dietary groups, the number of phasic contractions and the response time were reduced compared to pre-feeding levels (Table 2). Phasic and tonic force production was unchanged. Scallops fed *T. iso* increased the time spent in tonic contractions. Although the significant differences formed a consistent pattern, the differences were not pronounced (p values between 0.01 and 0.05), due in part to the fairly small sample tested. The % recuperation of initial performance during the post-feeding tests did not differ between the dietary groups (data not shown).

3.3 Mitochondrial oxidative capacities

Mitochondria isolated from the male and female gonad portions of scallops from both dietary treatments, had respiratory control ratios (RCR, state 3/state 4) greater than 3. The time required to transfer from state 3 to state 4 was considerably shorter for mitochondria from female than male gonad, with those from female gonad taking 1–2 min and those from male gonad taking from 3–8 min. For scallops fed *T. iso*, muscle mitochondria had RCR values above 4 whereas those from scallops fed *C. calcitrans* were rarely coupled (i.e. RCR well below 3). Muscle mitochondria from scallops fed *T. iso* made the transition from states 3 to 4 in 2–4 min.

The maximal rates of pyruvate oxidation (state 3) by mitochondria isolated from muscle, male gonad and female gonad differed markedly between scallops fed the two dietary treatments (Fig. 1). For muscle and female gonad, mitochondria isolated from scallops fed *T. iso* had approximately twice the

Table 1. The impact of feeding with monospecific algal diets on the initial response of *Argopecten purpuratus* to 10 min of contact with the starfish, *Myenaster gelatinosus*. The performance of each scallop was measured shortly after arrival from Tongoy Bay (pre-treatment) and after 8 weeks of laboratory feeding (post-feeding). Values are shown as X ± SD (n = 11 and 12 for scallops fed *C. calcitrans* and *T. iso*, respectively). Results shown are only for scallops for which a pre-treatment and a post-feeding escape response were measured. For one of the scallops fed *T. iso*, the initial recording was lost, decreasing the number to 12. Parameters that were significantly affected by the feeding treatment were identified by paired t-tests (* $p < 0.05$).

INITIAL ESCAPE RESPONSE OF *ARGOPECTEN PURPURATUS*			
	Unit	Pre-treatment	Post-feeding
Scallops fed *Chaetoceros calcitrans* n = 11			
Total phasic contractions		32.9 ± 9.5*	25.4 ± 10.4
Phasic contractions, first minute		15.4 ± 7.6	14.6 ± 5.8
Total tonic contractions		12.9 ± 6.6	11.1 ± 5.7
% Tonic contraction, first minute		71 ± 25	87 ± 14
Tonic duration	s	527 ± 45*	565 ± 22
Maximal phasic force	N	10.6 ± 1.0	10.2 ± 0.7
Mean phasic force	N	7.5 ± 1.0	7.6 ± 0.6
$Force_{max}$, tonic	N	4.5 ± 1.0	4.5 ± 0.7
Response time	s	537 ± 86*	379 ± 188
Minimum time interval between phasic contractions	s	0.5 ± 0.4	1.7 ± 3.3
Scallops fed *Isochrysis galbana* (*T. iso*) n = 12			
Total phasic contractions		29.9 ± 8.7	25.2 ± 9.5
Phasic contractions, first minute		13.6 ± 5.5	14.7 ± 6.4
Total tonic contractions		8.5 ± 3.7	9.3 ± 5.1
% tonic, first minute		60 ± 26*	83 ± 13
Tonic duration	s	503 ± 63*	569 ± 24
$Force_{max}$, phasic	N	10.3 ± 1.6	10.5 ± 1.1
Mean phasic force	N	7.7 ± 1.5	7.9 ± 1.2
$Force_{max}$, tonic	N	4.6 ± 0.7	4.9 ± 1.3
Response time	s	440 ± 177	332 ± 189
Minimum time interval between phasic contractions	s	0.6 ± 0.6	0.7 ± 0.6

protein specific capacities than those from scallops fed *C. calcitrans*. The mitochondria isolated from the male gonad did not differ between scallops fed *T. iso* and *C. calcitrans*. Non-phosphorylating rates (state 4) did not differ with dietary treatment (not shown).

3.4 Flux through respiratory chain complexes

Flux through complexes I–IV in mitochondria isolated from muscle and female gonad was lower for scallops fed *C. calcitrans* than for those fed *T. iso* (Fig. 2). Muscle mitochondria showed an impact of diet on flux through complex IV, with flux again being lower in mitochondria from scallops fed *C. calcitrans* (Fig. 3). Flux through complex IV was approximately 5 fold state 3 rates both in scallops fed *T. iso* and *C. calcitrans*. In mitochondria from female gonad of scallops fed *T. iso*, flux through complex IV was also 5 fold state 3 rates, while in those fed *C. calcitrans*, it was approximately 10 fold. In mitochondria from male gonad, flux through complex IV was 10 fold state 3 rates for both diets.

3.5 Fatty acid composition of mitochondrial phospholipids

Our analyses confirmed that fatty acid compositions were specific to the microalgae (Table 3). FA in *C. calcitrans* were dominated by 14:0, 16:0, 16:1*n*-7, 16:3*n*-4 and 20:5n-3 while the main FA in *T. iso* were 14:0, 16:0, 18:1*n*-9, 18:2*n*-6, 18:3*n*-3, 18:4*n*-3 and 22:6*n*-3. Despite these differences in FA composition, 7–8 weeks of feeding with these diets had minimal effects on the FA composition of mitochondrial phospholipids from muscle, male or female gonad (Table 4). A slight imprint of diet was only apparent in muscle and male gonads, affecting the monounsaturated FA (MUFA) 16:1*n*-7 and 18:1*n*-9, and the 18 carbon polyunsaturated FA (PUFA) 18:3*n*-3 and 18:4*n*-3.

The 20 carbon MUFA, which were absent or present only in trace amounts in the diets, were clearly apparent in the mitochondrial phospholipids of muscle, female gonad and male gonad. In particular, 20:ln-7 attained 5% in mitochondria isolated from male gonads. For PUFA, the same observation can be made for 22:4(*n*-9)*Δ*13*trans* and 22:4*n*-6, 22:5*n*-6, 22:5*n*-3 for scallops fed C. *calcitrans*.

3.6 Tissue oxidative capacity

To assess whether the overall tissue aerobic capacity followed similar patterns as mitochondrial capacities, we measured the mass specific activities of citrate synthase in muscle, male gonad and female gonad. Scallops fed *T. iso* had higher activities of citrate synthase in muscle (1.8 ± 0.3 U g^{-1} wet mass) than those fed *C. calcitrans* (1.5 ± 0.2 U g^{-1}). No dietary effects were apparent in male or female gonad. Scallops fed *C. calcitrans* tended to have a lower activity in male gonad compared to those fed *T. iso* (34.7 ± 0.3 and 40.0 ± 5.6 U g^{-1},

Table 2. Impact of monospecific algal diets on repeat escape responses of *Argopecten purpuratus*. Scallops were exposed to a second period of 10 min of contact with their starfish predator after 15 min of recuperation from their first test. The performance of each scallop was measured shortly after arrival from Tongoy Bay and after 8 weeks of laboratory feeding. Values are shown as X ± SD (n = 11 and 13 for scallops fed *C. calcitrans* and *T. iso*, respectively). Results shown are only for scallops for which a pre-treatment and a post-feeding escape response were measured. Parameters that were significantly affected by the feeding treatment were identified by paired t-tests (* $p < 0.05$).

REPEAT ESCAPE RESPONSES OF *ARGOPECTEN PURPURATUS*			
	Unit	Pre-treatment	Post feeding
Scallops fed *Chaetoceros calcitrans* n = 11			
Total phasic contractions		17.4 ± 10.0*	10.2 ± 10.4
Phasic contractions, first minute		6.8 ± 2.9*	4.6 ± 4.4
Total number of tonic contractions		7.0 ± 5.7	4.6 ± 5.5
% Tonic, first minute		83 ± 24	96 ± 7
Tonic duration	s	567 ± 47	534 ± 159
Maximal phasic force	N	10.6 ± 1.2	9.7 ± 1.6
Mean phasic force	N	8.0 ± 1.8	6.1 ± 4.1
Maximal tonic force	N	4.3 ± 1.3	4.2 ± 1.6
Response time	s	477 ± 213*	217 ± 277
Minimum time interval between phasic contractions	s	1.9 ± 2.2	0.6 ± 0.3
# individuals responding		11/11	8/11
Scallops fed *Isochrysis galbana (T. iso)* n = 13			
Total phasic contractions		16.9 ± 10.8*	9.0 ± 8.0
Phasic contractions, first minute		7.1 ± 5.0*	3.5 ± 3.4
Total number of tonic contractions		6.2 ± 4.1*	2.0 ± 1.5
% tonic, first minute		84 ± 23*	97 ± 7
Tonic duration	s	557 ± 61*	596 ± 7
Maximal phasic force	N	10.22 ± 0.9	9.7 ± 1.1
Mean phasic force	N	8.0 ± 1.3	7.6 ± 2.6
Maximal tonic force	N	4.9 ± 1.2	4.8 ± 1.3
Response time	s	492 ± 129*	330 ± 236
Minimum time interval between phasic contractions	s	0.8 ± 0.6	3.0 ± 7.1
# individuals responding		13/13	12/13

respectively), but differences were not significant. In the female gonad, these activities were 3.9 ± 0.3 and 3.9 ± 0.8 U g^{-1}, respectively.

4 Discussion

Feeding *Argopecten purpuratus* mono-specific algal diets modified performance at all levels we examined, ranging from initial and repeat escape response performance to mitochondrial oxidative capacities, to flux through respiratory chain complexes and to tissue activities of citrate synthase. Although scallops fed *I. galbana*, variety *T. iso* fared better than those fed *C. calcitrans*, scallops fed either mono-specific diet had diminished escape responses and repeat performance relative to recently harvested scallops. Mitochondrial oxidative capacities were higher in scallops fed *T. iso* than in those fed *C. calcitrans*, with the latter having reduced mitochondrial oxidative capacities in muscle and female gonad and lower muscle activities of citrate synthase. As the FA composition of mitochondrial phospholipids was not markedly changed by diet, presumably these changes in mitochondrial capacity are caused by other dietary deficiencies or regulatory modifications. While reduced repeat performance was found for scallops held in the lab and fed monospecific diets, the reduced responsiveness of scallops fed *C. calcitrans* may reflect the decreased muscle oxidative capacity compared to scallops fed *T. iso*.

Feeding *A. purpuratus* either *C. calcitrans* or *T. iso* did not reduce escape performance by impeding neuromuscular communication, as the mono-specific diets did not change the response during the first minute of contact with the predator. A strong initial response (phasic contractions in the first minute) requires rapid integration of sensory information to activate the adductor muscle. The force with which scallops reacted to contact with the predator was also unchanged by feeding with mono-specific diets. That maximal and mean phasic and tonic forces were unchanged by diet indicates that fibre recruitment was not modified by dietary quality under laboratory holding conditions. Nonetheless, feeding with *C. calcitrans* reduced the number of phasic contractions in the initial response. The number of phasic contractions made by a scallop during an escape response is primarily a function of phosphoarginine and ATP levels at the start of the escape response (Bailey et al. 2003). Our results suggest that feeding *A. purpuratus* with *C. calcitrans* reduced resting levels of arginine phosphate in the adductor muscle, thereby reducing the number of phasic contractions during the initial escape response.

After feeding on monospecific diets, *A. purpuratus* increased their use of tonic contractions during both initial and repeat escape tests. Tonic contractions have varied roles, ranging from maintaining a constant valve opening during routine ventilation, to closing the valves in attempts to avoid predation, to providing a respite for a fatigued phasic adductor muscle. While, logically, a high number of phasic contractions

Table 3. Fatty acid composition in microalgae expressed as mol% (mean of three samples of each algal suspension). The FA for which the levels contrasted markedly between the microalgae are shown in bold.

FATTY ACIDS	TOTAL LIPIDS	
	C. calcitrans	*T. iso*
14:0	10.1	13.8
16:0	10.6	13.6
18:0	1.2	0.4
16:1n-7	33.4	6.3
18:1n-9	0.6	15.2
18:1n-7	0.6	1.4
20:1n-11	0.0	0.0
20:1n-9	0.0	0.0
20:1n-7	0.0	0.0
16:2n-7	4.7	0.0
16:2n-4	5.4	0.0
16:3n-4	14.3	0.0
18:2n-6	0.5	7.1
18:3n-3	0.1	9.5
18:4n-3	0.4	15.6
20:4n-6	0.6	0.2
20:5n-3	11.1	0.8
22:4(n9)Δ13*trans*	0.0	0.0
22:4n-6	0.0	0.1
22:5n-6	0.0	1.7
22:5n-3	0.0	0.2
22:6n-3	1.4	11.2
Total SFA	23.1	28.3
Total MUFA	36.4	23.4
Total PUFA	39.5	47.6
Total n-4	19.7	0.1
Total n-6	1.5	10.1
Total n-3	13.5	37.4
n-3/n-6	9.0	3.7
22:6n-3/20:5n-3	0.1	14.5

should decrease the time spent in tonic contractions, the rapidity of phasic contractions can make it difficult to detect this inverse correlation. Effectively, the time spent in tonic contractions during the initial escape response increased in scallops fed *T. iso*, even though the number of phasic contractions did not change. In *Placopecten magellanicus*, tonic contractions allow partial metabolic recuperation of the phasic adductor muscle and are initiated only after the energetic status of the phasic adductor has declined (Pérez et al. 2008). This metabolic recuperation increases the adenylate energy charge by reducing AMP levels, but leaves arginine phosphate levels unchanged. Tonic contractions may therefore prolong the period over which the phasic muscle can respond, without actually increasing the number of phasic contractions. The use of tonic contractions during the escape response increased more in scallops fed *T. iso* than in those fed *C. calcitrans*. This may have helped the scallops fed *T. iso* to maintain the numbers of phasic contractions during their initial escape response. Possibly, scallops fed *T. iso* are better able to sense the energetic status of the phasic adductor muscle and to initiate tonic contractions at the appropriate times.

As the repeat escape response tests were carried out 15 min after the initial test, they provide an indication of the scallops' capacity for aerobic recuperation. Oxygen uptake increases markedly after exhaustion in *P. magellanicus*, *Chlamys deliculata*, and *Chlamys islandica* and takes considerable time to return to basal levels (Thompson et al. 1980; Mackay and Shumway 1980; Tremblay et al. 2006; Kraffe et al. 2008). We assume that maximal rates of oxygen uptake occur directly after exhaustive exercise in *A. purpuratus* as in other scallops. After 15 min of recuperation *A. purpuratus* were able to mount fairly intense escape responses, but both dietary groups reduced the use of phasic contractions and shortened the response time during repeat tests, suggesting a loss of aerobic capacity.

The sensitivity of behavioural parameters to dietary treatment increased as a function of their dependence on aerobic processes. The initial escape response was least affected by dietary treatment, with scallops fed *C. calcitrans* showing a reduction in the total number of phasic contractions and those fed *T. iso* relying more on tonic contractions. The initial escape response is largely powered by arginine phosphate, with anaerobic glycolysis contributing towards the end of the escape response (Thompson et al. 1980; Chih and Ellington 1986; Bailey et al. 2003). The weakened repeat performance of the experimental scallops may be due to modifications of muscle mitochondria. Oxygen uptake after an escape response must involve muscle mitochondria, as oxidative phosphorylation is needed to return metabolic conditions to resting levels. Muscle mitochondria from scallops fed *C. calcitrans* had lower oxidative capacities, lower flux through complex I–IV and through complex IV and were not coupled, in contrast to muscle mitochondria from scallops fed *T. iso*. Feeding scallops *C. calcitrans* reduced muscle citrate synthase activity relative to that in scallops fed *T. iso*, suggesting that not only the capacity of individual mitochondria was modified, but also the overall number of mitochondria in muscle. Accordingly, fewer scallops fed with *C. calcitrans* responded during repeat tests than those fed *T. iso*.

As mitochondrial isolation is a terminal procedure, we could not compare the changes in mitochondrial capacities with the feeding treatment. However, an idea of the mitochondrial capacities of the scallops before the feeding treatment is provided by our preliminary experiments using scallops that had been freshly harvested from Tongoy Bay. The oxidative capacities of muscle mitochondria from these animals were similar to those for scallops fed *T. iso* (16 ± 3 natom O min^{-1} mg^{-1} protein, $n = 2$ pooled preparations). Mitochondria from male gonad had considerably higher capacities (20.5 natom $\pm$ 6 O min^{-1} mg^{-1} protein, $n = 3$) than those fed either algal diet while mitochondria from female gonad had two-fold the oxidative capacity of those fed *T. iso* (30 natom $\pm$ 6 O min^{-1} mg^{-1} protein, $n = 2$). Thus the deficiencies caused by mono-specific algal diets appeared to have their greatest impact on mitochondria from male and female gonad. Gamete quality would likely suffer as a consequence.

Table 4. Fatty acid composition of total phospholipids from mitochondria isolated from muscle, female gonad and male gonad of *Argopecten purpuratus* fed for 7–8 weeks on monospecific algal diets *Chaetoceros calcitrans* or *Isochrysis galbana* (*T. iso*). Results are expressed as mol%. Values are shown as X ± SD ($N = 3$ different mitochondrial preparations for each diet). FA proportions that were affected by the feeding treatment were identified by *t*-tests (* $p < 0.05$).

FATTY ACIDS	MUSCLE		FEMALE GONAD		MALE GONAD	
	C. calcitrans	*T. iso*	*C. calcitrans*	*T. iso*	*C. calcitrans*	*T. iso*
14:0	1.0 ± 0.2	1.4 ± 0.1	1.6 ± 0.2	1.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.1
16:0	17.8 ± 1.4	19.2 ± 1.0	12.8 ± 0.8	13.0 ± 0.9	20.6 ± 0.3	20.3 ± 1.0
18:0	8.1 ± 0.2	7.9 ± 0,1	10.9 ± 1.2	11.2 ± 1.6	10.4 ± 0.7	10.0 ± 0.3
16:1n-7	1.0 ± 0.1	0.4 ± 0.0*	2.6 ± 0.5	2.6 ± 0.4	1.2 ± 0.1	0.7 ± 0.1*
18:1n-9	0.7 ± 0.0	0.9 ± 0.0*	1.4 ± 0.3	1.5 ± 0.2	0.9 ± 0.0	1.1 ± 0.1*
18:1n-7	3.6 ± 0.3	3.4 ± 0.4	1.6 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.7 ± 0.1*
20:1n-11	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:1n-9	2.2 ± 0.1	2.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.4	2.0 ± 0.1	2.2 ± 0.1
20:1n-7	2.1 ± 0.2	2.1 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	5.1 ± 0.2	4.8 ± 0.1
18:3n-3	0.2 ± 0.0	0.4 ± 0.1*	0.6 ± 0.3	0.4 ± 0.3	0.2 ± 0.0	0.4 ± 0.1*
18:4n-3	0.4 ± 0.1	0.8 ± 0.2*	1.1 ± 0.4	1.1 ± 0.5	0.3 ± 0.1	0.7 ± 0.1*
20:4n-6	2.9 ± 0.2	2.4 ± 0.2*	4.0 ± 0.6	4.0 ± 0.8	2.5 ± 0.4	1.9 ± 0.1
20:5n-3	18.9 ± 0.5	19.0 ± 0.8	21.4 ± 1.3	21.2 ± 0.8	17.8 ± 2.0	17.6 ± 0.7
22:4(n-9)*Δ*13*trans*	3.1 ± 0.4	2.9 ± 0.4	4.4 ± 0.1	4.7 ± 0.4	2.4 ± 0.2	2.3 ± 0.4
22:4n-6	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
22:5n-6	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.5	1.7 ± 0.3	0.7 ± 0.1	1.1 ± 0.1*
22:5n-3	3.9 ± 0.4	3.9 ± 0.3	4.6 ± 0.3	4.4 ± 0.4	14.3 ± 1.8	14.7 ± 0.6
22:6n-3	23.6 ± 0.6	22.6 ± 1.0	20.9 ± 0.8	19.8 ± 0.9	11.9 ± 0.7	13.3 ± 1.0
Others [a]	7.5 ± 0.1	7.3 ± 0.1	5.6 ± 0.9	6.1 ± 0.3	5.7 ± 0.1	5.5 ± 0.5
Total SFA	28.3 ± 1.6	29.9 ± 1.1	27.0 ± 1.2	27.6 ± 1.0	32.9 ± 0.9	33.1 ± 1.0
Total MUFA	10.1 ± 0.4	9.7 ± 0.4	9.6 ± 0.3	9.5 ± 0.4	11.5 ± 0.4	10.7 ± 0.3*
Total PUFA	60.9 ± 1.4	59.8 ± 1.2	62.7 ± 1.5	61.9 ± 0.6	55.0 ± 1.1	56.6 ± 1.0

[a] Others: Total of 17 fatty acids detectable (iso15:0, ant15:0, iso17:0, 15:0, 17:0, 20:0, 22:0, 16:1n-9, 16:1n-5, 18:1n-5, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-3, 21:5n-3), none of which were more than 1.0%.

Scallops fed *T. iso* seem better able to maintain capacities of mitochondria from muscle and female gonad than those fed *C. calcitrans*.

As tissue masses and condition did not differ between dietary groups, caloric limitation does not seem to have caused the differences in performance. Phasic and tonic force production was not decreased by the dietary treatment under laboratory conditions, suggesting that myofibrillar content and muscle size were not changed. Rather, the data are compatible with the suggestion that dietary effects primarily modified mitochondrial capacities. Among the mechanisms implicated in such differences, we first hypothesised that differences in FA would have modified mitochondrial membrane composition and therefore changed the activities of membrane bound enzymes such as those involved in oxidative phosphorylation. Membrane lipids are primarily composed of phospholipids whose FA composition is regulated even when the FA composition of the diet is drastically changed. Despite the differences in lipid composition of *C. calcitrans* and *T. iso*, the FA composition of mitochondrial phospholipids was virtually identical in scallops fed these mono-specific diets. This suggests that adult *A. purpuratus* possess high homeostatic capabilities that minimise the effect of exogenous FA on phospholipid composition. Presumably, *A. purpuratus* can modulate the incorporation of FA even if the dietary supply is high, as observed for other bivalves (Pirini et al. 2007; Delaporte et al. 2003; Soudant et al. 1998). Even though we have not identified the exact mechanism(s) and factor(s) affecting mitochondrial capacities, differences between the phytoplankton in the content of other compounds such as vitamins or antioxidants could have led the functional capacities of mitochondria to differ.

Our results indicate that behavioural and mitochondrial assays can reveal differences in the status of scallops after an experimental treatment, such as the dietary trials reported here. Measurement of the scallop escape response is particularly practical as it can be easily repeated, allowing evaluation of changes in individual performance with a dietary treatment. However, the variability of the escape response indicates that greater numbers of individuals should be examined to strengthen the conclusions (Guderley et al. 2008a). The strong response of mitochondrial capacities to dietary treatment suggests a mechanistic basis for the behavioural changes. Mitochondrial assays, while less convenient than behavioural measurements in scallops, could be useful to evaluate physiological status of culture species, such as oysters or mussels, that lack strong behavioural responses. Future investigations feeding scallops with natural phytoplankton while holding

them in the laboratory could reveal whether the changes we observed were induced by diet or by laboratory holding.

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