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Growth of postlarval sea scallops, *Placopecten magellanicus*, on microalgal diets, with emphasis on the nutritional role of lipids and fatty acids

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Abstract

Culture of the sea scallop, *Placopecten magellanicus*, is constrained by a reliable supply of high-quality postlarvae, yet little is known about the diets and essential nutrients required to maximize growth and survival during these vulnerable stages. Therefore, post-settlement sea scallops were exposed to binary microalgal diets consisting of a flagellate: *Pavlova lutheri*, *Pavlova* sp. (Pav, CCMP 459) or *Tetraselmis striata* (Plat-P) and a diatom: *Chaetoceros muelleri*, *Thalassiosira weissflogii* or *Fragilaria familica* for 28–30 days. The combination Pav 459/*C. muelleri* provided a superior diet for sea scallop postlarvae, yielding a growth rate of up to 28 μm day^{-1}. However, when these algae were offered singly, a 32% (Pav 459) and 64% (*C. muelleri*) decrease in growth rates was observed, indicating that both species made a significant contribution to the success of the mixed diet. The two species are characterized by unique signatures of $n-6$ polyunsaturated fatty acids (PUFAs): *C. muelleri* has high levels of arachidonic acid (AA), and Pav 459 (in contrast to *P. lutheri*) has high levels of $n-6$ docosapentaenoic acid (DPA). DPA was selectively incorporated into tissues of scallops fed all binary diets except the one rich in DPA (Pav 459/*C. muelleri*). These results, coupled with a marked increase in $n-6/n-3$ ratios between the diet and tissues, provide evidence that $n-6$ PUFAs may play an important and previously underestimated role in scallop nutrition. The diet of *T. striata* and *C. muelleri*, which yielded the lowest growth rate (8.1 μm day^{-1}) contained dietary docosahexaenoic acid (DHA; 22:6$n-3$) concentrations 4.5–7× lower than any other binary treatment. Low DHA levels may thus explain the poor performance of this diet. Tissue protein and triacylglycerol concentrations reflected diet performance, with higher values generally associated with more successful diets. This work

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suggests that DHA, as well as the $n-6$ PUFAs AA and DPA, may be essential for optimizing growth of sea scallop postlarvae.

Keywords: Scallop growth; Microalgal diets; Lipids; Polyunsaturated fatty acids

1. Introduction

The giant scallop, *Placopecten magellanicus*, is one of the most important commercial scallop species accounting for more than 50% of scallop landings worldwide (Halvorson et al., 1995). There is also great potential for sea scallop culture (Dadswell and Parsons, 1991), but the industry is largely constrained by the availability and affordability of high-quality spat. Natural spatfalls are often limited and unreliable (Dadswell and Parsons, 1992), and hatchery raised spat are not only expensive, but often incur high and unexplained mortalities in larval and post-settlement stages. Observed mortalities may be a result of nutritional deficiencies, as little is known about the dietary needs of these developmental stages. Diets used in scallop hatcheries have generally been adopted from those used for other bivalves with more established culture practices (i.e., clams and oysters), yet the performance of algal diets is often highly species- and stage-specific. Previous work conducted on scallops (Pectinidae) has focused largely on larval (Whyte et al., 1989; Delaunay et al., 1993; Thompson et al., 1994; Soudant et al., 1998a; Nevejan et al., 2003a,b) and juvenile or adult stages (Pierson, 1983; Soudant et al., 1996; Parrish et al., 1999; Martinez et al., 2000; Navarro et al., 2000) with little attention given to animals immediately post-settlement. In *P. magellanicus*, the stage from settlement to field deployment ($\sim$ 300 μm to 3 mm) can take $\sim$ 5 months, and therefore, maintaining adequate growth and survival during this period is crucial to culture success.

As sea scallops are restricted to relatively deep and cold waters <20 °C (Naidu, 1991), their physiological tolerances to environmental factors differ from those of scallop species found in warmer and shallower waters. An inverse relationship between temperature and degree of membrane unsaturation has been established (Sinensky, 1974), suggesting that sea scallops may have a high requirement for polyunsaturated fatty acids (PUFAs) to maintain membrane fluidity at lower temperatures. A direct relationship between one particular PUFA, eicosapentaenoic acid (EPA, 20:5$n-3$), and membrane fluidity has been demonstrated in sea scallops (Hall et al., 2002), illustrating not only the general necessity for PUFAs, but also the need for specific fatty acids to meet various metabolic functions. As most bivalves are unable to synthesize significant amounts of long-chain PUFAs via elongation and/or desaturation of other fatty acids, they are reliant on dietary sources to meet these fatty acid requirements (Langdon and Waldock, 1981; Waldock and Holland, 1984). Thus, sea scallops are particularly vulnerable to nutritional deficiencies.

The importance of $n-3$ PUFAs, such as EPA and docosahexaenoic acid (DHA, 22:6$n-3$), has been identified in several bivalve species such as *Crassostrea gigas* (Langdon and Waldock, 1981), *Ostrea edulis* (Enright et al., 1986; Berntsson et al., 1997) and *Pecten maximus* (Marty et al., 1992). These fatty acids play a role in membrane

function, as precursors of biologically active eicosanoids such as prostaglandins and/or as an energy substrate. The specific PUFA requirements of postlarval scallops remain unknown, and therefore, hatcheries often feed multicomponent diets in an attempt to meet nutritional requirements. However, algal culture costs are high, typically 30% of total hatchery costs (Coutteau and Sorgeloos, 1993), and it is advantageous to limit the number of algal species cultured. To this end, the objectives of this research were (1) to identify cost-effective algal diets which maximize survival and growth of *P. magellanicus* postlarvae, (2) to relate diet characteristics to scallop growth, nutritional condition and especially lipid/fatty acid concentration and composition and (3) to ascertain the lipid/fatty acid requirements of sea scallops at this developmental stage and thus set the stage for subsequent more targeted algal and supplementation trials to further test our hypotheses on specific PUFA requirements in this species.

2. Materials and methods

2.1. Scallops

Early post-settlement sea scallops, (*P. magellanicus;* initial shell height 350–380 μm) were air-shipped within the same day from PecNord, Québec, to Halifax, Nova Scotia (NS), Canada, in plastic containers filled with chilled seawater. Animals were immediately transported to the National Research Council (NRC) of Canada's Aquaculture Research Station (ARS), Sambro, and placed in a 700-l recirculating downweller system containing 0.22 μm filtered seawater at 30‰ and 14 °C. Scallops were maintained on a mixed microalgal diet for 2 days before switching to experimental diets.

2.2. Microalgal diets

Six species of microalgae were tested as five binary diets in a first feeding trial (Experiment I). A subsequent trial (Experiment II) tested the top performing mixed diet from Experiment I against its two constituents in unialgal suspensions. These included the flagellates: *Pavlova lutheri*, obtained from the Center for the Culture of Marine Phytoplankton (CCMP), West Boothbay Harbor, ME, strain 1325 (MONO); *Pavlova* sp., CCMP 459 (Pav 459); and *Tetraselmis striata* (strain Plat-P, from the National Marine Fisheries Service, Milford, CT) and the diatoms: *Chaetoceros muelleri*, CCMP 1316 (CHGRA); *Fragilaria familica*, a local isolate from Mahone Bay, NS which has not been previously tested as a bivalve diet; and *Thalassiosira weissflogii*, CCMP 1336 (ACTIN). *C. muelleri* and *P. lutheri* are both commonly used in bivalve hatcheries. A monospecific diet of *T. weissflogii* was shown to be an excellent diet for sea scallops 0.75–1.4 mm (Ryan, 1999) and is larger than the commonly used *Thalassiosira pseudonana* (3H) and thus above the size threshold for 100% gill retention efficiency by adult pectinids (Bricelj and Shumway, 1991). A unialgal diet of Pav 459 was shown to support higher growth rates of sea scallop veliger larvae than three other commonly used microalgae, *P. lutheri* included (Feindel, 2000), and *T. striata*, although deficient in DHA, has proven an excellent diet for post-settlement Eastern oysters, *Crassostrea virginica* (Wikfors et al., 1996).

In Experiment I, all five diets consisted of one diatom and one flagellate. The three flagellates were tested in combination with CHGRA, and each of the three diatoms was combined with *P. lutheri*. *P. lutheri*/CHGRA served as a reference diet, as both species are commonly used in bivalve hatcheries. This experimental design further allowed for within-flagellate and within-diatom species comparisons. The biochemical composition of the mixed diet was calculated from that of its individual constituents (Table 2) based on an equal, 50:50 contribution to total particle volume.

For Experiment I, microalgae were grown either in batch culture in 20-l carboys (Pav 459, 20 °C; *T. striata*, 16 °C; CHGRA, 20 °C; *F. familica*, 16 °C) or in temperature-controlled 200-l photobioreactors in semicontinuous culture (*P. lutheri*, 21 °C; ACTIN, 18 °C) on f/2 media (−Si for flagellates; Guillard, 1975). In Experiment II, Pav 459 was grown in batch culture as above, and CHGRA was grown in the photobioreactor at 20 °C. Mass culture of Pav 459, at scales larger than 20-l carboys, has proved unreliable to date and requires further investigation. Photobioreactors were harvested at 80% of their volume twice weekly, and refilled with pasteurized seawater and fresh f/2 medium including Si for diatoms. All species were grown on 24-h light with CO_2 with the exception of *T. striata* which was grown on 14:10 L/D without the addition of CO_2. Delivery of CO_2 was made directly into the air line and regulated by a pH controller (Cole-Parmer model P-05656-00) as needed to maintain culture pH between 7.8 and 8.3. All algal cultures were harvested in late exponential growth phase.

2.3. Experimental systems

For Experiment I, ~ 150,000 postlarval scallops were divided equally by volume into five 400-l recirculating downweller systems; for Experiment II, ~ 25,000 scallops were divided equally into three systems. Each system consisted of a 350-l reservoir tank from which water was pumped at a flow rate of 10–12 l min^{-1} (Aquatic Ecosystems Mag Drive MD 2) into a "basket" located on the exterior of a 50-l tank held above the reservoir. The upper tank contained two identical downwellers (40.6 cm in height, 15.2 cm in diameter) containing a base of 140 μm Nitex square mesh. Water from the "basket" flowed through the two downwellers and into the upper tank. An overflow pipe was located 30.5 cm above the floor of the upper tank, and excess water was allowed to flow back to the reservoir tank. The stocking density of each downweller was ~ 83 and ~ 14 animals cm^{-2}, for Experiments I and II, respectively. All experimental systems were held in a temperature-controlled environmental chamber. Replication of tanks was not possible due to the scale of these experiments; however, identical maintenance and sampling protocols were followed for all treatments, and algal concentration, light and ambient temperature were kept under tight control. Constant experimental conditions are evidenced by the comparable growth rates attained on the Pav 459/CHGRA diet in Experiments I and II (see Results).

Scallops were exposed to one of five experimental diets in Experiment I, and one of three diets in Experiment II at ca. 2.15×10^6 μm^3 ml^{-1} for 28 (Experiment I) or 30 (Experiment II) days. Diets were offered at total cell concentrations equivalent in volume to 50 cells μl^{-1} of *Isochrysis galbana* clone T-Iso, which provides an optimal food level for growth of juvenile (0.5–5 mm) bay scallops (Lu and Blake, 1996). Algal concen-

trations were maintained within ≤ 20% of the desired level by daily batch addition, and seawater in the experimental tanks was replaced twice a week to minimize fouling. All cell counts were determined on a Coulter Multisizer II fitted with a 100-μm aperture (Beckman Coulter, Fullerton, CA). Weekly sampling was conducted for scallop shell height (umbo to the distal shell edge) and % mortality, whereas sampling of scallop tissues for lipids, proteins, dry weight (DW) and ash-free dry weight (AFDW) was conducted only at the start and end of the experiment. Prior to sampling, scallops were starved overnight in 0.22-μm filtered seawater to purge gut contents, and for Experiment I, scallop samples were sieved prior to final sampling to remove empty shell debris and biodeposits. The material that passed through the sieve was examined to confirm that it contained no live scallops, and samples of shell debris were taken for DW and AFDW analysis to determine their potential contribution to final samples. Algal sampling for proteins, lipid, DW and AFDW was conducted weekly. Only growth trajectories from Experiment II will be presented and discussed as a more detailed analysis will be presented in a subsequent paper including a comparison of other diets containing *Pavlova* spp.

2.4. Growth and survival

For percent survival and shell height determinations, a subsample of scallops was removed from each downweller, placed on a graduated petri dish and scanned at 10–25 × magnification using an analog camera (Pulnix model TMC7-DSP) coupled with a dissecting microscope. All video was recorded on a Sony DVCAM (DSR-V10), and calibrated using a stage micrometer. Fifty live scallops per downweller were measured from each tank at each sampling date and averaged for mean shell height determination.

Shell heights were determined using Optimas 6.5 image analysis software (Media Cybernetics, Silver Spring, MD). Tissue dry weights were obtained by filtering triplicate scallop samples of 0.1 ml (~ 130–2200 scallops, depending on time and treatment), measured in a graduated Eppendorf tube, onto pre-combusted, pre-weighed Whatman GF/C filters, rinsed with 2 ml of 3.4% ammonium formate to remove salts and oven dried at 80 °C for 24 h. Ash weight was determined after combustion at 480 °C for 24-h. The number of live individuals in each triplicate sample of 0.1 ml was determined microscopically to calculate the biomass (DW and AFDW) per individual and to provide a number to volume conversion factor for other analyses where scallops were sampled on a volume basis. Mortalities were determined by recording the number of live, "new" dead, and "old" dead from each downweller based on video observations. "New" dead scallops were the approximate size of live scallops but showed no movement and/or presence of intact tissues. "Old" dead scallops were smaller, showed no movement, gut contents or internal tissues. Duplicate counts of 200 total scallops (dead and live) per downweller were taken for mortality estimates, converted to percent data, and then averaged.

2.5. Lipid analysis

For lipid analysis, scallop samples of 0.3–0.5 ml were placed into glass tubes pre-rinsed with methanol and chloroform. Tubes were immediately placed into liquid nitrogen, and then purged with N_2 gas. Samples were stored in 3 ml of chloroform, under nitrogen

at −80 °C until analysis. The volume of algae sampled varied with culture concentration and corresponded to a minimum of ~0.6 mg lipid. Samples for algal lipids were filtered onto pre-combusted Whatman GF/C filters and treated as above. Multiple blanks were prepared for both scallop tissue and algal samples.

Lipid samples were shipped overnight, on dry ice, to Memorial University of Newfoundland for lipid analysis. Both algae and scallop lipids were extracted in chloroform–methanol (2:1) following a modified Folch procedure (Folch et al., 1957) as described by Parrish (1999). Lipid classes were determined using the Chromarod-Iatroscan TLC/FID system. They were quantified in a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) using lipid standards (Sigma), and summed to determine total lipid concentrations. Fatty acids were quantified from bulk lipid extracts following derivatization for 1.5 h at 85 °C with 1.5 ml 10% BF_3/MeOH. Fatty acid methyl esters (FAME) were run on a Varian 3400 Gas Chromatograph with an Omegawax 320 column (Supelco, Bellefonte, PA). Peak detection was done at 220 °C and identified with Varian Star Chromatography Software 4.02 with known standards (PUFA 1 and 37 Component FAME Mix, Supleco Canada). A Varian Saturn mass spectrometer was used for the confirmation of fatty acids for which standards were not available (e.g., 22:5$n-6$).

2.6. Protein analysis

Triplicate samples of 0.15 ml of scallops were placed into cryovials and immediately frozen in liquid nitrogen after excess water was removed. They were stored at −80 °C until analysis. For organic carbon (C) and nitrogen (N) determinations, tissues were lyophilized, powdered, weighed on a Cahn electrobalance and run on a Carlo-Erba 1108 CHN analyzer (Rodano, Italy) using acetanilide as the standard. For determination of algal C/N, 2–10 ml of algal cultures were filtered onto pre-combusted GF/C filters, dried at 80 °C for 24-h and stored in desiccant. Nitrogen values were converted to milligrams of protein using a conversion factor of 5.8 (Gnaiger and Bitterlich, 1984).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was used to investigate differences in final shell height ($n=100$) and scallop biomass ($n=3$). Linear regressions were fitted to shell height data to determine scallop growth rates, and differences among growth rates were tested using analysis of covariance (ANCOVA). One-way ANOVAs were conducted to determine differences in bulk biochemical properties of algae ($n=5$) and scallop tissues ($n=3$). A multivariate analysis of variance (MANOVA) was conducted to determine differences in lipid and fatty acid profiles among algal species and scallop tissues prior to conducting any multiple comparison tests. A two-way ANOVA was used to examine mortality data with diet type and time as factors. Other two-way ANOVAs were conducted to examine fatty acid ratios with diet type and organism (algae or scallop tissues) as factors. All percent and ratio data were arcsine transformed prior to analysis to normalize the data, and all multiple comparisons were carried out using Tukey's multiple comparison test. All analyses were carried out using SYSTAT 9.0 (SPSS, Chicago, IL). The significance value of all analyses was set at $P<0.01$, and where no significant difference

was detected, they were retested at $P < 0.05$. Unless otherwise noted, results are reported at $P < 0.01$.

3. Results

3.1. Scallop growth and mortalities

After 4 weeks of feeding, growth rates were clearly different between the five treatments in Experiment I, with the Pav 459/CHGRA combination yielding a superior mean growth rate of 28.1 μm day^{-1} (ANCOVA; $F_{4,1994} = 4448.3$; $P < 0.001$; Fig. 1A) and mean scallop dry and organic weights ~2 times greater than the reference diet of *P. lutheri*/CHGRA (Table 1), which resulted in a mean growth rate of 19.4 μm day^{-1}. The second highest performing diet was the combination of *P. lutheri* and the diatom *F. familica*, a species previously untested as food for bivalves. The combined diet of *T. striata* and CHGRA yielded poor performance, with the lowest growth rate (8.1 μm day^{-1}), and a final mean shell height half that of the Pav 459/CHGRA diet (Fig. 2), and dry and organic weights 5× and 8× lower, respectively, than the Pav 459/CHGRA diet. Furthermore, weekly instantaneous growth rates on the *T. striata*/CHGRA diet showed a marked decline over the 4-week experiment (Table 1). The range among growth rates was much greater in scallops exposed to diets composed of differing flagellate species (*P. lutheri*/CHGRA, Pav 459/CHGRA, *T. striata*/CHGRA) than among diets containing *P. lutheri* and differing in diatom species (Fig. 1A).

After sieving, the contribution of dead shell to final sample AFDW was low, ranging from 5% to 12%, except for the *T. striata*/CHGRA treatment that yielded a dead shell contribution of 23%. In Experiment II, the mixed diet of Pav 459/CHGRA was also the highest performing diet yielding a growth rate of 24.9 μm day^{-1} (Fig. 1B), comparable to that obtained in Experiment I, and a 31% and 64% decrease in growth rate was observed for scallops offered unialgal diets of Pav 459 and CHGRA, respectively.

Substantial new mortalities (20–31%) were observed during the first week of the feeding trial in all treatments (attributable to shipping stress), which decreased during the final 3 weeks, ranging from 3.4% to 9.4% per week. A two-way ANOVA conducted on the final 3 weeks of the feeding trial indicated both a significant effect of diet ($P < 0.05$) and time ($P < 0.001$) on new mortalities, with an overall decrease in new mortalities during week 4. The mean weekly new mortalities of scallops exposed to the *P. lutheri*/*F. familica* and *T. striata*/*C. muelleri* diets were higher (7.2% and 6.9%) than those offered the *P. lutheri*/*C. muelleri* treatment (4.6%). No other differential mortalities among treatments were detected.

3.2. Microalgal biochemical composition

Experimental diets were offered to scallops in equivalent biovolume concentrations (see Section 2.2). However, total diet DW and AFDW were also comparable, varying by < 25% among treatments. *P. lutheri*/CHGRA and *T. striata*/CHGRA, yielded the lowest and highest biomass concentrations with values of 0.710/0.946 mg DW l^{-1} and 0.637/0.805

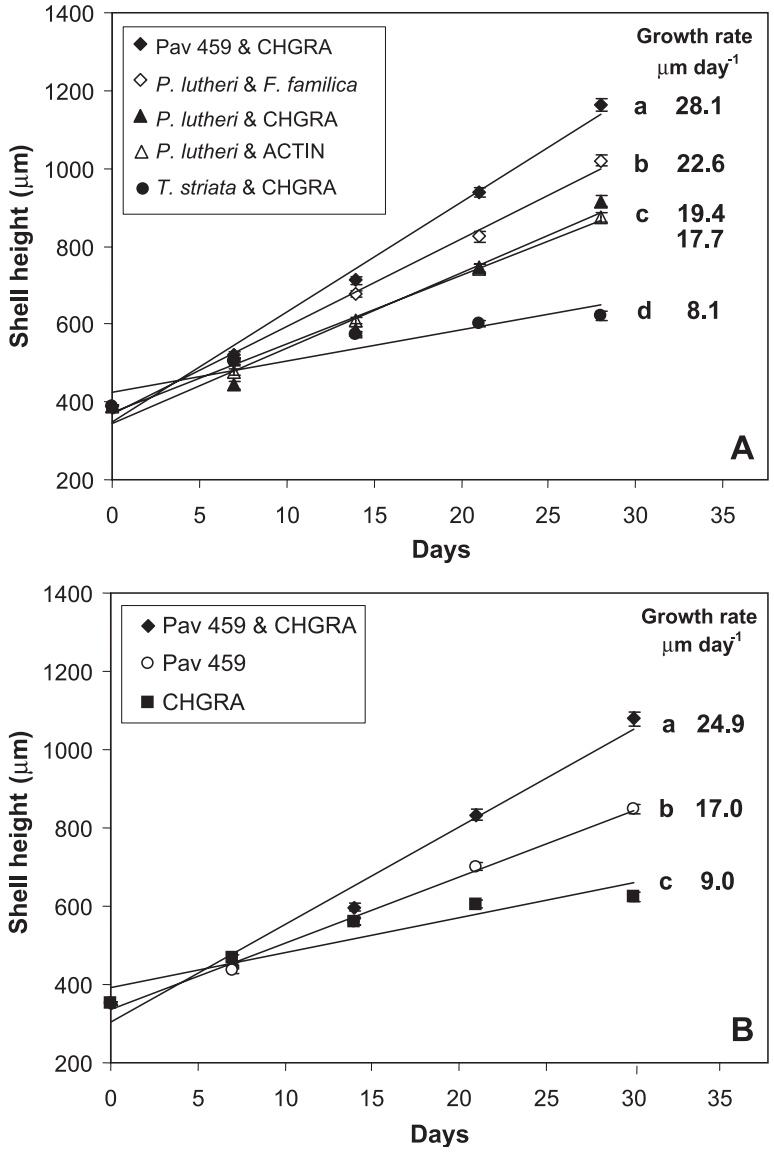

Fig. 1. Shell growth trajectories of *P. magellanicus* postlarvae fed different algal diets. Values represent mean shell height ± standard error. Linear regression equations (calculated from individual shell height data) are as follows: (A) Experiment I: Pav 459 and *C. muelleri* (CHGRA) ($y = 28.13x + 371.1$, $R^2 = 0.990$); *P. lutheri* and *F. familica* ($y = 22.59x + 366.7$, $R^2 = 0.994$); *P. lutheri* and CHGRA ($y = 19.39x + 344.0$, $R^2 = 0.973$); *P. lutheri* and *T. weissflogii* (ACTIN) ($y = 17.68x + 371.1$, $R^2 = 0.995$) and *T. striata* and CHGRA ($y = 8.11x + 424.2$, $R^2 = 0.891$); (B) Experiment II: Pav 459 and CHGRA ($y = 24.92x + 303.6$, $R^2 = 0.98$); Pav 459 ($y = 16.97 + 334.9$, $R^2 = 0.99$) and CHGRA ($y = 8.98 + 392.6$, $R^2 = 0.89$). Letters represent significant differences in growth rate determined by ANCOVA ($P < 0.001$).

Table 1
Growth characteristics for scallop postlarvae including initial and final dry and organic weights (mean ± S.E.) and weekly instantaneous growth rates, *k* (based on shell height) for Experiment I

	Initial	Pav 459/ CHGRA	*P. lutheri*/ *F. familica*	*P. lutheri*/ CHGRA	*P. lutheri*/ ACTIN	*T. striata*/ CHGRA
Individual scallop biomass (μg scallop^{-1})						
Dry weight	8.2 ± 0.4	135.6 ± 6.0^{a}	114.0 ± 1.2^{b}	75.8 ± 2.0^{c}	80.2 ± 4.1^{c}	26.4 ± 0.2^{d}
Organic weight	2.0 ± 0.1	33.6 ± 1.3^{a}	24.1 ± 0.7^{b}	16.0 ± 0.3^{c}	16.2 ± 1.2^{c}	4.4 ± 0.1^{d}
Instantaneous growth rates (k)						
Week 1	–	4.17	3.82	1.92	2.99	3.73
Week 2	–	4.52	4.11	3.97	3.48	1.85
Week 3	–	3.92	2.84	3.42	2.81	0.68
Week 4	–	3.08	3.04	2.97	2.34	0.51
Mean ± S.E.		3.93 ± 0.31^{a}	3.45 ± 0.31^{a,b}	3.07 ± 0.44^{a,b}	2.91 ± 0.24^{a,b}	1.69 ± 0.74^{b}

Different letters represent differences between final weights, $P<0.01$ and mean instantaneous growth rates, $P<0.05$.

mg AFDW l^{-1}, respectively. There were no significant differences in protein concentrations between the six algal species tested (ANOVA; $P>0.05$; Table 2). There was, however, a significant difference in the total lipid concentration (ANOVA; $F_{5,24}=13.9$; $P<0.001$; Table 2), most notably CHGRA contained 2.5 times more lipid than *F. familica* (295 vs. 117 mg lipid g AFDW $^{-1}$).

P. lutheri contained the highest levels of phospholipids, which accounted for more than half of the total lipid in this species, yet high levels (~ 45%) were also found in Pav 459 and *F. familica* (Table 2). A significant difference was detected in the calculated phospholipid concentrations of the mixed algal diets ($P=0.037$) which ranged from 58.1 to 90.0 mg g AFDW^{-1}, although a Tukey's multiple comparison test failed to show individual diet differences. *C. muelleri* was characterized by triacylglycerol (TAG) levels

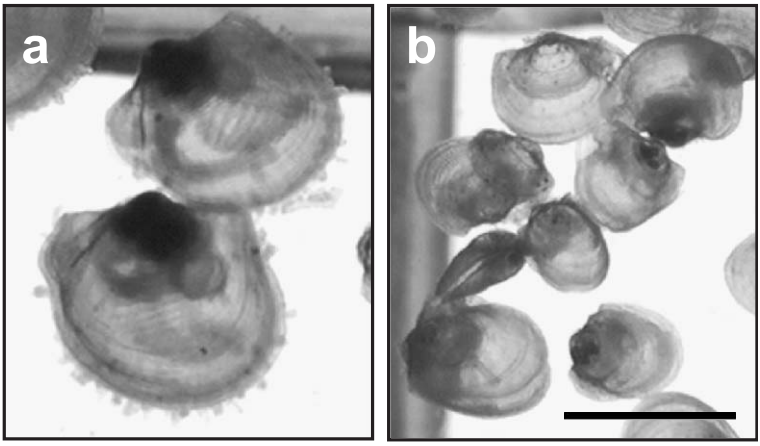

Fig. 2. Video micrographs of scallops at the end of the 4-week Experiment I exposed to (a) the highest Pav 459/ CHGRA and (b) lowest *T. striata*/CHGRA performing diets. Scale bar = 1 mm.

Table 2
Cell size (equivalent spherical diameter, ESD), dry weight, ash-free dry weight and biochemical characteristics (lipid and protein concentration, lipid class and fatty acid composition) of microalgal species used in diets for Experiment I

	Pav 459	*P. lutheri*	*T. striata*	CHGRA	ACTIN	*F. familica*
Size and biomass						
ESD (μm)	3.8 ± 0.1	4.0 ± 0.1	9.2 ± 0.2	5.1 ± 0.1	11.7 ± 0.1	5.2
Dry weight (pg cell^{-1})	13.7 ± 0.9	14.7 ± 1.4	273.8 ± 51.3	30.7 ± 2.4	401.4 ± 62.1	24.0 ± 2.7
AFDW (pg cell^{-1})	11.9 ± 0.7	13.4 ± 1.0	229.2 ± 37.9	26.9 ± 1.4	317.5 ± 52.9	18.4 ± 2.1
% ash	13.1	8.8	16.3	12.4	20.9	23.3
Bulk biochemical properties (mg g AFDW^{-1})						
Total protein	611.4 ± 19.6	504.5 ± 10.8	480.4 ± 61.7	499.9 ± 38.3	544.4 ± 55.4	423.6 ± 29.7
Total lipid	240.2 ± 16.5^{a,b}	234.2 ± 32.2	195.7 ± 21.8^{b}	295.4 ± 25.4^{a}	176.4 ± 23.1^{a,b}	116.7 ± 17.1^{b}
Total fatty acid	121.9 ± 7.3^{b}	124.9 ± 15.7^{b}	92.7 ± 11.3^{b}	224.1 ± 24.4^{a}	104.5 ± 9.7^{b}	72.7 ± 9.8^{b}
Lipid classes (% of total lipid)						
Triacylglycerols	0.6 ± 0.2^{b}	0.5 ± 0.2^{b}	0.2 ± 0.1^{b}	54.1 ± 7.2^{a}	18.8 ± 8.4^{b}	7.2 ± 0.7^{b}
Free fatty acids	2.8 ± 1.7^{b}	4.2 ± 1.2^{a,b}	0.9 ± 0.4^{b}	3.5 ± 1.3^{b}	5.3 ± 2.4^{a,b}	14.1 ± 2.2^{a}
Alcohols	2.6 ± 0.3^{a,b}	5.2 ± 0.5^{a}	0.4 ± 0.2^{c}	0.1 ± 0.0^{c}	0.2 ± 0.1^{c}	1.6 ± 0.7^{b,c}
Sterols	7.3 ± 0.3^{a}	5.5 ± 0.7^{a,b}	1.8 ± 0.1^{c}	2.7 ± 0.4^{c}	1.8 ± 0.8^{c}	4.2 ± 0.8^{b,c}
Acetone mobile polar lipids	40.4 ± 1.3^{a,b}	31.1 ± 4.1^{b}	66.2 ± 1.2^{a}	16.8 ± 4.9^{b}	39.3 ± 17.6^{b}	23.7 ± 1.0^{b}
Phospholipids	44.6 ± 1.8^{a,b}	52.0 ± 2.2^{a}	29.7 ± 1.1^{b,c}	19.8 ± 3.2^{c}	32.4 ± 14.5^{b,c}	45.9 ± 2.6^{a,b}
Fatty acid composition (% of total fatty acid)						
14:0	16.7 ± 0.4	12.6 ± 0.4	2.5 ± 0.3	12.7 ± 0.8	6.6 ± 0.4	15.6 ± 0.5
16:0	8.2 ± 0.3	10.2 ± 1.1	14.0 ± 0.4	14.7 ± 0.9	11.2 ± 2.0	4.5 ± 0.5
17:0i	0.6 ± 0.0	0.4 ± 0.0	2.7 ± 0.3	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
17:0ai	0.2 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	1.9 ± 0.3	2.4 ± 0.6	0.5 ± 0.1
Sum SAT	27.9 ± 0.8^{a,b}	26.8 ± 0.8^{b}	25.6 ± 0.8	32.8 ± 1.3^{a}	23.9 ± 1.1^{b}	24.1 ± 0.5^{b}
16:1$n-7$	7.2 ± 0.4	14.9 ± 1.3	1.3 ± 0.5	31.1 ± 2.0	26.4 ± 7.5	21.0 ± 1.4
16:1$n-5$	3.4 ± 0.2	0.2 ± 0.1	–	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.0
18:1$n-9$	0.2 ± 0.0	0.5 ± 0.0	6.9 ± 0.5	0.5 ± 0.0	0.7 ± 0.4	1.0 ± 0.7
18:1$n-7$	0.3 ± 0.0	3.1 ± 0.6	1.3 ± 0.1	0.8 ± 0.2	1.8 ± 0.6	6.7 ± 0.4
20:1$n-11$	–	–	4.5 ± 0.5	–	–	–
Sum MUFA	12.8 ± 0.4^{b}	20.7 ± 2.3^{a,b}	18.9 ± 0.9^{a,b}	34.2 ± 1.9^{a}	32.6 ± 6.8^{a}	32.1 ± 1.1^{a}
16:2$n-4$	1.6 ± 0.1	1.6 ± 0.1	0.1 ± 0.1	2.5 ± 0.1	2.8 ± 0.2	2.4 ± 0.1
16:3$n-4$	0.3 ± 0.0	1.8 ± 1.0	–	5.3 ± 1.4	17.9 ± 4.7	8.5 ± 0.4
16:4$n-3$	–	–	18.4 ± 0.6	–	–	0.1 ± 0.0
18:2$n-6$	1.1 ± 0.4	0.5 ± 0.3	7.0 ± 0.9	0.7 ± 0.1	0.6 ± 0.1	2.9 ± 0.8
18:3$n-3$	2.7 ± 0.5	1.0 ± 0.2	14.0 ± 1.5	0.1 ± 0.1	–	–
18:4$n-3$	5.0 ± 2.4	5.7 ± 1.9	7.7 ± 0.8	0.8 ± 0.7	0.2 ± 0.1	0.8 ± 0.1
20:4$n-6$ (AA)	1.8 ± 0.2^{b}	0.3 ± 0.2^{c}	0.2 ± 0.1^{c}	7.4 ± 1.0^{a}	0.9 ± 0.2^{b,c}	1.4 ± 0.2^{b}
20:5$n-3$ (EPA)	30.0 ± 0.8^{a}	28.3 ± 1.3^{a}	6.6 ± 0.2^{c}	12.0 ± 1.4^{c}	16.2 ± 2.5^{b,c}	22.9 ± 1.2^{a,b}
22:5$n-6$ (DPA)	7.8 ± 0.4^{a}	0.6 ± 0.1^{b}	–	0.1 ± 0.0^{c}	–	–
22:6$n-3$ (DHA)	8.0 ± 0.4^{a,b}	10.6 ± 1.6^{a}	0.2 ± 0.1^{d}	1.9 ± 0.7^{c,d}	3.4 ± 0.7^{b,c}	1.4 ± 0.8^{c,d}
Sum PUFA	59.3 ± 0.6^{a}	52.6 ± 1.8^{a}	55.5 ± 1.5^{a}	33.0 ± 2.7^{b}	43.6 ± 7.8^{a,b}	43.8 ± 1.4^{a,b}
Sum $n-3$	46.2 ± 1.3	45.7 ± 3.4	47.5 ± 2.4	15.0 ± 2.6	20.0 ± 3.1	25.7 ± 1.2
Sum $n-6$	11.1 ± 1.1	2.1 ± 0.6	7.7 ± 1.0	9.4 ± 1.1	1.9 ± 0.3	5.9 ± 0.7

Table 2 (*continued*)

	Pav 459	*P. lutheri*	*T. striata*	CHGRA	ACTIN	*F. familica*
Fatty acid composition (% of total fatty acid)						
$n-3/n-6$	$4.4 \pm 0.5^{b,c}$	28.3 ± 6.0^{a}	$6.6 \pm 0.9^{b,c}$	1.7 ± 0.4^{c}	$12.7 \pm 3.4^{a,b}$	$4.7 \pm 0.7^{b,c}$
DHA/EPA	0.27 ± 0.01^{a}	0.11 ± 0.05^{a}	0.02 ± 0.02^{c}	$0.15 \pm 0.04^{a,b}$	$0.20 \pm 0.02^{a,b}$	$0.06 \pm 0.04^{b,c}$
DPA/AA	4.57 ± 0.35	2.99 ± 0.69	0.09 ± 0.09	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
EPA/AA	17.9 ± 2.1	144.2 ± 28.6	38.3 ± 10.7	1.6 ± 0.2	26.2 ± 8.2	17.3 ± 2.0

All measurements expressed as mean ± S.E., $n=5$. Different letters indicate significant differences ($P<0.01$), there was no significant difference in protein concentration ($P>0.05$). Differences in algal biomass between species were not tested.

Only lipid classes and fatty acids contributing >2% in at least one species are reported.

Other identified lipid classes were hydrocarbons, steryl/wax esters, methyl esters, ethyl ketones, methyl ketones, glyceryl ethers and diacylglycerols.

Other identified fatty acids were 15:0i, 15:0, 16:0i, 16:0ai, 17:0i, 17:0, 18:0, 19:0, 20:0, 22:0, 23:0, 24:0, 14:1, 15:1, 16:1$n-9$, 17:1, 18:1$n-11$, 18:1$n-5$, 20:1$n-9$, 20:1$n-7$, 22:1$n-11$(13), 22:1$n-9$, 24:1, 16:4$n-1$, 18:2$n-4$, 18:3$n-6$, 18:3$n-4$, 18:4$n-1$, 20:2a, 20:2b, 20:2$n-6$, 20:3$n-6$, 20:3$n-3$, 20:4$n-3$, 21:5$n-3$, 22:4$n-6$ and 22:5$n-3$.

For fatty acids, multiple comparison tests were only conducted on ΣSaturates (SAT), ΣMonoenes (MUFA), ΣPUFA, AA, EPA, DPA, DHA, $n-3/n-6$ and DHA/EPA as additional comparisons were constrained by the MANOVA.

– non-detectable.

exceeding those of all other algae tested (54% vs. 0.2–18.8%), which was reflected in all CHGRA-containing diets (20–26% vs. 4–9%). *T. striata* and Pav 459 contained the highest proportions of acetone mobile polar lipids (AMPL) found in any of the species tested (66.2% and 40.4%, respectively; Table 2), *F. familica* was the only alga characterized by high levels of free fatty acids (14% vs. < 5.3%), and the two *Pavlova* species contained the highest levels of sterols (5.5–7.3%). Sterol concentrations of algal diets showed a significant positive linear relationship with scallop growth rate ($R^2=0.82$; $P<0.05$; Fig. 3), as the Pav 459/CHGRA and *T. striata*/CHGRA diets had the highest (1.56 mg g $AFDW^{-1}$) and lowest (0.22 mg g $AFDW^{-1}$) concentrations, respectively.

Particular emphasis is given in this study to the four long-chain PUFAs 20:5$n-3$, 22:6$n-3$, 20:4$n-6$ and 22:5$n-6$ in both algal diets and scallop tissues. Both DHA and EPA have been shown to be essential fatty acids in bivalves, and AA plays a role as a precursor of bioactive molecules such as prostaglandins. Docosapentaenoic acid, $n-6$ DPA is the only other long-chain PUFA contributing >2% to either diets or tissues (Tables 2 and 3), and together, these four fatty acids contribute 69–82% of the total PUFA concentrations found in scallop tissues (Table 3). Ratios of these four PUFAs were examined to determine enrichment patterns between diets and tissues and to identify biochemical indices of dietary nutritional value and/or scallop condition.

Both CHGRA and Pav 459, which yielded the highest scallop growth in combination, possess a unique profile of these four PUFAs. *C. muelleri* was characterized by high levels (7.4%) of arachidonic acid (AA, 20:4$n-6$), and absolute concentrations of this PUFA (16.7 mg g $AFDW^{-1}$) were ~ 8–80 times higher than in any of the other species tested, as was reflected in the fatty acid concentration of all three diets containing CHGRA (Fig. 4a). *Pavlova* sp. 459 contained relatively high levels (7.8%) of docosapentaenoic acid (DPA, 22:5$n-6$), which accounted for < 1% of the fatty acid composition of all other

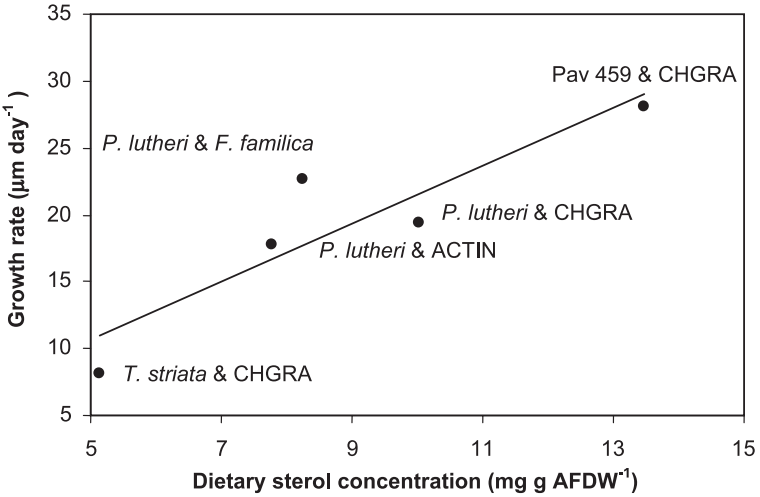

Fig. 3. Relationship between dietary sterol concentration and mean scallop growth rate; $y = 2.17x - 0.16$, $R^2 = 0.82$, $P < 0.05$). Removal of the *P. lutheri*/*F. familica* diet from this relationship increased the coefficient of determination to 0.96, $P < 0.05$.

algal species and provided a unique fatty acid signature for this strain. The absolute concentrations of DPA in Pav 459 (9.7 mg g AFDW^{-1}) were one to two orders of magnitude higher than those of the five other algal species tested ($\leq$ 0.7 mg g AFDW^{-1}; Fig. 4a).

T. striata was not only low in the total concentration of long-chain ($\geq C_{20}$) PUFAs (6.5 vs. 19–58 mg g AFDW^{-1} for all other algal species) but was also deficient in DHA, containing only 0.2% of this essential PUFA (Table 2) and DHA concentrations below those of all other algal species tested (0.20 vs. 1.05–13.42 mg g AFDW^{-1}). The EPA content was also relatively low in *T. striata* (6.6% vs. 12.0–30.0%), with concentrations 2.7–6.0 × lower than those in the other microalgae (6.1 vs. 16.5–36.5 mg g AFDW^{-1}). DHA and EPA levels were highest in the two *Pavlova* species both on a concentration and % composition basis, but Pav 459 had significantly higher levels of DPA and AA than *P.*

Notes to Table 3:

All values expressed as mean ± S.E., $n = 3$. Different letters represent statistical differences among final treatments, bulk biochemical properties and lipid classes $P < 0.05$ (except for TAG which was significant at $P < 0.01$) and fatty acid composition $P < 0.01$.

Only lipid classes and fatty acids contributing >2% in at least one experimental treatment are reported.

Other identified lipid classes were hydrocarbons, steryl/wax esters, methyl esters, ethyl ketones, methyl ketones, glyceryl ethers, alcohols and diacylglycerols.

Other identified fatty acids were 15:0i, 15:0ai, 15:0, 16:0i, 16:0ai, 17:0i, 17:0ai, 17:0, 20:0, 23:0, 14:1, 15:1, 16:1$n-9$, 16:1$n-5$, 18:1$n-11$, 18:1$n-5$, 20:1$n-9$, 20:1$n-7$, 22:1$n-9$, 16:2$n-4$, 16:3$n-4$, 16:4$n-3$, 16:4$n-1$, 18:2$n-4$, 18:3$n-6$, 18:3$n-4$, 18:4$n-1$, 20:2a, 20:2$n-6$, 20:3$n-6$, 20:3$n-3$, 20:4$n-3$, 21:5$n-3$ and 22:5$n-3$.

For fatty acids, multiple comparison tests were only run on ΣSaturates (SAT), ΣMonoenes (MUFA), ΣPUFA, AA, EPA, DPA, DHA and $n-3/n-6$ as additional comparisons were constrained by the MANOVA.

–non-detectable.

lutheri (Table 2). The two *Pavlova* species also differ in their $n-3/n-6$ ratios, with *P. lutheri* having the highest and Pav 459 having one of the lowest ratios (28.3 and 4.4, respectively; Table 2). The PUFA profiles of all five diets exhibited similar patterns when calculated on both an AFDW and per volume basis.

Table 3
Biochemical composition of scallop tissues (protein, lipid and fatty acid concentration, as well as lipid class and fatty acid composition), both initial and after 28 days exposure to different algal diets in Experiment I

	Initial	Pav 459/ CHGRA	*P. lutheri*/ *F. familica*	*P. lutheri*/ CHGRA	*P. lutheri*/ ACTIN	*T. striata*/ CHGRA
Bulk biochemical properties (mg g AFDW^{-1})						
Total protein	406.0 ± 49.9	538.7 ± 10.1^{a}	474.8 ± 15.1^{a,b}	381.2 ± 9.0^{b,c}	464.3 ± 18.9^{b}	349.9 ± 22.4^{c}
Total lipid	59.8 ± 16.2	53.8 ± 6.5^{a}	35.5 ± 5.7^{a,b}	63.1 ± 13.1^{a}	51.9 ± 9.6^{a,b}	13.0 ± 5.1^{b}
Total fatty acid	47.9 ± 11.3	37.8 ± 5.2^{a,b}	21.3 ± 3.9^{a,b}	50.7 ± 5.1^{a}	32.5 ± 6.3^{a,b}	6.7 ± 2.9^{b}
Lipid classes (% of total lipid)						
Triacylglycerols	49.4 ± 4.6	31.6 ± 3.0^{a}	6.7 ± 1.1^{b,c}	26.9 ± 1.5^{a}	15.5 ± 1.2^{a,b}	1.6 ± 1.6^{c}
Free fatty acids	2.7 ± 1.2	1.7 ± 1.1	3.8 ± 2.0	2.2 ± 0.3	0.3 ± 0.3	3.2 ± 1.6
Sterols	–	7.2 ± 0.4	11.9 ± 0.7	7.7 ± 0.9	9.3 ± 0.9	14.2 ± 5.6
Acetone mobile polar lipids	5.2 ± 3.8	11.1 ± 2.81	16.3 ± 3.0	14.1 ± 1.9	16.3 ± 1.5	17.6 ± 8.0
Phospholipids	39.3 ± 3.1	45.7 ± 1.2^{a,b}	59.1 ± 6.6^{a}	45.1 ± 0.9^{a,b}	56.3 ± 1.0^{a,b}	32.8 ± 16.7^{b}
Fatty acid composition (% of total fatty acid)						
14:0	7.7 ± 0.5	5.3 ± 0.1	5.5 ± 0.1	5.0 ± 0.3	4.6 ± 0.1	2.4 ± 0.3
16:0	13.8 ± 0.2	11.4 ± 0.2	11.3 ± 0.2	12.5 ± 0.3	12.5 ± 0.3	12.1 ± 0.3
18:0	2.9 ± 0.1	3.3 ± 0.1	5.2 ± 0.1	3.9 ± 0.3	4.6 ± 0.2	6.8 ± 0.2
Sum SAT	26.7 ± 0.7	22.0 ± 0.4	23.9 ± 0.1	23.7 ± 0.3	24.4 ± 0.4	23.7 ± 1.8
16:1$n-7$	7.4 ± 0.1	8.8 ± 0.1	4.6 ± 0.1	10.6 ± 0.8	5.1 ± 0.1	4.6 ± 0.5
17:1	0.2 ± 0.2	0.6 ± 0.5	3.2 ± 0.3	2.2 ± 0.6	1.0 ± 1.0	2.5 ± 1.3
18:1$n-9$	5.4 ± 0.7	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.6 ± 0.7	2.3 ± 0.0
18:1$n-7$	5.8 ± 0.7	5.1 ± 0.0	8.7 ± 0.2	8.7 ± 0.2	8.3 ± 0.5	7.1 ± 0.3
20:1$n-11$	–	1.3 ± 0.1	2.8 ± 0.1	1.2 ± 0.6	1.7 ± 0.9	2.9 ± 1.5
Sum MUFA	20.6 ± 0.7	18.9 ± 0.6^{b}	22.8 ± 0.2^{a,b}	26.2 ± 1.1^{a}	20.4 ± 1.3^{a,b}	23.6 ± 1.1^{a,b}
18:2$n-6$	4.2 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.6 ± 0.3	1.1 ± 0.1
18:3$n-3$	3.3 ± 0.1	1.0 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	1.6 ± 0.1
18:4$n-3$	7.1 ± 1.1	2.9 ± 0.1	6.4 ± 0.5	4.1 ± 0.7	5.4 ± 1.6	6.4 ± 0.4
20:4$n-6$ (AA)	2.3 ± 0.1	8.8 ± 0.1^{b}	2.8 ± 0.0^{c}	8.1 ± 0.1^{b}	1.9 ± 0.1^{d}	10.5 ± 0.4^{a}
20:5$n-3$ (EPA)	9.8 ± 0.1	19.4 ± 0.2^{a}	14.6 ± 0.2^{b}	15.4 ± 0.2^{b}	18.1 ± 0.1^{a}	9.2 ± 0.3^{c}
22:5$n-6$ (DPA)	4.5 ± 0.3	10.1 ± 0.1^{a}	4.5 ± 0.2^{b}	3.0 ± 0.3^{c}	4.5 ± 0.2^{b}	2.9 ± 0.1^{c}
22:6$n-3$ (DHA)	17.1 ± 0.3	10.4 ± 0.2^{c}	20.9 ± 0.4^{a}	14.2 ± 0.8^{b}	19.2 ± 0.6^{a}	13.8 ± 0.4^{b}
Sum PUFA	52.7 ± 1.1	59.3 ± 0.5^{a}	53.3 ± 0.1^{a,b}	50.1 ± 1.0^{b}	55.2 ± 1.2^{a,b}	52.7 ± 1.7^{a,b}
Sum $n-3$	39.8 ± 0.8	36.0 ± 0.3	43.9 ± 0.1	35.7 ± 0.7	45.3 ± 0.1	34.1 ± 1.0
Sum $n-6$	12.8 ± 0.5	21.0 ± 0.2	8.6 ± 0.1	12.4 ± 0.3	7.3 ± 0.4	16.3 ± 0.1
$n-3/n-6$	3.1 ± 0.1	1.7 ± 0.0^{d}	5.1 ± 0.1^{b}	2.9 ± 0.0^{c}	6.2 ± 0.3^{a}	2.1 ± 0.1^{d}
DHA/EPA	1.7 ± 0.0	0.5 ± 0.0	1.4 ± 0.0	0.9 ± 0.1	1.1 ± 0.0	1.5 ± 0.0
DPA/AA	2.0 ± 0.1	1.2 ± 0.0	1.6 ± 0.1	0.4 ± 0.0	2.3 ± 0.0	0.3 ± 0.0
EPA/AA	4.3 ± 0.0	2.2 ± 0.0	5.3 ± 0.1	1.9 ± 0.0	9.4 ± 0.2	0.9 ± 0.0
DHA + DPA	21.6 ± 0.5	20.6 ± 0.3	25.3 ± 0.6	17.2 ± 1.1	23.7 ± 0.5	16.7 ± 0.5
EPA + AA	12.1 ± 0.2	28.0 ± 0.2	17.3 ± 0.2	23.5 ± 0.2	20.0 ± 0.3	19.7 ± 0.7

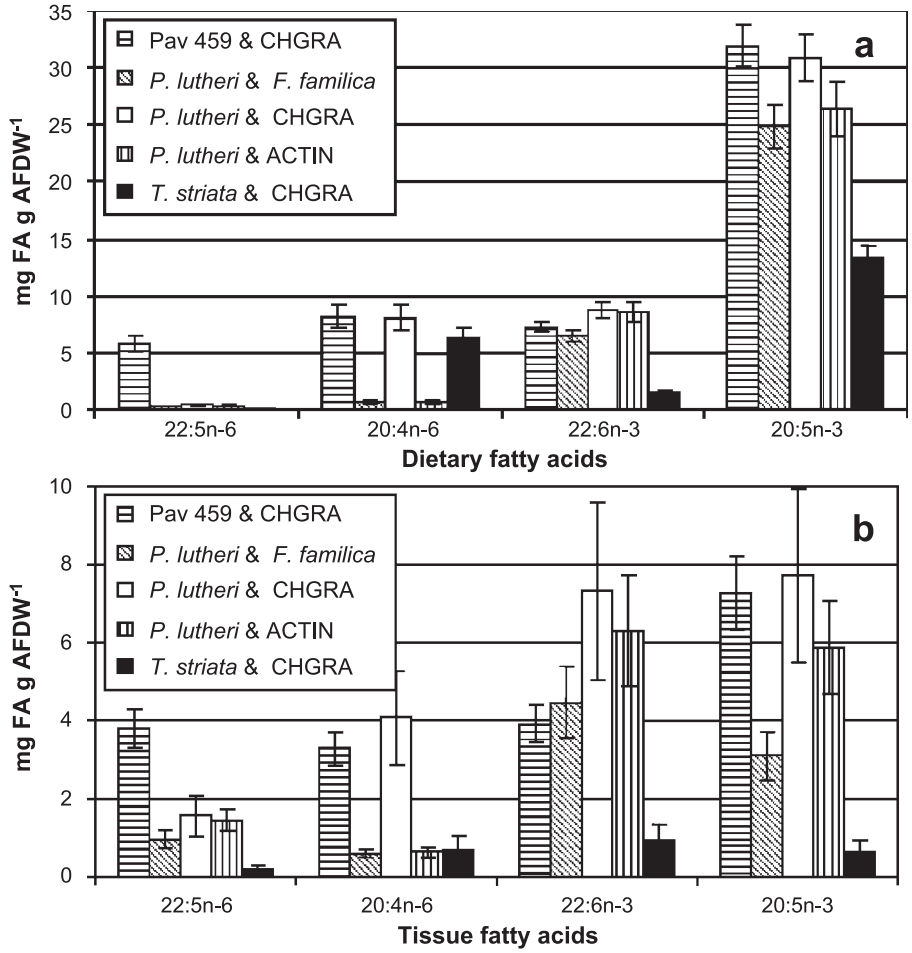

Fig. 4. Concentrations (mean ± S.E.) of four selected polyunsaturated fatty acids (PUFAs) on a per ash-free dry weight (AFDW) basis from Experiment I in (a) five experimental diets offered to scallops; (b) scallop tissues after 4 weeks of exposure to experimental diets.

3.3. Scallop biochemical composition

Protein concentration in scallop tissues reflected diet ranking in terms of scallop growth: postlarvae on the Pav 459/CHGRA diet exhibited the highest protein concentration (538.7 μg g AFDW^{-1}) while those exposed to *T. striata*/CHGRA had the lowest protein concentration (by 35%), and all other diets tested yielded intermediate concentrations ranging from 381.2 to 474.8 μg g AFDW^{-1} (Table 3). Total lipid concentrations of scallop tissues offered the *T. striata*/CHGRA diet were significantly lower than those of any other CHGRA-containing dietary treatment, although no difference was observed among the other four diets (ANOVA; $F_{4,10} = 5.3$; $P < 0.05$; Table 3). A difference in total

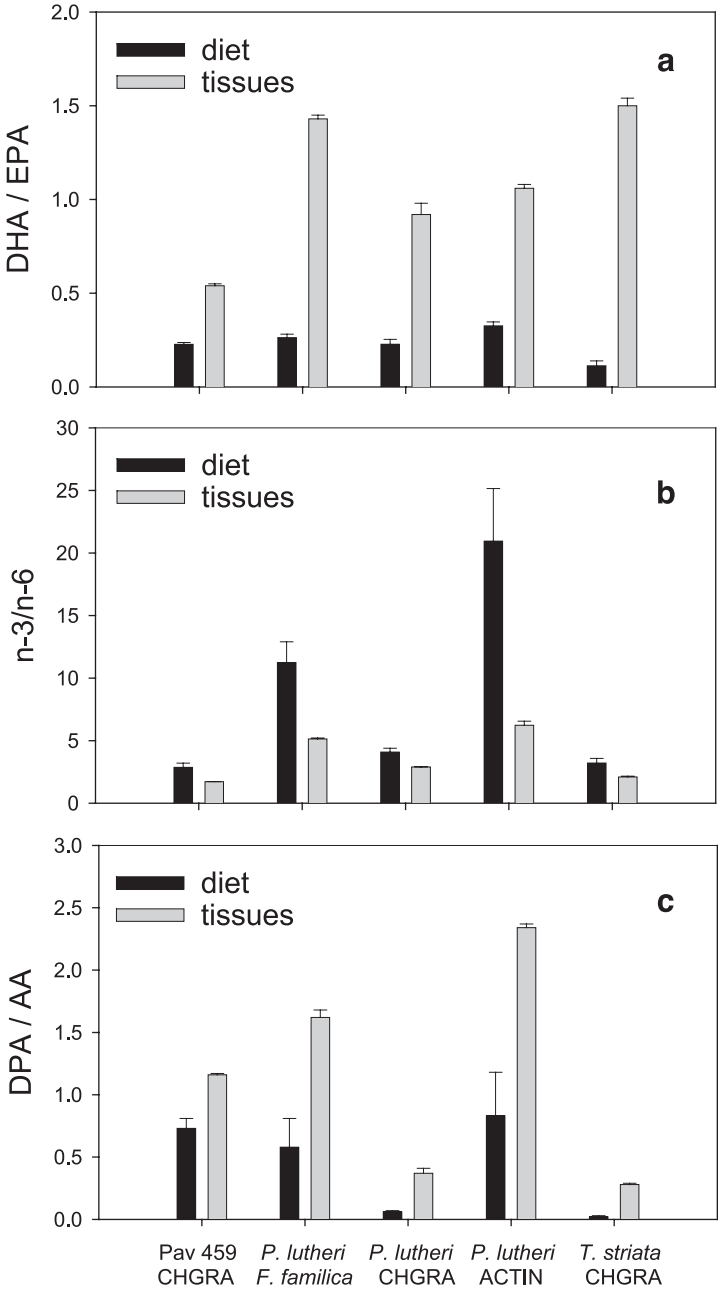

Fig. 5. Comparison of proportions of (a) docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA), (b) $\Sigma n-3/\Sigma n-6$ fatty acids and (c) $n-6$ DPA docosapentaenoic acid/arachidonic acid (AA) in diets and scallop tissues from Experiment I. Dietary values presented are calculated from the mean concentration of weekly algal samples, and data for scallop tissues are based on PUFA concentrations after feeding on experimental diets for 4 weeks. Error bars as in Fig. 3.

fatty acid concentration was only observed between the highest (Pav 459/CHGRA; 37.8 mg g AFDW^{-1}) and lowest (*T. striata*/CHGRA; 6.7 mg g AFDW^{-1}) performing diets (ANOVA; $F_{4,10}=4.4$; $P<0.05$; Table 3).

Scallops exposed to diets containing either of the two *Pavlova* species had the highest percentage of TAG (26.9–31.6%), whereas animals exposed to the *T. striata*/CHGRA diet showed significantly lower levels (1.6%) of this lipid class. There was a slight but significant difference in tissue phospholipid levels ($P<0.05$); scallops fed the *F. familica*-containing diet had higher levels than those fed the *T. striata*/*C. muelleri* diet (59.1% vs. 32.8%). Ratios of TAG/sterol and TAG/phospholipid largely reflect diet performance, with higher tissue values associated with faster growing animals, with the exception of the *P. lutheri*/*F. familica* diet (calculated from Table 3; $P<0.05$). There were no significant differences among the relative proportions of any other lipid class in scallop tissues ($P<0.05$; Table 3).

3.4. Comparison of dietary and tissue fatty acids

Fatty acid profiles of scallop tissues largely reflected those of the diets. For example, high levels of AA were observed in the animals exposed to diets containing the AA-rich CHGRA (Table 3), and high levels of DPA were reflected in scallops exposed to the DPA-rich Pav 459 (Fig. 4b). The *T. striata*/*C. muelleri* diet was characterized by low concentrations of both DHA and EPA (Fig. 4a), and the n-3 concentrations in the tissues of scallops fed this diet were greatly reduced relative to those scallops fed other diets (2.3 vs. 9.4–18.2 mg g AFDW^{-1}).

Although scallop tissues reflected some of the fatty acid characteristics of their diet, there were also pronounced shifts in fatty acid composition indicative of selective

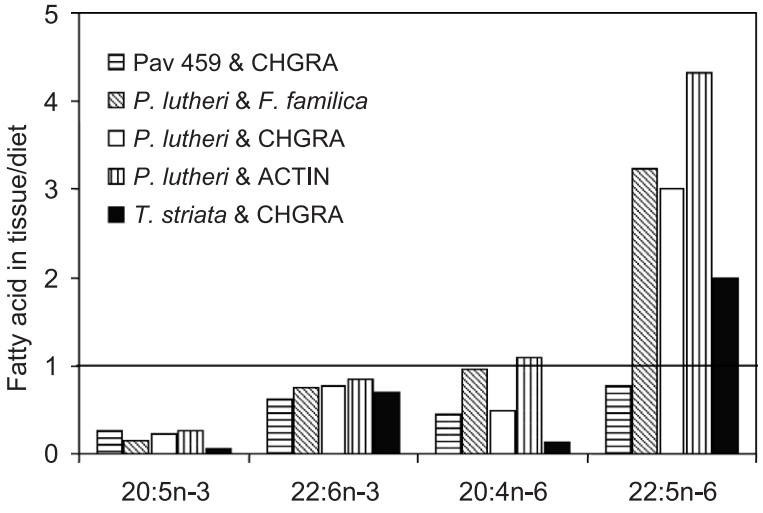

Fig. 6. Relative proportions (based on mg g AFDW^{-1}) of four major polyunsaturated fatty acids found in scallop tissues compared to experimental diets after 4 weeks of feeding during Experiment I. Values greater than 1 represent enrichment within the tissues compared to the diet offered.

incorporation/catabolism. In all treatments, there was a highly significant increase in the DHA/EPA ratio in scallop tissues relative to the diets (two-way ANOVA; $P < 0.001$; Fig. 5a). This apparent enrichment was greatest in the *T. striata*/CHGRA diet (14 ×), which had the lowest DHA/EPA ratio. Lower $n-3/n-6$ ratios were observed both in diets and tissues of experimental treatments containing CHGRA (Fig. 5b), but overall $n-3/n-6$ ratios were higher in diets than in corresponding tissues, indicating either selective utilization/elimination of $n-3$ fatty acids or selective incorporation/retention of $n-6$ fatty acids in scallop tissues (Fig. 5b). Higher DPA/AA ratios also occurred in tissues relative to the algal diet in all treatments (Fig. 5c).

A marked decrease in EPA concentrations in tissues relative to the diet was observed in all cases; a decrease in AA levels in tissues relative to the diet was only observed in diets containing the AA-rich CHGRA (Fig. 6). A substantial enrichment of DPA (up to four-fold) was observed in scallop tissues in all treatments with the exception of scallops fed the high DPA Pav 459/CHGRA diet.

4. Discussion

4.1. Effect of microalgal diets on scallop growth

This study has identified the Pav 459/CHGRA combination as a superior diet for sea scallop postlarvae, achieving growth rates not unlike those reported for postlarvae in the wild (30–45 μm day^{-1}; Parsons et al., 1993) and comparable or superior to the highest values obtained in hatcheries: a maximum of 9 μm day^{-1} for postlarvae fed diets of one to three algal species (Gillis, 1993) and 3–37 μm day^{-1} for four to six component diets (Ryan, 1999). This study also suggests that the high nutritional value of the Pav 459/CHGRA diet is largely related to high concentrations of specific long-chain PUFAs (see Sections 4.3 and 4.4). *C. muelleri* also contains high proportions of carbohydrate (49% of organic weight; Ben-Amotz et al., 1987), which has been positively related to bivalve growth and condition (Enright et al., 1986; Whyte et al., 1990). Other studies, however, have shown that carbohydrate plays a negligible role as an energy substrate in postlarval stages, as it comprises a minor (< 2%) fraction of total organics (García-Esquivel et al., 2001), and in contrast to protein and lipid, was not significantly correlated with growth (Wikfors et al., 1992). Thus, carbohydrate was not included in biochemical analysis conducted in the present study, but cannot be ruled out as a contributing factor explaining the elevated growth rates attained on the Pav 459/CHGRA combination, versus a diet of Pav 459 alone. Dietary digestibility and amino acid composition are two other factors potentially influencing growth which were not measured in this study. However, previous studies have shown only minor variation in amino acid composition between algal species, leading Knauer and Southgate (1999) to conclude that amino acid composition is likely not a major factor in determining bivalve growth on different diets. Potential digestibility differences among algal species are discussed below and require additional research.

Despite differences in culture methods, the long-chain PUFA profiles of the microalgal species tested were generally comparable to those previously reported for the diatom and flagellate species used in this study (Volkman et al., 1989, 1991; Napolitano et al., 1990;

Feindel, 2000; J. Craigie, NRC/IMB unpub. data for *T. weissflogii* and *F. familica*). Scallop growth was not related to either total lipid or total fatty acid concentration of the microalgal diets. Algal protein concentration did not significantly differ across diet treatments ($P>0.05$) and was therefore not considered a factor contributing to observed differences in scallop growth, although dietary protein content was positively related to growth performance in *Argopecten purpuratus* postlarvae (1.8 mm SH) (Uriarte and Farias, 1999).

Dietary biochemical composition does not explain the high growth performance of animals exposed to the *F. familica*/*P. lutheri* combination. *F. familica* was characterized by the lowest total lipid and fatty acid concentrations, low levels of DHA, an undetectable level of DPA, and only moderate proportions of EPA and AA. However, lipid extracts from *F. familica* cultures also contained elevated levels of free fatty acids (14% vs. 0.9–5%), and when lipolytic degradation is high, it can alter both lipid class and fatty acid composition of algae (Berge et al., 1995; Budge and Parrish, 1999) and may partially confound attempts to relate lipid class or fatty acid composition to growth.

Dietary success of the *P. lutheri*/*F. familica* diet may also be related to the digestibility of *F. familica*, as differential digestibility of diatoms has been reported in postlarval abalone (Kawamura et al., 1995), or to differences in food capture related to morphological constraints of sea scallop postlarvae at these early developmental stages. Prior to gill reflection (~ 1 mm SH in *P. magellanicus*), suspension feeding is unlikely to be very effective, and alternate feeding methods, such as pedal feeding, may be employed until the gill is fully functional (Veniot et al., 2003). The *F. familica* diet was particularly "sticky," adhering to the mesh downweller base, and the resulting microalgal film may have provided a superior algal delivery method for scallops of this particular size range.

One of the major assumptions for comparing the biochemical composition of the diet and scallop tissues in this study is that both algal species are being incorporated at equal rates, which assumes equal capture, ingestion and digestion and thus no particle selectivity based on algal size or food quality. Although adult scallops only capture particles greater than ~ 6 μm with 100% efficiency (reviewed by Bricelj and Shumway, 1991), recent work using paired fluorescent beads has shown that particles within the size range used in this present study can be effectively ingested by postlarval sea scallops (Anderson, 2003).

4.2. Lipid classes

There was a positive relationship between dietary sterol concentration and scallop growth rate (Fig. 3). As sterols play a structural role in cellular membranes (Nes, 1974) and bivalves are generally considered to have a limited ability to synthesize sterols (Walton and Pennock, 1972; Holden and Patterson, 1991), dietary sterol concentrations and composition may be particularly important to sea scallops. *C. muelleri* is characterized by high levels of cholesterol (46–48% of total sterol; Copeman et al., unpubl. data; Tsitsa-Tzardis et al., 1993), and although only binary diets including CHGRA contained significant levels of cholesterol, it was present in the scallop tissues of all treatments in this study. The *F. familica*/*P. lutheri* diet was characterized by the highest percentages (~ 18%) of 24-methylenecholesterol, which has been positively related to post-settlement oyster growth (Wikfors et al., 1996) and could play a role in explaining the high growth

rates attained by scallops on this diet. Preferential accumulation/retention of cholesterol and stigmasterol has been previously noted in the tissues of larval *P. maximus* (Soudant et al., 1998b), whereas in this study, marked enrichment of cholesterol, brassicasterol and campesterol was observed in scallop tissues relative to the diets (Copeman et al., unpubl. data). Additional research on the nutritional role of sterols in bivalves is required using diets specifically selected for this purpose.

Dietary TAG concentrations did not appear to influence diet performance, but rather reflected elevated levels in diets containing the fatty acid-rich CHGRA. There was, however, a positive relationship between the TAG composition in scallop tissues and growth rate, except for the *P. lutheri*/*F. familica* diet, indicating that the fastest growing scallops were able to convert proportionally more energy into storage material as neutral lipids which generally provide the main energy reserve. Excluding the *P. lutheri*/*F. familica* diet, TAG/sterol and TAG/phospholipid ratios in scallop tissues were an indicator of dietary success, with higher values corresponding to faster growing animals, as has been demonstrated for *P. magellanicus* larvae (Pernet et al., 2003).

4.3. n−3 fatty acids

In the present study, the *T. striata*/CHGRA diet proved unsuitable for sea scallop postlarvae, yielding growth rates almost four times lower than those of the highest performing diet. However, a unialgal diet of *T. striata* (Plat-P) supported good growth of *C. virginica* postlarvae, relative to a unialgal diet of *Isochrysis* sp. (T-Iso), which was attributed to the high EPA concentration of *T. striata*, even though this species contained non-detectable levels of DHA (Wikfors et al., 1996). Langdon and Waldock (1981) hypothesized that either DHA or EPA was necessary for good oyster growth, but not necessarily the presence of both, which may explain why *T. striata* is a successful species for oyster culture. However, it is possible that sea scallops have more stringent DHA requirements than oysters to ensure high growth performance. Poor success of this diet could also be attributed to poor cell digestibility of *T. striata*. *Tetraselmis suecica* was not readily digested by *P. maximus* larvae, as determined by epifluorescence of gut contents (Le Pennec and Rangel Davalos, 1985). Thus, low digestibility coupled with lower essential PUFA concentrations, especially of DHA, may explain the depressed growth and poor condition observed in sea scallops fed this diet. However, a monospecific diet of *C. muelleri*, which is also DHA-deficient, supported a low growth rate (9.0 μm day^{-1}) comparable to that of the *T. striata*/CHGRA diet (8.1 μm day^{-1}), suggesting that DHA-deficiency is likely responsible for the poor performance of sea scallops on both diets.

Although DHA and EPA are considered important for bivalve nutrition (Trider and Castell, 1980; Langdon and Waldock, 1981; Enright et al., 1986; Marty et al., 1992), it is possible that the DHA requirements of sea scallops outweigh those of EPA. Soudant et al. (1998a) proposed that DHA plays a structural role in *P. maximus* larvae, and it has been suggested that this role cannot be fulfilled by EPA (Feindel, 2000). However, an interchange between DHA and DPA, and also between EPA and AA, in scallop lipids is suggested by results of the present study. The sums of DHA + EPA, AA + EPA and DPA + DHA were quite similar across diets (18–19% coefficient of variation, CV), whereas the sum of the proportions of AA + DPA remained relatively variable (44%

CV), suggesting less homology between this fatty acid pair. Additionally, the CV for the percent fatty acid of DHA + DPA in scallop tissues (18.5%) was lower than that of either DHA (27.1%) or DPA (59.4%) across diet treatments. Similarly, the combined CV of EPA + AA was lower than that of either PUFA constituent (19.2% vs. 25.5% and 59.7%). Therefore, these two C_{20} (EPA and AA) and C_{22} (DHA and DPA) PUFAs may be substituting for each other in the same phospholipids.

4.4. n−6 fatty acids

The mixed diet of Pav 459/CHGRA yielded the highest growth rates of sea scallop postlarvae in both experiments and outperformed unialgal diets of both Pav 459 and CHGRA. Pav 459 alone supported higher growth rates of *P. magellanicus* veliger larvae than other unialgal diets, including *P. lutheri* (Feindel, 2000). *Pavlova* sp. 459 is characterized by an exceptionally high percentage of $n-6$ DPA, compared to other algae tested in this study. Marked enrichment of DPA (2–4.3 ×) was observed in the tissues of scallops exposed to all diets except for the DPA rich Pav 459/CHGRA combination (Fig. 6). Likewise, enrichment of DPA was reported in *P. maximus* female gonads (Soudant et al., 1996) and in the polar lipids of *P. maximus* larvae (Delaunay et al., 1993). DPA may serve to maintain membrane fluidity or structural integrity (Soudant et al., 1996), which would make it particularly important in species adapted to cold water. Since elongation and desaturation activities for long-chain PUFAs are low or absent in bivalve species (De Moreno et al., 1976; Waldock and Holland, 1984; Delaunay et al., 1993), selective retention of DHA and DPA could be facilitated by other mechanisms, such as acyltransferases, associated with TAG and phospholipid production in scallops (Marty et al., 1992).

Growth rates for sea scallop postlarvae fed a unialgal diet of Pav 459 were reduced by 31% from those attained on the mixed Pav 459/CHGRA diet (Fig. 1B). As CHGRA is characterized by high concentrations of AA, and Pav 459 contains high concentrations of the other three long-chain PUFAs, this decreased growth rate may be related to AA deficiency in the unialgal Pav 459 diet. Thus, AA, in addition to DPA, may be necessary to sustain high growth rates of sea scallop postlarvae. Arachidonic acid is a known precursor in prostaglandin production and can increase prostaglandin release in mussels, *Modiolus demissus* (Freas and Grollman, 1980). Prostaglandins in molluscs are known to influence important functions such as ion regulation, egg production, and spawing (reviewed by Stanley-Samuelson, 1987; Deridovich and Reunova, 1993). Therefore, eicosanoids derived from AA may also play an important functional role in sea scallop postlarvae.

4.5. Fatty acid ratios

A large relative increase in DHA/EPA values between diet and scallop tissues was observed, indicating either selective DHA incorporation/retention or selective elimination/utilization of EPA in the tissues (Fig. 5a). Preferential incorporation of DHA at the expense of EPA has been previously observed in the polar lipids of *P. maximus* larvae, and it has been suggested that DHA may be an integral structural component of cell membranes (Soudant et al., 1998a), which may therefore explain the preferential retention of DHA (Delaunay et al., 1993). However, Hall et al. (2002) described a positive relationship

between the proportion of EPA in the gills of adult sea scallops and membrane fluidity, but no correlation between DHA and membrane fluidity. Although finfish rely on DHA to regulate membrane fluidity, this might not be the case in bivalves, and the specific role of DHA in scallops remains poorly understood. Furthermore, DHA enrichment relative to the diet does not occur in all marine organisms, as observed in turbot (Rainuzzo, 1993), indicating that relative enrichment of DHA in scallop tissues may be a response to species-specific needs.

In the $n-6$ polar fraction of *P. maximus* larvae, there was a decrease of AA associated with an increase in DPA, which reflected the dietary proportions of these two fatty acids (Soudant et al., 1998a). In this study, high DPA/AA ratios were also observed in bulk lipids of scallop tissues relative to the diet (Fig. 5c), but this is likely more than a simple reflection of dietary levels. Active incorporation of DPA into scallop tissues (Fig. 6) could contribute to the increase in the DPA/AA ratio found in tissues relative to the diet. It is also possible that decreasing levels of AA could be associated with eicosanoid production in scallops, as AA is converted into other bioactive compounds (Delaunay et al., 1993), although the reductions in AA concentrations involved may not have been detectable.

Eicosanoids produced from AA are more active than those formed from EPA and compete for the same enzymes in marine fish (Sargent et al., 1999), and presumably shellfish, although this has never been directly addressed. A minimum dietary EPA/AA ratio of 4.2 was suggested for turbot larvae, and a level of 20 had no deleterious effect, although higher values were not tested. The authors contend that excess dietary EPA relative to AA is not deleterious, whereas excess AA can be detrimental, inducing stress as a result of elevated eicosanoid production. Dietary ratios in the present study ranged from 2.2 to 55.6 (calculated from Table 2), such that the lowest performing diet, *T. striata*/CHGRA, had an EPA/AA ratio (2.2) lower than the minimum suggested above. This low ratio is not a result of excessively high AA concentrations, as all diets containing CHGRA have elevated concentrations of AA, but a relatively low concentration of EPA (Table 2). If the range of EPA/AA ratios determined to be critical for turbot larvae has any relevance to postlarval sea scallops, the low EPA/AA ratio of the *T. striata*/CHGRA diet might offer another explanation for its poor performance.

Algal diets with low $n-3/n-6$ ratios have been considered "good" diets for oyster larvae (Webb and Chu, 1983), and Feindel (2000) found that the $n-6$ long-chain PUFAs, DPA and AA, were most strongly correlated with growth of larval *P. magellanicus*. Marty et al. (1992) found an increase in the ratio of $n-6/n-3$ fatty acids between diets and tissues in the polar lipids of *P. maximus* larvae, and this therefore suggests that $n-6$ fatty acids play an important role in this organism. Evidence of a requirement for $n-6$ fatty acids in scallops is further supported in the present study by (1) the superior growth rates of *P. magellanicus* postlarvae possibly associated with high dietary concentrations of DPA and AA in the Pav 459/CHGRA diet and (2) the increase of $n-6/n-3$ fatty acids found in scallop tissues relative to the diet (Fig. 5b). Therefore, we propose that the importance of $n-6$ fatty acids in bivalve nutrition has been largely overlooked. Future lipid supplementation trials, including partitioning of PUFAs between polar and neutral lipid fractions, will be conducted to more accurately define the fatty acid requirements of sea scallop postlarvae.

5. Conclusions

The binary Pav 459/CHGRA diet yielded scallop growth rates superior to any other binary diet tested and comparable to the mid-upper range of sea scallops offered four to six component hatchery diets. Therefore, the Pav 459/CHGRA diet provides an excellent diet for low-cost implementation in sea scallop hatcheries. Our results suggest that the high nutritional value of this diet may be linked to high concentrations of $n-6$ fatty acids DPA and AA, contributed by Pav 459 and CHGRA, respectively. Marked enrichment of DPA in scallop tissues relative to the available diet indicates that this fatty acid may play an essential role in these organisms, and that the importance of $n-6$ fatty acids in pectinids, and bivalves in general, requires further investigation. Although *T. striata* was shown to be an excellent diet for juvenile oysters, the *T. striata*/*C. muelleri* diet was unsatisfactory for sea scallop growth, potentially due to its DHA-deficiency. The inability to grow on diets low in DHA in this study suggests that sea scallops may have a more stringent DHA requirement than Eastern oysters, although the specific role of DHA remains largely unknown.

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