Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Aquaculture 262 (2007) 410–418
Aquaculture
www.elsevier.com/locate/aqua-online

Effect of *Rhodomonas salina* addition to a standard hatchery diet during the early ontogeny of the scallop *Pecten maximus*

Réjean Tremblay [a,*], Simon Cartier [a], Philippe Miner [b], Fabrice Pernet [c], Claudie Quéré [b], Jeanne Moal [b], Marie-Louise Muzellec [d], Michel Mazuret [b], Jean-François Samain [b]

[a] *Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, QC, Canada G5L 3A1*
[b] *DRV/A, Laboratoire de Physiologie des mollusques, IFREMER Centre de Brest, Plouzané, France 29280*
[c] *Institut de Recherche sur les Zones Côtières, Université de Moncton, Shippagan, NB, Canada E8S 1J2*
[d] *Ecloserie du Tinduff, Comité Local des Pêches Maritimes du Nord-Finistère, Plougastel-Daoulas, France 29470*

Received 7 August 2006; received in revised form 6 October 2006; accepted 6 October 2006

Abstract

The main objective of this study was to identify algal diets that maximize the survival and growth and alter the biochemical content of *Pecten maximus* larvae with the aim of improving metamorphosis. We also evaluated the potential of the cryptophyceae *Rhodomonas salina* as a food source for these larvae. Two flagellates, *Isochrysis aff. galbana* (T) and *Pavlova lutheri* (P), and two diatoms, *Chaetoceros gracilis* (C) or *Skeletonema costatum* (S), were tested as two ternary diets, namely PTC and PTS. PTC and PTS were compared with diets that also included *R. salina* (R). The addition of *R. salina* and the replacement of *C. gracilis* by *S. costatum* in the traditional hatchery diet seem to be two interesting alternatives for increasing the productivity of larval scallop culture and improving the metamorphosis rate. With these two diets, larval growth increased and metamorphosis was observed to occur more rapidly. Moreover, our results showed that the addition of *R. salina* significantly improved the overall condition of the larvae by promoting an increase in organic matter and total lipids. This accumulation of lipids during ontogeny seems to promote larvae to grow and to complete metamorphosis more rapidly than with the other diets. The level of polyunsaturated fatty acids in the algae could also at least partially explain the results obtained, since the PUFA level of *C. gracilis* was about half those of *S. costatum* and *R. salina*.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Scallop; Nutrition; Metamorphosis; Energetic content; Algal fatty acids and sterols

1. Introduction

The success of larval scallop culture and the recruitment of postlarvae in natural populations depends on the accumulation of endogenous energy reserves to support larval development and the metamorphic period. Metamorphosis consists of a series of events involving major tissue reorganization to transit from a pelagic to a benthic way of life. During this period, bivalve larvae are limited in their ability to feed on exogenous particulates and rely on endogenous sources of energy. Neutral lipids and proteins account for >80% of the energy budget of metamorphosing bivalves; carbohydrates are usually considered to be of minor importance (Holland and Spencer, 1973; Gallager et al., 1986; Whyte et al., 1992;

* Corresponding author. Institut des Sciences de la Mer, UQAR, 310 allée des Ursulines, Rimouski, Québec, Canada G5L 3A1. Tel.: +1 418 723 1986x1705; fax: +1 418 724 1842.
E-mail address: rejean_tremblay@uqar.qc.ca (R. Tremblay).

0044-8486/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2006.10.009

Videla et al., 1998; Garcia-Esquivel et al., 2001). However, these findings do not rule out the potential of dietary carbohydrate in sustaining the energetic needs of bivalve larvae during early ontogeny. Indeed, larvae of the scallop *Patinopecten yessoensis* fed a diet rich in carbohydrate exhibited a higher nutritional condition compared to those fed low carbohydrate diets (Whyte et al., 1989).

Microalgal polysaccharides are of nutritional significance since the digestive efficiency of marine invertebrates depends on the type of polysaccharide ingested (Kristensen, 1972). For instance, one of the main digestive enzymes of *Pecten maximus* larvae is amylase, suggesting that starch is likely a preferred carbohydrate for this species. *Rhodomonas salina* is known to be rich in starch. Shumway et al. (1985) showed that ingested *R. salina* were absorbed in a majority of the bivalves they studied. Some preliminary results showed that *P. maximus* larvae fed *R. salina* exhibited an increase (by 1.8 times) in organic matter compared to those fed other diets (Robert et al., 1994). Although the use of *R. salina* in a routine diet for different invertebrate species or for juvenile bivalves has been previously reported (Enright et al., 1986; Brown et al., 1998; McCausland et al., 1999), the potential of *R. salina* to improve the growth rate and metamorphosis in larval bivalves has not yet been investigated.

The objectives of the current research were (1) to optimize the classical ternary algal diets used in *P. maximus* hatchery practices with the aim of maximizing larval survival and growth and improving biochemical content before settlement, and (2) to evaluate the addition of the cryptophyceae *R. salina* to the larval diet and to examine the related larval performance during metamorphosis.

2. Material and methods

2.1. Animal maintenance and algal production

Adult scallops, *P. maximus* (about 150 g whole wet weight), were collected from the Bay of Brest and kept in a flow-through seawater system at 15 °C for 30 days and continuously fed with a mixture of *Isochrysis aff. galbana*, *Pavlova lutheri* and *Chaetoceros gracilis* at the Écloserie du Tinduff near Brest (Northern Brittany, France). Scallops were induced to mass spawn by thermal shock following the method of Gruffydd and Beaumont (1970). Two days after fertilization, D-stage larvae were collected on a 45 μm square mesh screen. Five days after fertilization, scallops were transferred to IFREMER's experimental hatchery located in Argenton, France. Nutritional experiments consisting of 5 treatments were conducted in triplicate in 2-L glass beakers at an initial density of 7 larvae ml^{-1}. Larvae were reared in a temperature-controlled room at 19–20 °C in 1-μm cartridge-filtered seawater at 34 ppt. Water was renewed every 2 or 3 days and bacterial growth was limited by the addition of the antibiotic thiamphenicol at 8 mg L^{-1}. Larval cultures were maintained until metamorphosis (21 days post fertilization, dpf), when survivors were harvested for biochemical analysis.

Microalgae were cultured in 18-L carboys with 1-μm filtered sterilized seawater enriched with Conway medium and silicate (40 mg L^{-1}) for diatoms. Algae were maintained at ~20 °C under continuous illumination from cool-white fluorescence lights at a light intensity of 1500 lux and were mixed with aeration (air: CO_2=98.5:1.5%). Diatoms were produced in batch and harvested in late exponential growth phase. Other species were produced following a continuous method and harvested the day before utilization (Robert et al., 1996a).

2.2. Experimental design

Two flagellates, *I. aff. galbana* (T) and *P. lutheri* (P), and two diatoms, *C. gracilis* (C) and *Skeletonema costatum* (S), were tested as two standard hatchery diets, namely PTC and PTS. These diets were compared to a new diet that included *R. salina* (R), namely PTCR. Larvae were fed once a day with a daily ration of one of three diets: 1:1:1 PTC or PTS at 45 cells μl^{-1} (~1.1 ng μl^{-1} dry weight, dw), or 1:1:1:1 PTCR at 60 cells μl^{-1} (~3.3 ng μl^{-1} dw). Two additional control diets were tested to account for the higher biomass of the PTCR diet: PTC*C, which consisted of a daily ration of 165 cells μl^{-1} of PTC (~3.3 ng μl^{-1} dw), and PTC*G, which consisted of an increasing daily ration of PTC, starting with 45 and increasing to 165 cells μl^{-1} (~1.1 to ~3.3 ng μl^{-1}dw from 5 to 21 dpf). The latter treatment was used to avoid an over saturation of the larval culture with algae at the beginning of the experiment. Algal cells were visually examined with a dissecting microscope at 40× and counted using a haemocytometer cell before feeding scallop larvae.

2.3. Growth and survival measurements

Growth and survival were estimated every 2 or 3 days until metamorphosis (21 dpf) for each experimental treatment (n=3 for each of the 5 diets). During the larval cycle, larvae were collected on a 45-μm mesh

and mortality was assessed by counting a sample of approximately 250 individuals. Mortality is expressed as a cumulative number of empty shells based on the total number of shells. From hatching to the beginning of the experiment (2 to 5 dpf respectively), only ca. 10% mortality occurred. Shell growth was calculated on a sample of 50 to 90 larvae per treatment ($n=3\times5$) using shell length measurements (anterior–posterior distance) made with image analysis software (SXM for MAC). Live larvae were recognized by the appearance of the double ring at the margin of the shell, corresponding to a peripheral groove on which the dissoconch shell will be attached (Doroudi et al., 1999). This criterion was used to assess the number of larvae that began metamorphosis (Gerard et al., 1989).

2.4. Ash-free dry weight

The ash-free dry weight of larvae was estimated to evaluate their level of organic matter. Larvae were ground, dried at 80 °C for 24 h to obtain the dry weight, and then heated at 450 °C for 4 h to determine ash weight; a Mettler M3 microbalance was used. The organic matter was calculated from the difference between the dry matter and the ash weight.

2.5. Biochemical analysis

Biochemical analyses of microalgae were carried out in triplicate for each separate microalgal culture of *C. gracilis*, *S. costatum* and *R. salina*. Since the flagellates *I. galbana* and *P. lutheri* were common to all three diets and their biochemical contents are given in several publications (Delaunay et al., 1993; Soudant et al., 1996, 2000; Brown et al., 1997), they were not characterized. Microalgal cultures (5 to 15 ml) were filtered onto 25 mm Whatman GF/F filters pre-combusted at 450 °C. The filters were placed in 15 ml glass tubes containing 6 ml of a mixture of chloroform and methanol (2:1, v:v) with 0.01% butylated hydroxytoluene (BHT) as an antioxidant, closed under nitrogen, shaken, and frozen at −20 °C for lipid extraction following the Folch procedure (Folch et al., 1957). Multiple procedure blanks were prepared. Prior to lipid extraction, samples were sonicated for 10 min in chloroform–methanol (2:1; v/v) using a sonicating bath at 5 °C. Lipid extracts were evaporated to dryness and recovered with three 500-μl washings of 98:2 chloroform:methanol.

Total lipids were transesterified under nitrogen using BF_3/CH_3OH for 10 min at 100 °C. Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph equipped with an on-column injector, a DB-Wax (30 m×0.25 mm; 0.25 μm film thickness) capillary column and a flame ionization detector. Hydrogen was used as the gas carrier. Fatty acids were identified by comparing their retention time with standards and quantified with tricosanoic acid (23:0) as an internal standard.

Sterols were obtained from the neutral lipid fraction as previously described (Marty et al., 1992). Neutral lipids were transesterified using sodium methoxyde (MeONa) at ambient temperature for 90 min. This method allows the protection of certain phytosterols such as 24-methylene cholesterol. Esterified sterols were analyzed using a gas chromatograph equipped with an on-column injector and a Restek Rtx65 fused silica capillary column (15 m×0.25 mm; 0.25 μm film thickness). Hydrogen was used as the gas carrier. Esterified sterol fractions were identified by comparing their retention times with standards and were quantified with cholestane as an internal standard.

One sample of 21-day-old larvae (ca. 6000–11000 ind.) was harvested from each tank onto 25 mm Whatman GF/F filters pre-combusted at 450 °C. Lipids from scallop larvae were extracted for total lipid quantification as previously described for micoalgae. Subsamples were used for colorimetric determinations of proteins (Lowry et al., 1951) and carbohydrates (Dubois et al., 1956). Larvae were rinsed with 10 ml of 37% ammonium formate then homogenized in 1 ml of nanopure water before analysis. Colorimetric assays were performed on a Uvikon® spectrophotometer.

2.6. Statistical analysis

Survivorship patterns were compared using the Life Test procedure from SAS 8.02 (SAS Institute Inc. 1999–2001). Multiple analyses of variance (MANOVAs) were conducted to determine differences in fatty acid and sterol profiles among algal species. MANOVAs on the fatty acid profiles considered total fatty acids (TFA), monosaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), EPA, DHA, and AA as dependent variables. A two-way analysis of variance (ANOVA) was conducted to determine differences in shell length and occurrence of double rings as a function of diet and day. A one-way ANOVA was performed to determine differences in organic matter as a function of dietary treatment. A one-way MANOVA was performed to determine differences in absolute (ng larva^{-1}) and relative (mass% of OM) concentrations of protein, lipid and carbohydrate in 21-day-old scallops. Where differences were detected, least-square differences (LSD) multiple comparison tests were used to determine which means

were significantly different. Residuals were screened for normality using the expected normal probability plot and further tested using the Kolmogorov–Smirnov test. Homogeneity of variance–covariance matrices was tested using the Levene test. Percentages were arcsine square-root transformed to achieve homogeneity of variances. Variance analyses were carried out using SPSS 13.0 for Windows (Chicago, IL). The significance value for all analyses was set at $P<0.05$.

3. Results

3.1. Microalgal lipid composition

Total fatty acids (TFA, in pg cell^{-1}) in *R. salina* were 2.2 and 2.5 times higher than those observed in *C. gracilis* and *S. costatum* respectively (Table 1). The diatom *C. gracilis* was lower in polyunsaturated fatty acids (PUFA) compared to *S. costatum* (by 1.9 times) and *R. salina* (by 2.2 times). Indeed, *C. gracilis* was characterized by elevated levels of saturated (SFA) and monounsaturated (MUFA) fatty acids, with these two fatty acid classes constituting ~72% of the TFA. The long chain PUFAs 20:5n-3 (EPA), 22:6n-3 (DHA) and 20:4n-6 (AA) collectively contributed 48.9, 36.8 and 24.2% of the total PUFA found in *C. gracilis, S. costatum* and *R. salina* respectively. The major long chain PUFA in *C. gracilis* was EPA while AA and DHA remained low. *S. costatum* was characterized by a high level of EPA, an intermediate level of DHA and a low level of AA. Finally, *R. salina* exhibited the lowest level of EPA, an intermediate level of DHA and the highest level of AA. As a consequence, the DHA/EPA ratio varied from 0.11 in *C. gracilis* and 0.38 in *S. costatum* to 0.75 in *R. salina.*

Total sterol concentration (in fg cell^{-1}) in *R. salina* was ~2 times lower than those observed in *S. costatum* and *C. gracilis* (Table 2). High sterol concentrations were characteristic of the microalgal species. For instance, *C. gracilis* had a high level of cholesterol and fucosterol and low levels of 24-methylene cholesterol, *iso-*

Table 1
Fatty acid composition of the diatoms *Chaetoceros gracilis* (CHGRA) and *Skeletonema costatum* (SKEL) and the flagellate *Rhodomonas salina* (RHODO), expressed as % of total fatty acids

Fatty acid	CHGRA	SKEL	RHODO
14:0	16.38	14.39	15.48
16:0	24.85	6.84	20.17
18:0	3.77	0.93	1.46
Σ SFA	**45.45**	**22.27**	**37.11**
16:1n-9	–	0.08	–
16:1n-7	25.31	13.94	1.11
16:1n-13t	0.61	1.14	0.52
18:1n-9	0.24	1.14	11.79
18:1n-7	0.96	1.14	1.68
Σ MUFA	**27.13**	**18.43**	**15.61**
16:2n-7	2.54	1.55	–
16:2n-4	1.93	2.53	–
16:3n-4	5.59	20.55	–
18:2n-6	0.18	2.46	15.01
18:3n-6	0.46	0.07	3.27
18:3n-3	–	0.99	7.98
18:4n-3	0.72	3.75	7.80
20:4n-6 (AA)	0.1	0.27	2.98
20:5n-3 (EPA)	10.71	13.80	4.78
22:6n-3 (DHA)	1.14	5.30	3.58
Σ PUFA	**24.31**	**52.64**	**46.77**
Σ n-3	**13.11**	**24.57**	**24.68**
Σ n-6	**1.09**	**3.35**	**22.06**
n-3/n-6	12.03	7.33	1.12
DHA/EPA	0.11	0.38	0.75
EPA/AA	107.1	51.1	1.60
TFA (pg·cell^{-1})	5.17	4.53	11.34

Also shown are the n-3/n-6, DHA/EPA and EPA/AA ratios as well as the total fatty acids (TFA; pg·cell^{-1}).

Table 2
Sterol compositions of the diatoms *Chaetoceros gracilis* (CHGRA) and *Skeletonema costatum* (SKEL) and the flagellate *Rhodomonas salina* (RHODO), expressed as % of total sterols

Sterol	CHGRA	SKEL	RHODO
Cholesterol	47.21	20.04	9.71
Brassicasterol	1.31	–	90.29
Desmosterol	–	4.04	–
Campesterol	–	28.04	–
24-methylene cholesterol	8.40	41.56	–
Stigmasterol	–	–	–
β-sitosterol	–	6.33	–
Fucosterol	38.18	–	–
Iso-fucosterol	4.90	–	–
Total sterols (fg·cell^{-1})	107.68	98.22	55.68

Table 3
Summary of ANOVAs showing the effect of day and diet on (a) shell length and (b) occurrence of double rings

Source of variation	*df*	MS	*F*-value	*p*
(a) Shell length				
Day	6	1.1×10^{4}	446	<0.001
Diet	4	2.5×10^{3}	99	<0.001
Day*Diet	24	256	10	<0.001
Error	70	25		
Corrected total	104			
*(b) Double ring**				
Day	1	0.478	239	<0.001
Diet	4	0.553	277	<0.001
Day*Diet	4	1.6×10^{-3}	0.818	0.529
Error	20	1.9×10^{-3}		
Corrected total	29			

*Arcsinus transformation for double ring occurrence was applied to normalize the data.

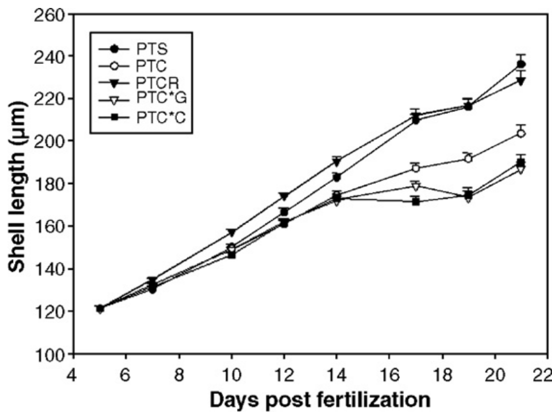

Fig. 1. Shell length of *P. maximus* larvae fed different diets as a function of the day post fertilization (mean±SD; $n=3$ replicate tanks per diet). See text for diet abbreviations.

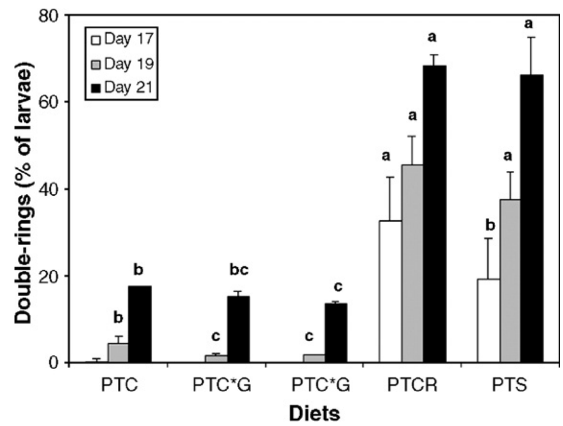

Fig. 3. Occurrence of double rings in *P. maximus* larvae fed PTC, PTC*G, PTC*C, PTCR or PTS a function of the number of days post fertilization (mean±SD; $n=3$ replicate tanks per diet). Different letters indicate significant differences. See text for diet abbreviations.

fucosterol and brassicasterol. In contrast, *S. costatum* exhibited a high level of 24-methylene cholesterol, intermediate levels of campesterol and cholesterol, and low levels of β-sitosterol and desmosterol. Finally, *R. salina* showed two major sterols, brassicasterol (>90% of total sterol) and cholesterol.

3.2. Scallop performance

Diet and day showed an interaction in their effect on the shell length of scallop larvae (Table 3 and Fig. 1). Larvae offered PTS and PTCR exhibited greater shell lengths than those fed all other diets ($P<0.001$), and the PTS and PTCR diets showed similar growth ($P=0.081$). These results are reflected by the growth rates, with values of 7.29±0.25 and 6.94±0.33 µm day^{-1} for PTS and PTCR compared to 5.04±0.53, 4.69±0.43 and 3.88±0.16 µm day^{-1} for PTC, PTCC and PTCG. No dietary effect was detected on scallop survival (Lifetest procedure, $P=0.966$). At the end of the experiment, cumulative survival varied between ~70 to 80% (Fig. 2). The occurrence of double rings varied as a function of day and diet (Table 3 and Fig. 3). The first double rings appeared at around 15 dpf in larvae fed the PTCR diet. The highest percentage of double rings at 17 dpf was recorded for the PTCR diet compared to the PTS diet ($P<0.05$); larvae from the PTC diet had no double rings at all. At 19 and 21 dpf, scallops fed PTCR and PTS were at a similar stage of development ($P=0.981$) and showed a higher level (~5×) of double rings than scallops fed the other diets ($P<0.003$). Occurrences of double rings were similar between larvae fed PTC, PTC*G and PTC*C ($P=0.862$). Finally, dissoconchs were first observed after 19 dpf in cultures fed PTCR and only after 21 dpf for those fed PTS. Cultures

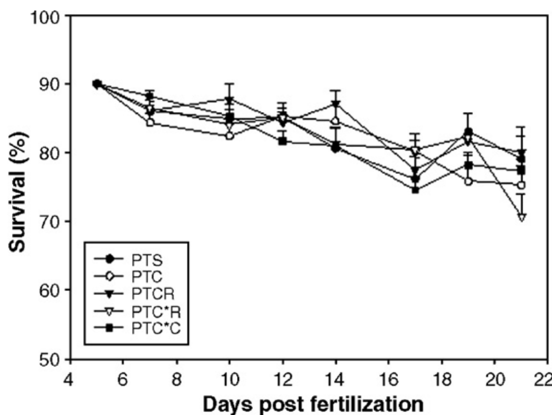

Fig. 2. Survival of *P. maximus* larvae fed different diets as a function of the day post fertilization (mean±SD; $n=3$ replicate tanks per diet). See text for diet abbreviations.

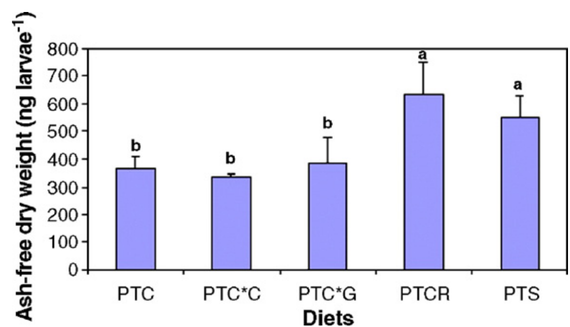

Fig. 4. Ash-free dry weight (ng larvae^{-1}) of *P. maximus* larvae fed PTC, PTC*G, PTC*C, PTCR, or PTS at 21 days post fertilization (mean±SD; $n=3$ replicate tanks per diet). Different letters indicate significant differences between diets. See text for diet abbreviations.

Table 4
Summary of MANOVAs showing the effect of diets on lipids, proteins and carbohydrates at (a) absolute (ng larva^{-1}) and (b) relative (%) values

Diet	*df*	MS	*F*-value	*p*
*(a) Absolute concentration**				
Lipids	4	7.03×10^{-2}	44.1	<0.001
Proteins	4	4.84×10^{-2}	19.3	<0.001
Carbohydrates	4	1.35×10^{-2}	1.8	0.198
Error lipids	10	1.59×10^{-3}		
Error proteins	10	2.51×10^{-3}		
Error carbohydrates	10	7.36×10^{-3}		
Corrected total lipids	14			
Corrected total proteins	14			
Corrected total carbohydrates	14			
*(b) Relative concentration**				
Lipids	4	1.86×10^{-3}	7.3	0.005
Proteins	4	1.84×10^{-3}	5.5	0.013
Carbohydrates	4	2.88×10^{-3}	3.9	0.057
Error lipids	10	2.52×10^{-4}		
Error proteins	10	3.36×10^{-4}		
Error carbohydrates	10	7.39×10^{-4}		
Corrected total lipids	14			
Corrected total proteins	14			
Corrected total carbohydrates	14			

*Logarithmic and arcsinus transformations, respectively, were applied on absolute (ng larva^{-1}) and relative (%) values to normalize the data.

fed other diets did not exhibit any dissoconchs during the experiment. Therefore, PTCR and PTS promoted higher growth rates and better metamorphic yields than the other diets tested.

3.3. Biochemical content

The ash-free dry weight of 21 dpf larvae varied between ~350 and 650 ng larva^{-1}, depending on the dietary treatment ($P=0.002$). Indeed, the top performing larvae fed PTCR and PTS exhibited ash-free dry weights nearly twice as high as larvae fed all other dietary treatments ($P<0.011$; Fig. 4). The absolute contents (amount per larva) of lipids and proteins in 21 dpf larvae were significantly influenced by the diet while carbohydrate content was not (Table 4). Indeed, larvae fed the top-performing PTCR and PTS diets showed an increase (~1.8×) in their lipid and protein contents compared to those fed other diets (Table 5, $P<0.001$). Larvae fed PTCR had higher lipid levels than larvae fed PTS ($P=0.003$); both these diets had lipid levels that were significantly higher than other diets ($P<0.010$). Larvae fed PTCR and PTS had similar proteins levels ($P=0.566$) that were again significantly higher than other diets. Relative concentrations of lipids, proteins and carbohydrates were ~32, 63 and 5% of OM, respectively (Fig. 5). Therefore, differences in the ash-free dry weight were mainly attributable to lipids and proteins since carbohydrates were scarce in 21 dpf larvae.

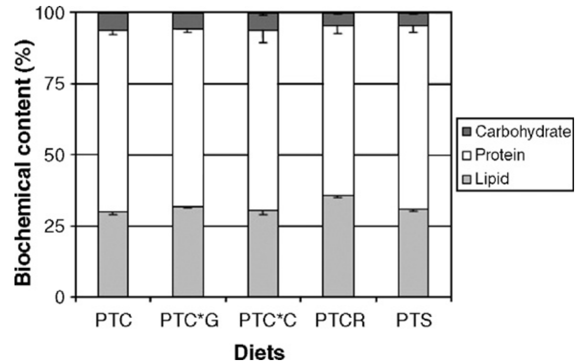

Fig. 5. Relative concentrations of lipids, proteins and carbohydrates of *P. maximus* larvae fed PTC, PTC*G, PTC*C, PTCR, and PTS at 21 days post fertilization (mean±SD, $n=3$ replicate tanks). See text for diet abbreviations.

4. Discussion

Based on growth rate, on the biochemical content of larvae and on metamorphic yield (estimated by the double ring rate), our results showed that PTCR and PTS were the best performing diets, followed by PTC and then PTC*C and PTC*G, for the culture of scallop *P. maximus* larvae. Larvae fed PTS or PTCR showed higher organic matter and total lipid and protein contents than larvae fed PTC. Moreover, larvae fed the PTCR diet had a higher total lipid and underwent metamorphosis earlier compared to larvae fed the PTS diet. Our results show that the addition of *R. salina* significantly improved the condition of the larvae by promoting the

Table 5
The biochemical content of larvae (ng larva^{-1}) at 21 dpf fed with PTC, PTC*G, PTC*C, PTCR and PTS (mean±SD)

	PTC	PTC*G	PTC*C	PTCR	PTS
Lipid	78.5±7.6^{a}	67.2±2.3^{a}	59.6±6.6^{a}	141.5±13.0^{b}	106.4±10.5^{c}
Protein	135.6±10.8 ^{a}	131.4±4.5 ^{a}	122.6±16.3 ^{a}	234.7±38.9 ^{b}	221.0±26.8 ^{b}
Carbohydrate	15.3±1.1^{a}	12.0±0.6^{a}	11.6±2.6^{a}	18.2±4.6^{a}	15.8±3.0^{a}

See text for diet abbreviations.
Values (±SD) with different superscript letters are significantly different ($P<0.05$).

enrichment of organic matter through proteins and total lipids. The starch content in the *R. salina* cells (~40% of total carbohydrates; present study) seemed beneficial to *P. maximus* larvae compared to others algal species; carbohydrate levels in diatoms (*C. gracilis* and *S. costatum*) and prymnesiophytes (*I. galbana* and *P. lutheri*) are generally under 15% (Brown et al., 1997). These carbohydrates could provide an energy source for metabolic demand during larval development. It may also be converted to and accumulated as lipids. This high level of organic matter in the form of proteins and lipids stored in organisms with shells of about the same size at metamorphosis seems to promote larvae to complete metamorphosis more rapidly. The biomass provided by the PTCR diet was approximately three times higher than that of the PTC diet (3.3 compared to 1.1 ng μl^{-1} dw) and was amplified by the larger volume of *R. salina,* which has cell sizes of 11.9 to 12.8 μm in diameter (Schiopu et al., 2006). In addition to the extra biomass provided by the addition of *R. salina* to the traditional larvae diet composed of *P. lutheri*, *I. aff. galbana* and *C. gracilis*, we observed a significant effect of *R. salina* on larval scallop culture. Larvae fed the adjusted PTC diet (on the basis of algal dry weight), that is, with a continuous or gradual addition of algae (PTC*C and PTC*G) to obtain biomasses similar to the PTCR diet, did not show superior growth or higher biochemical content than larvae fed the PTC diet. In fact, lower growth was observed with these diets even though there was a higher availability of organic matter. These results suggest a negative effect of a high concentration of small algal cells on the feeding activity of scallop larvae, as has been reported for mussel larvae (Sprung, 1984, 1989), probably due to the saturation of the particulate filtration system. Lu and Blake (1996) observed that *I. galbana* cell concentrations higher than 40 cells μl^{-1} saturated the ingestion rate of larval bay scallops, *Argopecten irradians*. In our experiment, we did not evaluate whether the 165 cells μl^{-1} of the PTC*C and PTC*G diets decreased the ingestion rate of larvae, but these high algal densities did not increase scallop larva mortality. The increased feed concentrations may cause water quality problems due to bacteria proliferation. Nevertheless, the use of antibiotics to ensure the success of *P. maximus* larval culture seems to limit this problem, as no more mortality was observed in these treatments. The use of antibiotics at a concentration of 8 mg L^{-1} has been demonstrated to be beneficial for the survival of scallop larvae with no impact on the growth rate (Nicolas et al., 1996; Robert et al., 1996a,b). The quantitative advantage for larval growth with *R. salina* in the algal diet may have resulted from the high level of digestible organic matter available. The results suggest a good ingestion and retention ability as well as efficient assimilation of this large microalgae by the *P. maximus* scallop larvae, as has already been demonstrated for adult sea scallops, *Placopecten magellanicus* (Shumway et al., 1985).

Qualitative differences between diets can contribute to performance. The PTS diet, which provided less algal dry weight than the PTCR diet, showed better growth than the PTC diet from day 12 until the end of the experiment and a growth pattern similar to PTCR at the end of the larval cycle (between 17 and 21 dpf). The PUFA levels in our diets could explain at least partially the growth and metamorphic results. Indeed, the PUFA level of *C. gracilis* in the PTC diet was half that of *S. costatum* in PTS and also lower, but to a lesser extent, than *R. salina* in the PTCR diet. Lipids, and more particularly the essential polyunsaturated fatty acids (PUFA), are generally considered to be the most important constituent of the algal diet for bivalve larvae (Whyte et al., 1989; Delaunay et al., 1993; Soudant et al., 1998; Nevejan et al., 2003; Pernet and Tremblay, 2004).

However, the success of the PTS diet is not in accordance with a previous study, where poor ingestion of *S. costatum* in a PTS diet was observed and related to lower growth compared to *P. maximus* larvae fed with *Chaetoceros calcitrans* (Soudant et al., 1998). According to published results on the biochemical compositions of microalgae species, the polyunsaturated fatty acid profile of *S. costatum* is similar to that of *C. gracilis,* showing a predominance of EPA with low levels of DHA, very low levels of AA and low variability between strains (Brown et al., 1989; Volkman et al., 1989; Dunstan et al., 1993). However, in our experiment, the DHA percentage composition was five-fold higher in *S. costatum* compared to *C. gracilis*. It has been previously observed that *P. maximus* larvae fed on diatoms only had a good growth rate but failed at metamorphosis (Delaunay et al., 1993). This was attributed to a high DHA requirement during this period of active changes in morphology and membrane building, as an EPA/DHA ratio of about 2 was highly controlled in polar lipids of *P. maximus* larvae. A higher percentage of DHA in the PTS diet would meet such a requirement better than the PTC diet and would improve the growth and success of *P. maximus* larval metamorphosis; survival was similar with other diets, as has been observed in *Crassostrea gigas* larvae (Volkman et al., 1989) and spat (Thompson and Harrisson, 1992). These high levels of DHA in *S. costatum* compared to values reported in the literature would reflect culture conditions. Many studies indicate that diatoms submitted to nutritive stress will significantly increase their lipid levels (Dunstan, 1993; Von Elert, 2002; Pernet et al., 2003). However,

high DHA levels may also be associated with a specific strain, which seems to be the case in our study. The good performance obtained when PTC was supplemented with *R. salina* in the PTCR diet could also result from a higher DHA/EPA ratio compared to the diet consisting of *C. gracilis* alone.

We observed that saturated fatty acids (SFA) seemed not to be a predominant factor influencing the growth of *P. maximus* larvae: the SFA in *C. gracilis,* included in the PTC diet, were twice as high as those from *S. costatum*. SFA provide more energy via β-oxidation than PUFA, and our results contradict an earlier study showing a superiority of diets containing higher amounts of SFA for *P. magellanicus* and *C. gigas* larvae (Langdon and Waldock, 1981). It is possible that the SFA effect can be detected when other limiting factors are optimized.

The sterol composition of microalgae could be important in determining the nutritional value for bivalves (Wikfors et al., 1996). In our study, we observed that the total amount of sterol was twice as high in *C. gracilis* and *S. costatum* compared to *R. salina*. Furthermore, the cholesterol concentration in *C. gracilis* was more than twice that in *S. costatum* and 5 times higher than concentrations in *R. salina*. Thus, in the diets studied, the level of sterols and more specifically cholesterol seemed not to be limiting for larval growth. Nevertheless, we observed that *S. costatum* sterol is composed mainly of 24-methylene cholesterol (nearly 42%) and that *R. salina* is very rich in brassicasterol (90%), two sterol classes used by oyster spat for the bioconversion of cholesterol (Soudant et al., 2000). Marine bivalves contain complex mixtures of C_{26} to C_{30} sterols (cholesterol $= C_{27}$), with each molecular species being characterized by a planar ring system with a 3β-hydroxyl group and a side chain of varying length. Furthermore, bivalves have a negligible capacity for the biosynthesis or conversion of sterols, although they are capable of selectively incorporating certain sterols (Soudant et al., 1996; Knauer et al., 1999). In *P. maximus,* the preferential incorporation of cholesterol over other sterols has been previously observed (Soudant et al., 1998).

In conclusion, our results suggest that a more efficient diet to improve larval growth of *P. maximus* and to attain metamorphosis more rapidly would be obtained by the addition of *R. salina* to a traditional hatchery diet composed of *I. galbana*, *P. lutheri* and *C. gracilis*. We also observed that the replacement of *C. gracilis* by *S. costatum* in a diet including *I. galbana* and *P. lutheri* seemed to improve larval growth of *P. maximus*. However, this last result seems to be associated with a specific *S. costatum* strain and particular culture conditions. Nevertheless, further investigations are being undertaken to better understand the effect of the addition *R. salina* on the kinetics of fatty acids, lipid classes, proteins and carbohydrates during larval development and for metamorphosis success and spat competency.

Acknowledgements

The authors would like to thank Tinduff technicians Alain Le Gall, Anne Doen and Régis Lasbleiz for providing scallop larvae. The authors are also grateful to Mathieu Cusson and Andréane Bastien for help with statistical analyses and Laure Devine for text revision.

References

Brown, M.R., Jeffrey, S.W., Garland, C.D., 1989. Nutritional Aspects of Microalgae Used in Aquaculture: A Literature Review. Report, vol. 205. CSIRO Marine Laboratories.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 15, 315–331.

Brown, M.R., McCausland, M.A., Kowalski, K., 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture 165, 281–293.

Delaunay, F., Marty, Y., Moal, J., Samain, J.-F., 1993. The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) larvae. J. Exp. Mar. Biol. Ecol. 173, 163–179.

Doroudi, M.S., Southgate, P.C., Mayer, R.J., 1999. Growth and survival of blackslip pearl oyster larvae fed different densities of microalgae. Aquac. Int. 7, 179–187.

Dubois, M., Gillies, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for the determination of carbohydrates and related substances. Anal. Chem. 28, 350–356.

Dunstan, G., 1993. Changes in the lipid composition and maximization of the polyunsaturated fatty acid content of three microalgae grown in mass culture. J. Appl. Phycol. 5, 71–83.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.-M., Jeffrey, S.W., 1993. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35, 155–161.

Enright, C.T., Newkirk, G.F., Craigie, J.S., Castell, J.D., 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J. Exp. Mar. Biol. Ecol. 96, 1–13.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497.

Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture 56, 81–103.

Garcia-Esquivel, Z., Bricelj, V.M., Gonzalez-Gomez, M.A., 2001. Physiological basis for energy demands and early postlarval mortality in the Pacific oyster, *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 263, 77–103.

Gerard, A., Salaun, M., Tritar, S., 1989. Critères de compétences des larves à la métamorphose chez *Pecten maximus*. Haliotis 19, 373–380.

Gruffydd, L.D., Beaumont, A.R., 1970. Determination of the optimum concentration of eggs and spermatozoa for the production of normal larvae in *Pecten maximus*. Helgol. Wiss. Meeresunters. 20, 486–497.

Holland, D.L., Spencer, B.E., 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L., during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assoc. U.K. 53, 287–298.

Knauer, J., Barrett, S.M., Volkman, J.K., Southgate, P.C., 1999. Assimilation of dietary phytosterols by Pacific oyster *Crassostrea gigas* spat. Aquac. Nutr. 5, 257–267.

Kristensen, J.H., 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 14, 130–142.

Langdon, C.J., Waldock, M.I., 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. J. Mar. Biol. Assoc. U.K. 61, 431–440.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lu, Y.T., Blake, N.J., 1996. Optimum concentrations of *Isochrysis galbana* for growth of larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say). J. Shellfish Res. 15, 635–643.

Marty, Y., Delaunay, F., Moal, J., Samain, J.-F., 1992. Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. J. Exp. Mar. Biol. Ecol. 163, 221–234.

McCausland, M.A., Brown, M.R., Barrett, S.M., Diemar, J.A., Heasman, M.P., 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 174, 323–342.

Nevejan, N., Saez, I., Gajardo, G., Sorgeloos, P., 2003. Energy vs. essential fatty acids: what do scallop larvae (*Argopecten purpuratus*) need most? Comp. Biochem. Physiol. 134B, 599–613.

Nicolas, J.-L., Corre, S., Gauthier, G., Robert, R., Ansquer, D., 1996. Bacterial problems associated with scallop *Pecten maximus* larval culture. Dis. Aquat. Org. 27, 67–76.

Pernet, F., Tremblay, R., 2004. Effect of varying levels of dietary essential fatty acid during early ontogeny of the sea scallop *Placopecten magellanicus*. J. Exp. Mar. Biol. Ecol. 310, 73–86.

Pernet, F., Tremblay, R., Demers, E., Roussy, M., 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. grown in a semicontinuous system. Aquaculture 221, 393–406.

Robert, R., Moal, J., Campillo, M.-J., Daniel, J.Y., 1994. The food value of starch rich flagellates for *Pecten maximus* (Linné) larvae. Preliminary results. Haliotis 23, 169–171.

Robert, R., Moal, J., Miner, P., Quéré, C., Connan, J.P., Daniel, J.Y., Le Coz, J.R., Samain, J.F., Mazuret, M., 1996a. Microalgae biomass from photobioreactor as food for fish and shellfish larvae (MANTA). European Community Program, Final Report. A1R1-CT92-0286, pp. 68–91.

Robert, R., Miner, P., Nicolas, J.-L., 1996b. Mortality control of scallop larvae in the hatchery. Aquac. Int. 4, 305–313.

Schiopu, D., George, S.B., Castell, J., 2006. Ingestion rates and dietary lipids affect growth and fatty acid composition of *Dendraster excentricus* larvae. J. Exp. Mar. Biol. Ecol. 328, 47–75.

Shumway, S.E., Cucci, T.L., Newell, R.I.E., Yentsch, C.M., 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91, 77–92.

Soudant, P., Marty, Y., Moal, J., Robert, R., Quere, C., Le Coz, J.R., Samain, J.F., 1996. Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143, 361–378.

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

Soudant, P., Sanles, M.V., Quere, C., Le Coz, J.R., Marty, Y., Moal, J., Samain, J.F., Sorgeloos, P., 2000. The use of lipid emulsions for sterol supplementation of spat of the Pacific oyster, *Crassostrea gigas*. Aquaculture 184, 315–326.

Sprung, M., 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). II. Food uptake. Mar. Ecol., Prog. Ser. 17, 295–305.

Sprung, M., 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: abundance, growth, mortality and food demands. Arch. Hydrobiol. 115, 537–561.

Thompson, P.A., Harrisson, P.J., 1992. Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. Mar. Biol. 113, 645–654.

Videla, J.A., Chaparro, O.R., Thompson, R.J., Concha, I.I., 1998. Role of biochemical energy reserves in the metamorphosis and early juvenile development of the oyster *Ostrea chilensis*. Mar. Biol. 132, 635–640.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219–240.

Von Elert, E., 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnol. Oceanogr. 47, 1764–1773.

Whyte, J.N.C., Bourne, N., Hodgson, C.A., 1989. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture 1989, 333–347.

Whyte, J.N.C., Bourne, N., Ginther, N.G., Hodgson, C.A., 1992. Compositional changes in the larva to juvenile development of the scallop *Crassadoma gigantea* (Gray). J. Exp. Mar. Biol. Ecol. 163, 13–29.

Wikfors, G.H., Patterson, G.W., Ghosh, P., Lewin, R.A., Smith, B.C., Alix, J.H., 1996. Growth of post-set oysters, *Crassostrea virginica*, on high-lipid strains of algal flagellates *Tetraselmis* spp. Aquaculture 143, 411–419.