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Influence of irradiance on the nutritional value of two phytoplankton species fed to larval Japanese scallops (*Patinopecten yessoensis*)

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Abstract Japanese scallop (*Patinopectin yessoensis* Jay) larvae grew faster and were larger after 18 d when fed a diet of high-light(HL)-grown *Chaetoceros simplex* or HL *Pavlova lutheri* relative to diets of the same phytoplankton species grown at low light (LL). When provided as saturating rations to larval scallop, these diets could be ranked: HL *C. simplex*>LL *C. simplex*>HL *P. lutheri*>LL *P. lutheri*. In both phytoplankton species, HL-grown cells contained more of the short-chain saturated fatty acid (FA), 16:0 than LL-grown cells. Scallop growth rates were a significant function of the amounts (mg g^{-1} dry wt) and the proportions (as percentage of total FAs) of the FAs 14:0 and 14:0+16:0 (total saturated FAs) in their diet. The proximate biochemical composition of HL- versus LL-grown phytoplankton showed no significant differences in protein, total lipid, carbon, carbohydrate or nitrogen per cell which were consistently associated with the greater nutritional value of HL cells. In spite of this high variability in proximate composition, the larval growth rate was a significant function of the average carbon content, nitrogen content and cell volume of the phytoplankton cells. Increased amounts of the "essential" polyunsaturated FAs 20:5 ω3 and 22:6 ω3 in the phytoplankton were negatively correlated with larval scallop growth rates. Thus HL-grown phytoplankton cells were nutritionally superior to LL-grown cells. This nutritional superiority seems to be determined by the fatty acid composition of the cells which, in turn, is controlled by variation in irradiance. The general tendency of predator FA profiles to resemble that of their prey was not observed in larvae fed *P. lutheri*. The much greater amounts of 18:4 ω3, 20:5 ω3, and 22:6 ω3 FA in *P. lutheri* relative to *C. simplex* were not evident in the scallop larvae fed these cells.

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Introduction

Variation in the biochemical composition of phytoplankton can affect the nutritional value of the phytoplankton for herbivores (Galleger and Mann 1981, Enright et al. 1986, Thompson et al. 1993). The biochemical composition of phytoplankton is significantly influenced by physical and chemical factors such as irradiance (Cohen et al. 1988, Sukenik et al. 1989), temperature (Thompson et al. 1992 a, b) and nutrient availability (Mortensen et al. 1988, Harrison et al. 1990), which vary in natural ecosystems or can be manipulated in hatcheries or laboratories. In spite of the potential for shifts in the biochemical composition of phytoplankton to influence the growth of aquatic herbivores, the subject remains poorly studied in natural ecosystems and is only partly resolved for a few species combinations in hatchery and/or laboratory systems.

Given the relatively low success of artificial diets to support the growth of larval bivalves (Langdon et al. 1985, Chu et al. 1987), most research and most bivalve hatcheries still rely upon live phytoplankton as food (De Pauw et al. 1983), however there exists little consensus on which biochemical constituents of phytoplankton are important in determining their food value to planktonic herbivores. A review of the literature on bivalve larvae (Webb and Chu 1982) suggested that good diets were those high in lipids, with balanced ratios of certain "essential" fatty acids. Others have emphasised the requirement for a diet with a balance of protein, lipid and carbohydrate (Whyte et al. 1989), while it has been shown that cells low in protein can constitute a superior diet for *Crassostrea gigas* (Utting 1986).

Recently it has been demonstrated that the proportions of short-chain saturated fatty acids (e.g. 14:0 and 16:0) in several species of phytoplankton could be correlated with their nutritional value for larval *C. gigas* (Thompson et al. 1993), suggesting that the nutritional value of phytoplankton may have a consistent basis related to their lipid composition.

It is undoubtedly true that many marine organisms require a dietary source of certain polyunsaturated fatty acids (PUFAs) because they do not synthesize some PUFAs at rates sufficient to meet their physiological needs (Watanabe et al. 1983). Such PUFAs are commonly called "essential" FA (EFAs). While field studies have shown that many zooplankton species sequester PUFAs (Corner and O'Hara 1986, Sargent and Hendersen 1986), there is remarkably little experimental evidence that diets containing more EFAs are nutritionally superior for herbivores. There is now growing evidence that the importance of dietary PUFAs may have been overgeneralized (Dickey-Collas and Geffen 1992, Thompson et al. 1993). There is very little information on the absolute or relative amounts of dietary PUFAs that are required for maximum growth rates of most marine invertebrates.

In the present study, we used monospecific diets of *Chaetoceros simplex* and *Pavlova lutheri* grown at high and low irradiance. By using variation in irradiance to manipulate the fatty acid composition of phytoplankton (Orcutt and Patterson 1974, Sukenik et al. 1989, Thompson et al. 1990) we have produced monospecific phytoplankton diets which vary in their FA composition (Thompson et al. 1993). We examine the possibility that the nutritional value of phytoplankton is associated with their content of various fatty acids, both short-chain saturated fatty acids and the EFAs, 20:5 ω3 or 22:6 ω3. Also variations in the carbon, nitrogen, protein, lipid, and carbohydrate content of the phytoplankton cells, as influenced by irradiance, were investigated as potential factors affecting the nutritional value of the phytoplankton cells.

Materials and methods

Algal culture and medium

Chaetoceros simplex Ostenfield (NEPCC #591), and *Pavlova lutheri* Droop (NEPCC #5) were obtained from the Northeast Pacific Culture Collection (NEPCC), Department of Oceanography, University of British Columbia. Cultures were grown in enriched natural seawater using the nutrient-enrichment solutions (ES) of Harrison et al. (1980). The medium was modified as in Thompson et al. (1991). Most of the procedures used are in Thompson and Harrison (1992) and Thompson et al. (1993).

All the 8-liter phytoplankton cultures were grown in computer-controlled turbidostats similar in design to those used by Ostgaard et al. (1987). High-light cultures were continuously illuminated by four Vita-lite^R fluorescent tubes (Fig. 1), and low-light cultures by one tube. The following irradiances were measured using a Biospherical^R 4 π meter at the center of the cultures at normal operating cell densities: saturating white-light culture (HL), 160 µmol photons $m^{-2} s^{-1}$; low white-light culture (LL), 19 µmol photons $m^{-2} s^{-1}$. Phytoplankton cultures were grown at 17 °C. Cell densities were maintained near 9×10^8 cells l^{-1}. Inflow nutrient concentrations were never limiting and these cells grew at the maximum rates for the prevailing conditions of light and temperature. The cultures were stirred at 60 rpm (0.6 gravity) with a 7.6 cm teflon-coated magnetic bar, and bubbled. The pH of most cultures was checked daily (range 8.2 to 8.5) and adjusted by variation in the ratio of CO_2 to air to ~8.2.

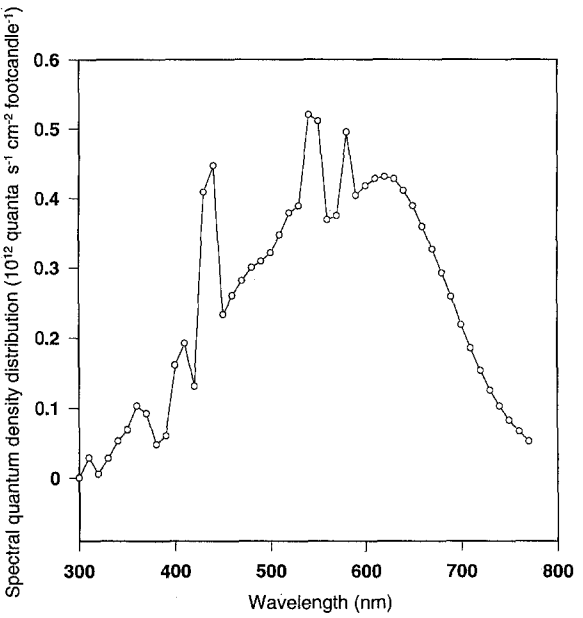

Fig. 1 Spectral quantum data for Vita-lite® fluorescent bulbs. Data and bulbs from Duro-test Corporation, North Bergen, New Jersey, USA

Determination of phytoplankton biomass in each culture was made once per day by measuring in vivo fluorescence with a Turner Designs^R Model 10 fluorometer, and cell counts utilising a Coulter Counter^R Model TAII equipped with a population accessory.

Biochemical composition of algae and of *Patinopecten yessoensis*

Samples for the determination of phytoplankton biochemical composition were collected every 3 d. The data from each phytoplankton culture over the duration of the experiment were pooled to provide a mean for each biochemical parameter for each treatment (=algal diet). Particulate organic carbon and nitrogen (POC and PON) subsamples (25 ml) were collected on precombusted 13 mm Gelman A/E glass-fiber filters (nominal pore size 1 µm) and were analyzed on a Carlo Erba CNS analyzer. Subsamples (100 ml) for total lipid were extracted in chloroform:methanol:water (Bligh and Dyer 1959) and analyzed by the lipid charring technique (Marsh and Weinstein 1966), using tripalmitin as a standard. Total lipid also contained chlorophyll *a*. Samples for determination of total polymeric carbohydrates were collected from the methanol:water fraction of the total lipid extraction, hydrolyzed in sealed borosilicate glass tubes containing 3 ml of 1 N H_2SO_4 for 20 h at 100 °C, and analyzed by the phenol-sulphuric acid technique of Dubios et al. (1956). Subsamples (50 ml) for total protein were collected on precombusted 25 mm GF/F filters and stored at −20 °C for later protein analysis by the modified Lowry technique (Lowry et al. 1951, Dortch et al. 1984).

Phytoplankton subsamples (3 to 5 liters) for fatty acid determinations were collected by centrifugation, freeze-dried, and sealed in precombusted glass bottles filled with nitrogen gas. At the end of the experiment, scallops were collected on 50 µm Nitex netting, rinsed briefly with distilled deionized water, freeze-dried and stored frozen under nitrogen gas. Prior to analysis, samples were frozen at −20 °C for periods of < 4 wk, or for longer periods at −80 °C. Samples were saponified and methylated as described in Whyte (1988). An internal standard was included with each weighed phytoplankton sample prior to saponification and methylation, allowing for the estimation of the absolute quantity of FAs per unit dry weight. FAs were analyzed on a Hewlett-Packard 5890A gas-liquid chromatograph fitted

with a Supelcowax 10 fused silica capillary column (30 m×0.32 mm i.d. 0.25 µm film) and identified by comparison with saturated and PUFA-1 methyl ester standards (obtained from Supelco Inc.) in accord with Ackman (1986). The shorthand notation used in fatty acid identification is *L:B ωX*, where *L* is the chain length, *B* is the number of double bonds, and *ωX* is the position of the double bond closest to the terminal methyl group.

Scallop culture

Patinopecten yessoensis Jay larvae were grown following the guidelines developed by Bourne et al. (1989). Recently fertilized scallop larvae from Island Scallop^R (Qualicum, British Columbia), were split into 8 groups (2 per treatment) each consisting of 10000 larvae in 5 liters of water. Unenriched natural seawater (collected from a minimum depth of 7 m, 28‰ S) for the scallop experiments was prefiltered, first through 25 µm, then through 1 µm cartridge filters and finally through a Gelman A/E glass-fiber filter. Water was changed every 3 d. To change the water, larvae were gently strained onto 100 µm Nitex netting, concentrated into 250 ml, and mixed. A 10 ml subsample was removed for growth and survival measurements and the remainder was added to 5 liters of filtered natural seawater at 15 °C. After each water change, all treatments (diets) received an equal number of phytoplankton cells, starting at 10000 and rising to 20000 phytoplankton cells ml^{-1}, over the duration of the experiment. Overnight losses of phytoplankton due to grazing were estimated from in vivo fluorescence, and returned to original density at 24 and 48 h. Every 64 h, the water was changed again and the scallops were provided with fresh phytoplankton. Antibiotics (50 $\mu g\,l^{-1}$ streptomycin and 30 $\mu g\,l^{-1}$ penicillin G) were also added at each water change to reduce the possibility of problems with bacterial contamination (Bourne et al. 1989). Scallops were bubbled with air injected near the bottom of the flasks. Experiments were terminated after 18 d.

Scallops were measured from umbo to opposite edge using a calibrated digitizing table and images projected from an inverted microscope. A minimum of 50 individuals per replicate were examined to determine mean size every 3 d. Growth rates were calculated as the slope of the least-squares linear regression of the natural log of scallop size versus time. Similarly, to estimate mortality, an initial sample containing a maximum of ~630000 individuals was examined, and towards the end of the experiments a minimum of 55 individuals was examined. The slope of a least-squares linear regression of the percentage of dead scallops versus time was used to estimate the mortality rate.

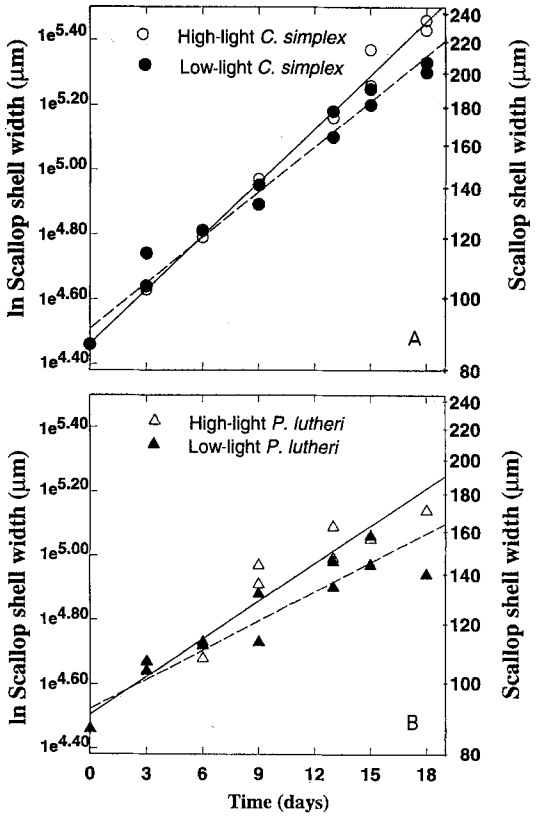

Fig. 2 *Patinopecten yessoensis.* Increases in mean size over time for larval scallop fed phytoplankton cells grown under different conditions. Scallop larvae were fed (A) high-light (HL)-grown *Chaetoceros simplex* (○), or low-light (LL) *C. simplex* (●), or (B) HL *Pavlova lutheri* (△) or LL *P. lutheri* (▲). Each data point is mean value of a minimum of 50 larvae. Least-square regressions are shown fitted to pooled data ($n=2$) for each treatment (continuous line = HL diet, dashed line = LL diet)

Results

Scallops

Patinopecten yessoensis fed HL (high-light)-grown *Chaetoceros simplex* grew from a mean size of 80 to 231 µm in 18 d at 15 °C (Fig. 2A). Scallops fed LL (low-light)-*C. simplex* were significantly smaller, with a mean size of only 202 µm at 18 d (Student's *t*-test, $P \leq 0.000$). Growth of larval scallops fed *C. simplex* showed no lag, and shell size increased exponentially during the 18 d period (Fig. 2A). The 95% confidence intervals on the slopes from the regressions did not overlap and the growth rates calculated for the scallops given HL versus LL *C. simplex* cultures were significantly different (Student's *t*-test, $P \leq 0.004$). Thus, the scallops fed diets of HL-grown *C. simplex* grew faster than those fed LL-grown *C. simplex* (Table 1) and were 13% larger by Day 18 (Fig. 2A). Scallops fed LL-grown *C. simplex* were larger and also grew faster (Student's *t*-test, $P \leq 0.02$) than those fed HL *Pavlova lutheri* (Table 1).

After 18 d, scallops fed HL *Pavlova lutheri* averaged 172 µm, while those fed LL *P. lutheri* were significantly smaller (Student's *t*-test, $P \leq 0.002$), with a mean size of 142 µm (Fig. 2B). Variability in the slope of the regression of scallop size versus time was high, evidently because scallops fed *P. lutheri* failed to grow exponentially towards the end of the experiment (Fig. 2B). Thus, the regression analysis failed to demonstrate a difference in the growth rates of scallops fed HL versus LL *P. lutheri* (Fig. 2B). However, by using the slopes from the regressions of scallops size versus time from each of the replicate experiments, it was possible to show that HL-grown *P. lutheri* supported significantly higher growth rates of scallops than LL-grown *P. lutheri* (Student's *t*-test, $P \leq 0.006$) (Table 1).

Variation in larval scallop survival was similar to variation in growth rates. Mortality was highest for scallops fed *Pavlova lutheri*, and significantly lower (Student's *t*-test, $P \leq 0.05$) for those fed *C. simplex* (Fig. 3). HL-grown *Chaetoceros simplex* diets yielded the lowest mortality rates (Fig. 3), although these were not significantly lower than those fed LL *C. simplex*.

Table 1 *Patinopecten yessoensis*. Growth and mortality responses of scallop larvae in relation to biochemical composition of diets: high-light (HL) or low-light (LL)-grown *Chaetoceros simplex*; HL or LL-grown *Pavlova lutheri*. Values are means ±1 standard deviation where $n \leq 3$, or means ± ranges where $n = 2$

Diet	Scallop results (d^{-1})	($\% d^{-1}$)	Biochemical composition of algal diets (pg $cell^{-1}$)					Cell vol (μm^3)
	Growth rate ($n=2$)	Mortality ($n=2$)	Carbon ($n=4$)	Nitrogen ($n=4$)	Protein ($n=4$)	Lipid ($n=4$)	Carbohydrate ($n=4$)	($n=14$)
C. simplex								
HL	0.055±0.0009[a]	1.2±0.4	15.9±6.5	2.5±1.0	8.2±1.4	4.7±0.4[b]	2.3±1.3	125.4±28
LL	0.047±0.0006	2.2±0.4	12.8±1.5	2.3±0.2	11.0±1.7	6.9±1.4	1.1±0.2	71.6±15[b]
P. lutheri								
HL	0.040±0.0019[a]	4.3±1.3	11.6±3.6[b]	1.3±0.1[b]	6.6±1.9[b]	5.9±0.8[b]	3.0±1.6	38.2± 6.4[b]
LL	0.028±0.0001	3.7±0.1	5.7±0.2	0.9±0.1	2.4±0.5	3.7±0.7	0.7±0.1	21.6± 2.1

[a] $P \leq 0.05$ (Student's *t*-test): growth rate significantly different from larvae receiving diet of the same phytoplankton species grown at the alternate irradiance

[b] $P \leq 0.05$ (Student's *t*-test): parameter significantly different from same phytoplankton species grown under the alternate irradiance

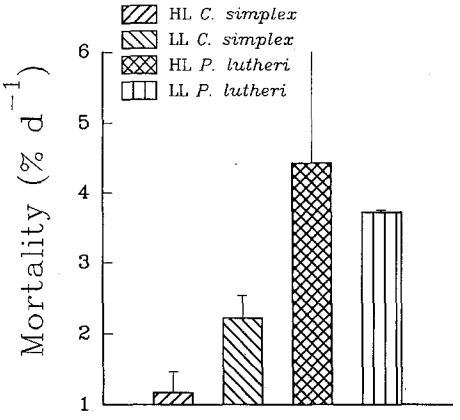

Fig. 3 *Patinopecten yessoensis*. Mortality rates of scallop larvae fed either *Chaetoceros simplex* or *Pavlova lutheri* grown under high (HL) or low (LL) light for 18 d. Bars show ½ range, $n=2$

Phytoplankton

Cultures of *Chaetoceros simplex* showed a tendency to develop short chains of 2 and sometimes up to 8 cells. This tendency was more pronounced in the HL culture. In both cases, the majority of cells were present as single cells of ~50 to 60 μm^3, with 10 to 25% of the cells in short chains, which dramatically increased the mean cell volume as measured by the Coulter counter. This problem also complicates the interpretation of the biochemical composition data when standardized to cell numbers for *C. simplex*, because one "cell" may actually be a chain of up to 8 cells (Table 1). HL-grown *C. simplex* contained significantly (Student's *t*-test, $P \leq 0.05$) less lipid per cell than LL-grown *C. simplex*, but they were otherwise statistically similar in carbon, nitrogen, protein, and carbohydrate $cell^{-1}$ (Table 1). Although quite variable, HL-grown *C. simplex* was significantly larger (Student's *t*-test, $P \leq 0.05$) than LL-grown *C. simplex*.

HL-grown *Pavlova lutheri* contained significantly more carbon, nitrogen, protein and lipid than LL-grown *P. lutheri* (Table 1). HL-grown *P. lutheri* cells were also larger than *P. lutheri* cells grown at LL (Table 1). Although sometimes statistically insignificant, all HL-grown cells had more carbon, nitrogen and carbohydrate than the same species grown at LL (Table 1). None of the significant changes in proximate composition were consistently associated with a diet of greater nutritional value for larval scallops (Table 1), yet larval growth rate was a significant function of the average cell volume, carbon and nitrogen content (Fig. 4). These relationships did not hold for carbon and nitrogen content standardized per unit cell volume.

Fatty acids

FA data are standardized per unit dry weight which removes the bias associated with chain formation in *Chaetoceros simplex*. Irradiance had a significant influence on the fatty acid composition of both *C. simplex* and *Pavlova lutheri* (Table 2). *C. simplex* cells grown under greater irradiance contained significantly more 14:0, 16:0, 16:1 $\omega 7$, 16:2 $\omega 4$(?), 16:4 $\omega 1$ and less 16:2 $\omega 7$ (Table 2). Although not significant (NS, $P > 0.05$), HL-grown *C. simplex* showed a tendency towards less 20:5 $\omega 3$ than LL *C. simplex* (Table 2). Previous research has shown the proportion of 20:5 $\omega 3$ to be negatively correlated with the light-limited growth rates of *C. simplex* (Thompson et al. 1990). Variability in FA content was higher in the present study than in previous studies (Thompson and Harrison 1992, Thompson et al. 1993), in part due to the standardization of results per unit dry weight.

The fatty acid composition of *Pavlova lutheri* was also influenced by irradiance. HL-grown *P. lutheri* cells also contained significantly more 16:0 than LL-grown cells (Table 2). A comparison of the FA profiles of *Chaetoceros simplex* and *P. lutheri* revealed some very strong differences. *P. lutheri* contained 54 to 58 mg g^{-1} PUFA, while *C. simplex* contained only 31 to 38 mg g^{-1} PUFA. Conversely,

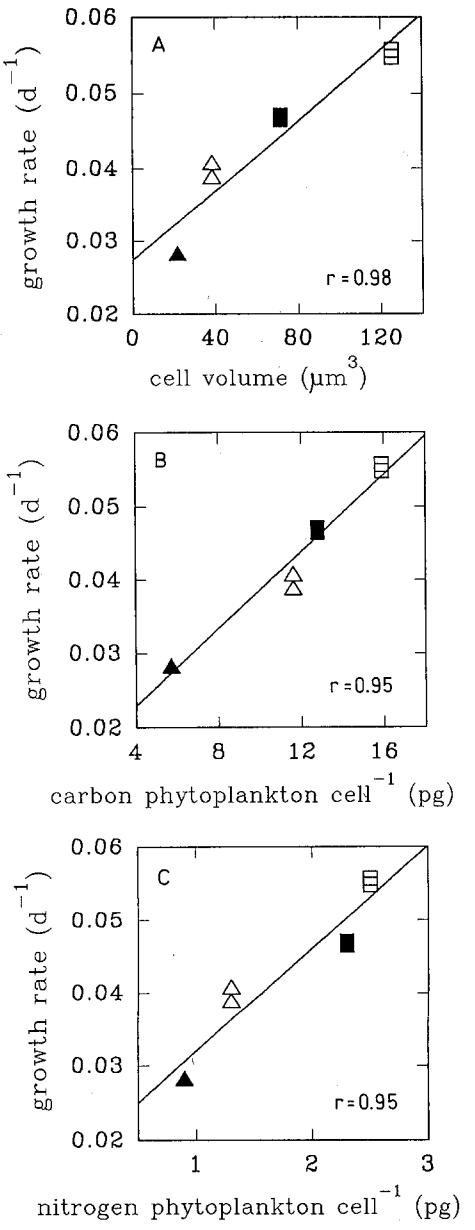

Fig. 4 *Patinopecten yessoensis.* Specific growth rates (d^{-1}) of larvae *P. yessoensis* as a function of diets of HL *Chaetoceros simplex* (□), LL *C. simplex* (■), HL *Pavlova lutheri* (△), or LL *P. lutheri* (▲). Lines are linear least-square fits

Table 2 *Chaetoceros simplex* and *Pavlova lutheri.* Major fatty acids (FA) (>1 mg g^{-1} dry wt) of algae grown under high (HL) or low (LL) light. Values [mg FA (g cell)$^{-1}$] are means ±1 SD (n=4). Fatty acids identified in accordance with Ackman (1986). PUFA: polyunsaturated fatty acids; SAT: saturated FA

FA	*C. simplex*		*P. lutheri*	
	HL	LL	HL	LL
14 : 0	32.2±4.9	23.2±3.4*	14.8±1.3	11.8±3.5
16 : 0	9.3±3.7	2.8±0.2*	19.2±3.2	12.5±2.4*
16 : 1 ω7	20.2±5.2	9.1±1.3*	21.7±6.5	14.9±3.0
16 : 1 ω5	1.2±0.2	1.4±0.2	0.1±0.1	0.0±0.0
16 : 2 ω7	3.1±0.2	4.2±0.5*	0.2±0.1	0.5±0.1
16 : 2 ω4(?)	3.3±0.6	5.6±1.2*	1.2±0.1	1.9±0.7
16 : 3 ω4	11.2±1.8	16.0±2.1	0.4±0.1	1.0±0.3
16 : 4 ω1	3.2±0.9	1.8±0.6*	0.0±0.0	0.2±0.1
18 : 1 ω9	0.2±0.0	0.3±0.0	1.3±0.7	0.4±0.1
18 : 1 ω7	0.4±0.1	0.4±0.1	1.4±0.3	1.4±0.3
18 : 2 ω6	0.2±0.1	0.3±0.0	2.9±1.3	0.8±0.2
18 : 3 ω3	0.0±0.0	0.1±0.1	1.3±0.6	1.6±0.4
18 : 4 ω3	1.6±0.5	1.1±0.3	10.3±2.8	13.5±5.9
20 : 5 ω3	12.2±9.2	16.9±9.4	29.2±2.9	30.8±1.5
22 : 5 ω6	0.0±0.0	0.0±0.1	1.1±0.2	1.2±0.5
22 : 6 ω3	2.0±0.4	2.0±0.6	11.2±1.5	9.4±3.7
Σ PUFA	30.9±13.9	37.9±13.1	53.5±8.1	57.8±12.6
Σ SAT	41.5± 8.6	26.0± 3.6*	34.0±5.5	24.3± 5.9

* $P \leq 0.05$ (Student's t-test): fatty acid composition of HL-grown cells significantly different from LL grown cells

HL *C. simplex* contained 42±9 mg g^{-1} saturated FA, while LL *P. lutheri* contained only 24±6 mg g^{-1} (Table 2).

Of the five FAs which were present in significantly greater amounts in HL- versus LL-grown *Chaetoceros simplex* (14:0, 16:0, 16:1 ω7, 16:2 ω4(?), 16:4 ω1), all were also present in greater proportions in scallops fed HL *C. simplex* (Tables 2 and 3). Faster growing scallops fed HL-grown *C. simplex* contained significantly (Student's t-test, $P \leq 0.05$) more in relative proportions of the FAs 16:1 ω7 and 18:2 ω6, and less 18:0, and 18:2 ω9 than those fed LL-grown *C. simplex* (Table 3). Scallops fed both HL- and LL-grown *C. simplex* contained 18% 20:5 ω3 and ~4.6% 22:6 ω3 (Table 3). When the proportions of 20:5 ω3 and 22:6 ω3 found in the scallops were compared with the proportions provided in their diets of *C. simplex*, 20:5 ω3 was approximately equal and 22:6 ω3 two times greater (Table 3). Other FAs present in greater proportions in the larval scallops relative to their diets of *C. simplex* included: 16:0, 18:0, 18:1 ω9, 18:1 ω7, 20:1 ω9, and 20:1 ω7 (Table 3).

The large amounts of PUFAs found in *Pavlova lutheri* were not evident in the scallops consuming these cells (Table 3). Diets consisting of *P. lutheri* contained 30 mg g^{-1} (26% of total FA) 20:5 ω3 and 10 mg g^{-1} (9% of total FA) 22:6 ω3, levels two to five times greater than in diets of *Chaetoceros simplex*. The relative proportions of these PUFAs were unexpectedly low in the scallops consuming *P. lutheri* at ≤2% 20:5 ω3 and 1.5% 22:6 ω3. Scallops fed *P. lutheri* showed several significant differences in their FA composition between those larvae fed HL versus LL-grown cells, also many significant differences from those fed *C. simplex* (Table 2). Unlike those scallop larvae fed *C. simplex*, there was very little correspondence between the FA composition of *P. lutheri* and the FA composition of larvae fed *P. lutheri*. For example, HL *P. lutheri* contained significantly more 16:0 than LL *P. lutheri*, yet scallop larvae fed HL *P. lutheri* contained a significantly lower proportion of this FA (cf. Tables 2 and 3). Scallop larvae fed *P. lutheri* contained relatively more 16:0, 18:0, 18:1 ω9, 18:1 ω7, 20:1 ω9, 20:1 ω11 and 22:1 ω11 than was contained in their diet (Table 3).

Considered over both phytoplankton species there were significant positive correlations (r=0.95, $P \leq 0.01$, and

Table 3 *Patinopecten yessoensis*. Major fatty acids (>1% of total fatty acids) of scallop larvae fed either *Chaetoceros simplex* or *Pavlova lutheri* grown under high (HL) or low (LL) light. Means ±ranges given as a percentage of total fatty acids for $n=2$. Fatty acids identified in accordance with Ackman (1986)

FA	Larvae fed *C. simplex*		Larvae fed *P. lutheri*	
	HL	LL	HL	LL
14 : 0	7.2±0.1	4.8±1.2[d]	3.6±0.2	4.5±0.1[a, b]
Unknown #	1.2±0.1	1.1±0.3	0.1±0.3	0.0±0.0[b]
15 : 0	0.5±0.1	0.6±0.0	0.7±0.1	1.1±0.1
16 : 0	14.3±0.5	13.7±1.2[c]	19.5±0.0	24.7±1.3[a, b, c]
16 : 1 ω9	0.6±0.1	1.0±0.1	0.8±0.1	1.5±0.3
16 : 1 ω7	7.1±0.1	4.9±0.4[a, d]	5.2±0.2	6.2±0.7[d]
16 : 2 ω7	2.0±0.0	1.6±0.2	0.0±0.0	0.0±0.0[b]
16 : 2 ω4(?)	1.2±0.0	0.8±0.5	0.2±0.1	0.0±0.0[b]
16 : 3 ω4	5.9±0.1	5.8±0.4[d]	0.6±0.4	0.4±0.1[b]
16 : 4 ω1	1.6±0.2	1.0±0.0[d]	0.0±0.0	0.0±0.0[d]
18 : 0	4.3±0.1	6.2±0.3[a, c]	5.0±0.4	7.0±0.6[a, c]
18 : 1 ω9	7.1±0.2	7.2±2.5[c]	19.8±2.7	22.5±1.5[b, c]
18 : 1 ω7	4.0±0.3	3.5±0.4[c]	3.5±0.1	4.2±0.0[a, c]
18 : 2 ω9	0.1±0.0	1.7±0.0[a]	0.6±0.0	0.7±0.0
18 : 2 ω7	0.0±0.0	0.0±0.0	0.0±0.0	2.3±0.9
18 : 2 ω6	3.5±0.4	1.0±0.1[a]	0.0±0.0	0.0±0.0[b]
18 : 2 ω4	0.6±0.1	0.9±0.9	3.6±4.4	0.0±0.0
18 : 4 ω3	1.0±0.1	0.5±0.5[d]	0.2±0.0	0.1±0.2[b]
20 : 1 ω11	1.1±0.1	1.0±1.3	2.2±0.1	2.3±0.3[c]
20 : 1 ω9	1.2±0.0	1.5±0.0[c]	2.6±0.2	2.8±0.2[b, c]
20 : 1 ω7	1.0±0.1	1.1±0.4[c]	0.3±0.0	0.1±0.1[b]
20 : 2 f	0.3±0.1	1.1±1.1	0.7±0.0	0.7±0.1
20 : 5 ω3	18.2±0.1	18.7±1.3	2.3±0.9	1.0±0.3[b, d]
22 : 1 ω11	0.0±0.0	0.0±0.0	2.1±0.2	2.0±0.4[b, c]
22 : 6 ω3	4.5±0.1	4.8±0.4[c]	1.6±0.1	1.4±0.3[b, d]
24 : 1 ω9	0.0±0.0	0.0±0.0	1.2±0.2	1.1±0.3[b, c]

[a] $P \leq 0.05$ (Student's t-test): fatty acid composition of larvae fed LL-grown phytoplankton significantly different from those fed HL-grown phytoplankton of same species

[b] $P \leq 0.05$ (Student's t-test): fatty acid composition of larvae fed *C. simplex* (HL+LL) significantly different from those fed *P. lutheri* (HL+LL)

[c] $P \leq 0.05$ (Student's t-test): proportion of this FA in larvae significantly greater than in diet

[d] $P \leq 0.05$ (Student's t-test): proportion of this FA in larvae significantly less than in diet

r=0.75, $P \leq 0.05$, respectively) between the amount of 14:0, and 14:0+16:0 (=total saturated FA) per phytoplankton cell and the growth rates of the larval scallops consuming those cells (Fig. 5 A). Trends within each species were consistent with these relationships. Similarly, there were negative correlations between the amounts of 20:5 ω3 (r=0.93, $P \leq 0.01$), 22:6 ω3 (r=0.78, $P \leq 0.05$), 20:5 ω3+22:6 ω3 (r=0.89, $P \leq 0.01$) and total PUFA (r=0.95, $P \leq 0.01$) per phytoplankton cell and the growth rates of the larval scallops fed those cells (Fig. 5 B).

The proportions of specific FA found in both groups of scallops fed either *Chaetoceros simplex* or *Pavlova lutheri* were correlated with their growth rates (Fig. 6). Faster growing scallops had the highest proportions of 14:0, 20:5 ω3 and 22:6 ω3 (Fig. 6 A) and the lowest proportions of 16:0, 18:1 ω9, and 20:1 ω9 (Fig. 6 B). Within diets consisting of one phytoplankton species, trends were not always consistent with the correlations observed over all

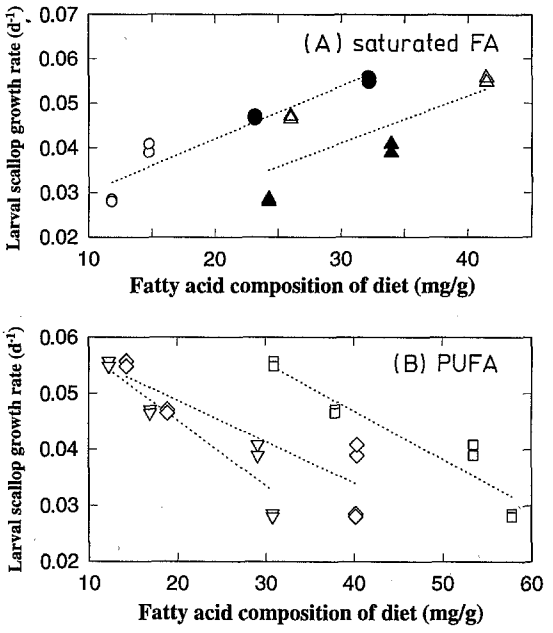

Fig. 5 *Patinopecten yessoensis*. Specific growth rates (d^{-1}) of scallop larvae as a function of amount of various fatty acids in their diets. (A) Saturated fatty acids; ○, ●: 14 : 0 in *Chaetoceros simplex* and *Pavlova lutheri* respectively, △, ▲: 14 : 0 + 16 : 0 in *C. simplex* and *P. lutheri*, respectively. (B) Polyunsaturated fatty acids; ▽: 20 : 5 ω3, ◇: 20 : 5 ω3 + 22 : 6 ω3; □: total polyunsaturated fatty acids. Dotted lines are linear least-square fits

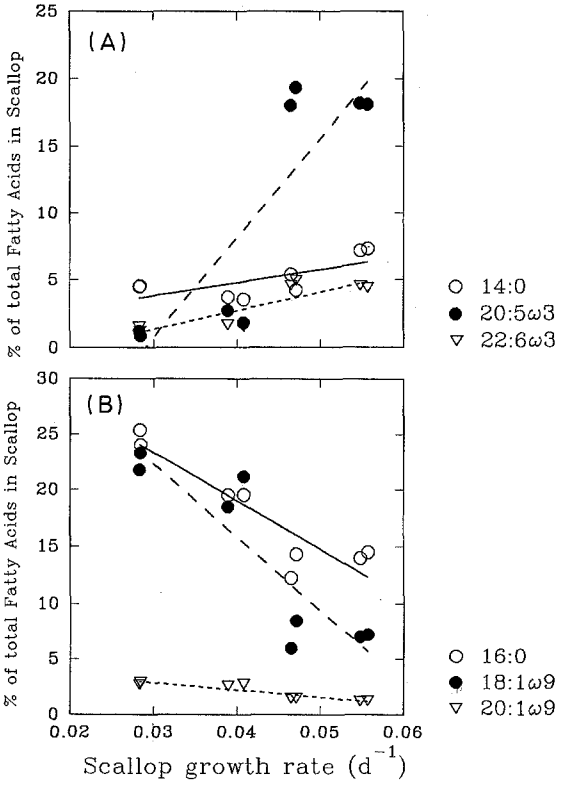

Fig. 6 *Patinopecten yessoensis*. Proportions of specific fatty acids in larval scallops fed either *Chaetoceros simplex* or *Pavlova lutheri*, correlated with larval growth rate. (A) Positive correlations with fatty acids 14 : (○), 20 : 5 ω3 (●), 22 : 6 ω3 (▽); (B) negative correlations with fatty acids 16 : 0 (○), 18 : 1 ω9 (●), 20 : 1 ω9 (▽)

diets. For example, the proportion of 20:5 ω3 and 22:6 ω3 was higher (NS) in scallop larvae fed LL *C. simplex* (growth rate = 0.47 d^{-1}) compared with those fed HL *C. simplex* (growth rate = 0.55 d^{-1}), yet the overall trend was for increasing amounts of these FAs with increasing larval growth rate.

Discussion

In early research using monospecific algal diets to raise bivalve larvae, it was reported that *Pavlova lutheri* (formerly *Monochrysis lutheri*) was a good food item for some bivalve larvae (Davis and Guillard 1958, Walne 1963), although some more recent work has shown it to be a poor food for larvae of the Pacific oyster (*Crassostrea gigas*) (Langdon and Waldock 1981). In our study, *P. lutheri* was a markedly inferior food item relative to *Chaetoceros simplex* for Japanese scallop (*Patinopecten yessoensis*) larvae. At the end of 18 d, larvae fed *C. simplex* where 28% larger than those fed *Pavlova lutheri*. Also, those larvae fed *C. simplex* had a substantially lower mortality than those fed *P. lutheri*. The nutritional value of both *C. simplex* and *P. lutheri* for scallop larvae was greater if they were grown at higher irradiances.

Due to the covariance of phytoplankton cell size, carbon, nitrogen and FA composition, plus the possible covariance of unmeasured factors, we cannot be certain why *Pavlova lutheri* proved to be such a poor food for larval scallops. Although significant relationships existed between the average carbon and nitrogen content phytoplankton $cell^{-1}$ and larval growth rate, they did not exist when these data were standardized per unit cell volume. Furthermore, no similar relationships existed for protein, lipid or carbohydrate $cell^{-1}$. Factors which were significant and consistently different between HL- and LL-grown cells of the same species and between *P. lutheri* and *C. simplex*, were cell size and FA composition. *P. lutheri* was smaller than *C. simplex* (present study), but is well within the size range of other phytoplankton species shown to be good foods for *Patinopecten yessoensis* (Bourne et al. 1989). *C. simplex* contained significantly more saturated FAs than *Pavlova lutheri* (present study). In similar studies, the greater content of saturated FAs in *Thalassiosira pseudonona, Chaetoceros simplex, C. gracilis, Phaeodactylum tricornutum* and *Isochrysis galbana* (Tahitian strain) was consistently associated with superior diets for *Crassostrea gigas*, while increases in cell size, carbon or nitrogen content were not (Thompson et al. 1993). In comparison, we note that our scallop larvae show a remarkably similar response to variation in FA composition of their diets (Fig. 7). The FA data shown (Fig. 7) are expressed as percent of total FA to be consistent with previous studies. This method of standardization also removes a large part of the potential bias associated with cell size and chain formation in *Chaetoceros simplex*. These results (Fig. 7) indicate that five species of phytoplankton grown under HL are a nutritionally superior diet relative to the same phytoplankters grown under LL for two different species of bivalve. Phytoplankton cells grown at HL generally have more saturated fatty acids (Orcutt and Patterson 1974, Sukenik et al. 1989, Thompson et al. 1990), are larger, and have greater carbon quotas (Yoder 1979, Thompson et al. 1991). For most marine phytoplankton the dominant saturated FAs are 14:0 and 16:0 (Ackman et al. 1968, Orcutt and Patterson 1975, Volkman et al. 1989), which provide the basic components of neutral lipids found in *Crassostrea gigas* larvae (Chu and Webb 1984). It is known that greater amounts of neutral lipid in adult oysters (Creekman 1977), eggs (Gallager and Mann 1986) or larvae (Holland and Spencer 1973, Waldock and Nascimento 1979, Gallager et al. 1986) are associated with increased larval vigor, growth and survival. In experiments with larvae of the Sydney rock oyster (*Saccostrea commercialis*), diets supplemented with cod-liver oil yielded by far the best growth rates and a high survival rate (Numaguchi and Nell 1991). Analysis of cod-liver oil showed it had the highest content of the FAs 14:0+16:0 relative to the five other die-

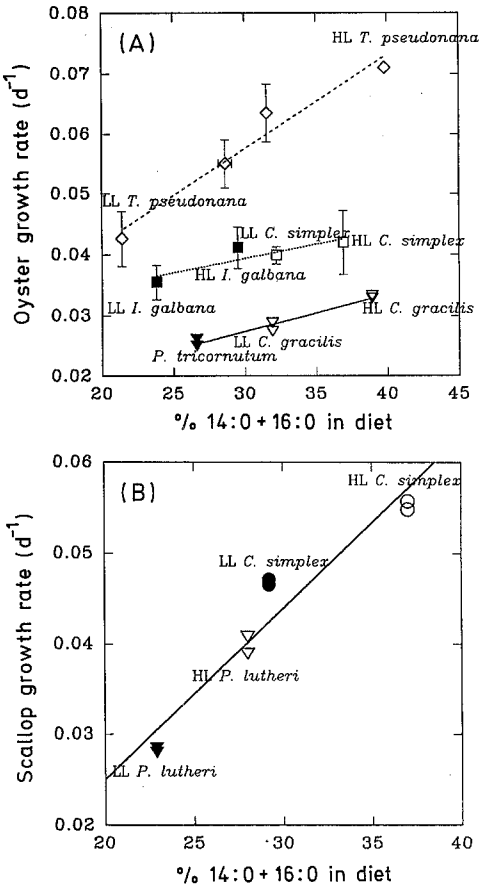

Fig. 7 *Crassostrea gigas* and *Patinopecten yessoensis*. Specific growth rates (d^{-1}) of larvae as a function of proportions of fatty acids 14:0 + 16:0 in their diets. (A) *C. gigas* fed *Thalassiosira pseudonana* (◇) (from Thompson and Harrison 1992), *Isochrysis* aff.*galbana* (■) or *Chaetoceros simplex* (□), and *C. gracilis* (▽) or *Phaeodactylum tricornutum* (▼) (data from Thompson et al. 1993). Where shown, error bars represent ±1 SD ($n \leq 3$). (B) *P. yessoensis* fed *C. simplex* (○, ●), or *Pavlova lutheri* (▽, ▼). HL: high light; LL: low light

tary supplements (Numaguchi and Nell 1991). One possible explanation of the nutritional superiority of diets high in saturated fats and containing only moderate amounts of polyunsaturated fats could be that the food energy is more efficiently released via β-oxidation of saturated fats (Lehninger 1982).

Pavlova lutheri contains considerably more PUFAs, particularly 18:4 ω3, 20:5 ω3 and 22:6 ω3, than *Chaetoceros simplex* (Thompson et al. 1990 and present study). These FAs, particularly the latter two, are important biochemical components of the scallop larvae (Whyte et al. 1989). Because FAs are usually incorporated intact rather than synthesized de novo, most studies with bivalves have shown that the FA composition of the predator resembles that of its prey (Langdon and Waldock 1981, Whyte et al. 1989, Thompson et al. 1993). Greater amounts of specific FA contained in HL *C. simplex* relative to LL *C. simplex* were reflected in *Patinopecten yessoensis* larvae fed these cells (present study). In double-label studies where it is possible to determine the amount of FA transferred from prey to predator, this transfer of intact FAs provided 98% of the FAs in the herbivorous zooplankters *Daphnia magna* and *D. pulex* (Goulden and Place 1990). Given that starved bivalve larvae show no growth (Thompson and Harrison 1992), the ability of *P. yessoensis* to grow, albeit slowly, for 18 d on a diet of *Pavlova lutheri* and yet fail to incorporate a similar FA profile suggests to us that *P. lutheri* somehow affects the lipid metabolism of this predator. Given that *P. lutheri* produces no known metabolites toxic to bivalves (Davis and Guillard 1958, Loosanoff and Davis 1963) and the evidence provided above, we suggest that the poor food value of *P. lutheri* is associated with its FA composition. Based on our research, we cannot separate whether diets high in saturated FAs are beneficial, or diets high in PUFAs are deleterious, but we suggest that the frequently assumed superiority of diets high in EFAs would benefit from a more thorough testing (cf. Dickey-Collas and Geffen 1992). In particular, the tendency to assume that a phytoplankton species such as *P. lutheri* may be a superior food item because it contains more EFAs (Langdon and Waldock 1981) may be unwarranted. For two species of marine bivalve larvae, increases in dietary EFAs were not beneficial (Thompson et al. 1993 and present study).

Several FAs were < 2 to >30 times more abundant in the scallop tissue than in their diets. The greatest increases were in 18 and 20 carbon ω9 and ω7 FAs, which may have been elongated from dietary 16:1 ω7 (Pascal and Ackman 1976, Klingensmith 1982, Whyte 1988). In oyster larvae, the proportions of these FAs were correlated with the proportion of 16:1 ω7 in their diets (Thompson and Harrison 1992), but this was not the case in the present study on scallops, where higher proportions of these 18 and 20 carbon ω7 and ω9 FAs were associated with slow growth in scallops. Because the FA composition of whole bivalve larvae seems to be partly determined by the FA composition of their diet (Waldock and Nascimento 1979, Whyte et al. 1989) and, in part, by the physiological condition of the larvae (Langdon and Waldock 1981), the generalized use of FA profiles as a measure of condition requires further research. The correlations between FA composition and larval groowth rate (Thompson and Harrison 1992, Thompson et al. 1993 and present study) provide evidence that FA composition can be a useful indicator of larval condition, particularly within one batch of larvae fed one or two species of phytoplankton. We suggest that selecting FAs which are largely synthesized de novo or must be modified from dietary FA rather than simply incorporated intact, such as the 18 and 20 carbon ω7 and ω9 FAs (Langdon and Waldock 1981, present study), should help in finding FAs which reflect larval condition rather than mimic the diet.

The fastest growing larvae contained moderately more 16:0, 18:0, and 22:6 ω3 than their diets. The FA 22:6 ω3 is not thought to be synthesized at an appreciable rate, suggesting that these scallops may selectively sequester this FA, as do other predators (Corner and O'Hara 1986). The greater amounts of 16:0 and 18:0 in the scallop larvae may also be selectively sequestered, but these FA can be synthesized de novo (Langdon and Waldock 1981) or elongated from the excess of dietary 14:0.

In conclusion, we believe the results obtained in this study may be useful to commercial bivalve hatcheries and may have some ecological significance. HL-grown phytoplankton cells were nutritionally superior to LH-grown cells. This nutritional superiority seems to be determined by the fatty acid composition of the cells which, in turn, is controlled by variation in irradiance. Further research is needed to determine whether other planktonic herbivores also respond to dietary FAs in a similar manner before the overall ecological significance can be properly assessed.

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