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Lipid requirements of the scallop *Pecten maximus* (L.) during larval and post-larval development in relation to addition of *Rhodomonas salina* in diet

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Abstract:

The main objective of this study was to evaluate the effect of the addition of *Rhodomonas salina* in the diet of *Pecten maximus* on growth, survival and metamorphosis success in relation to biochemical content. Food quality is an essential factor in the success of larval, post-larval and juvenile development. A diet rich in polyunsaturated fatty acids present an advantage for growth, survival and metamorphosis success. Larvae and post-larvae fed with the diet containing *R. salina* showed a higher accumulation of arachidonic acid (20:4n−6) and generally a lower concentration of docosahexaenoic acid (22:6n−3). Addition of *R. salina* in diet was related with higher level of eicosapentaenoic acid (20:5n−3) in larval stages, but lower level in post-larvae. Addition of *R. salina* seemed to be advantageous during larval stages by a higher accumulation of triacylglycerol associated with an earlier appearance of metamorphosis. The composition of sterol observed in larvae fed with an addition of *R. salina* showed a high level of brassicasterol. Advantage of the preferential accumulation of brassicasterol with the addition of *R. salina* is not clear but other studies suggest that brassicasterol can replace cholesterol in some functions. Our results suggest that addition of *R. salina* at larval stages could be advantageous in the aquaculture hatchery by a more rapid passage of *P. maximus* in the nursery.

Keywords: Nutrition; Biochemical characteristics; Fatty acids; Sterol profiles

1. Introduction

Successful seed production in aquaculture of marine bivalves depends to a large extent on the acquisition of energy reserves to support larval growth and transition through metamorphosis. Most invertebrate larvae primarily rely on lipids to fulfil their energetic needs whereas protein catabolism occurs when the organism is short on lipid reserves (Holland, 1978). Provision of sufficient lipid reserve is particularly important for successful completion of metamorphosis, a critical period during which bivalves are limited in their ability to feed on exogenous particulates (Baker and Mann, 1994) and undergo an energetically-demanding tissue reorganization to transit from a planktonic to a benthic way-of-life.
Growth and survival of marine bivalves also rely on the acquisition of essential fatty acids (EFA) for incorporation into membrane phospholipids (Soudant et al., 1998b; Pernet and Tremblay, 2004). EFA functions can be divided into two broad areas. On one hand, the n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (22:6n-3) is involved in maintaining the structural and functional integrity of biological membranes due to its unique structure, which facilitates rapid conformational changes in membrane proteins (Feller et al., 2002; Turner et al., 2003). On the other hand, C_{20} PUFAs, such as eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6), represent precursors of eicosanoids, a group of highly biologically active hormones that includes prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids (Smith and Murphy, 2003). Eicosanoid production is associated to stressful situations, with excess eicosanoid production having been recorded under pathological conditions in fish (Bell and Sargent, 2003).
Finally, dietary success supporting bivalve larvae growth has been also related to sterol composition of food (Soudant, 1998a). Sterols are ubiquitous lipid components of all eukaryotic organisms. In animal tissues, sterols are associated with a number of functions, and one of the most important is their role as structural components of cellular membranes (Nes, 1974). The ability to synthesize or bioconvert sterols de novo is generally low or absent, vary among the bivalve species, and imply that a dietary supply of sterols is necessary for bivalve growth (Voogt, 1975). Consequently, the qualitative and quantitative variability of the sterol compositions in microalgae used in hatchery (Gladu et al., 1991) will have repercussions on phytosterol, and particularly on the cholesterol composition in larvae, and will modify growth performances (Soudant et al., 1998a).
Although EFA and sterols requirements in bivalve larvae have been the subject of many studies (Delaunay et al., 1993; Nevejan et al., 2003; Soudant et al., 1998b; Pernet et al., 2005; Soudant et al., 2000) very few authors investigated their requirements during metamorphosis. Additionally, most of these previous studies focused on traditional standard hatchery diets which generally include *Isochrysis* sp., *Chaetoceros* sp. and *Pavlova* sp. However, addition of the PUFA-rich *Rhodomonas salina* in the diet of sand dollars increases larval growth, survival and metamorphic success (Schiopu et al., 2006). More recently, Tremblay et al. (2007) showed that addition of *R. salina* in the diet of *Pecten maximus* increased organic content and lipid reserves in pre-metamorphic scallops. Here we propose to evaluate the effect of the addition of *Rhodomonas salina* on growth, survival and lipid composition during the entire larval ontogeny of *P. maximus*, including the metamorphic period.

2. Material and methods

Experiments were realized in 2005 and repeated in 2006 in similar conditions, but with a higher level of temporal sampling, to define more precisely the dynamic of ontogenic changes between diets.

2.1 Animal maintenance

Adult scallops *Pecten maximus* were collected from the bay of Brest and kept in a flow-through seawater system at 15°C for 60 days and continuously fed with a mixture of *Isochrysis aff. galbana* (T-ISO), *Pavlova lutheri* and *Skeletonema costatum*. Spawning was induced by thermal stimulation (Gruffydd and Beaumont, 1970) and has been realized at the experimental hatchery of IFREMER, Brest. Experiments consisting of two nutritional treatments were conducted in triplicate 150-l tanks cylinder-cone shape until metamorphosis, at an initial density of 8 larvae·µl^{-1}. The seawater (20°C) was filtered at 1 µm, treated with U.V., stirred by aeration and renewed every two or three days. Bacterial growth was limited by addition of the antibiotic thiamphenicol at 8 ppm (Robert et al., 1996). At the onset of settlement, around 27 days post-fertilization (dpf), evidenced by the appearance of eyespot and "double-rings" shell edges over 50% of the population, the post-larvae were collected on a 150 µm mesh screen and transferred at the experimental hatchery of Argenton, IFREMER. For post-larvae rearing, the lack of availability in nursery has limited to use of only two replicates chosen randomly by nutritional treatments. Post-larvae were maintained in downweller with 130 µm mesh screen. Each downweller had scallops from one larval tank to follow the same stock through ontogeny. Each downweller contained 100 000 post-larvae and was in a raceway tank of 120 L (2m x 0.5m x 0.12 m). The seawater was pre-filtered at 5µm at a flow-rate of 100 L/h and temperature controlled at ≈ 15-16 °C. Mesh screen size was adjusted to shell size and post-larval rearing was effective until 41 dpf in 2005 and 45 pdf in 2006 (around 1mm post larvae length).
Microalgae from CCAP (U.K.) were cultured in 1 µm filtered-sterilized sea-water enriched with f/2 medium with addition of silicates for diatoms (Guillard, 1975). Briefly, algae were maintained at ≈ 20°C under continuous irradiance from cool-white fluorescence lights and mixed with aeration (air/CO_2; 98.5-1.5%) as described in Tremblay et al. (2007). The algae *Isochrysis galbana*, *Pavlova lutheri* and *Rhodomonas salina* were produced in continuous culture by opposite to a batch-culture for *Chaetoceros calcitrans.* The renewal rate for continuous culture was 25% for *P. lutheri* and 40% for T-ISO and *R. salina.* Algal cells were visually examined under a dissecting microscope and counted using a haemocytometer cell.

2.2 Experimental design

Two different microalgae mixtures were used as nutritional treatments for *P. maximus* larvae. The first was a standard diet namely PTC (*Pavlova lutheri* (P), *Isochrysis galbana* (T), *Chaetoceros calcitrans* (C)) and the second was an experimental diet namely PTCR (*Pavlova lutheri, Isochrysis galbana*, *Chaetoceros calcitrans* and *Rhodomonas salina* (R)). PTC served as a reference diet, commonly used in bivalve hatcheries (Delaunay et al., 1993). Larvae were fed daily with 15 cells·µl^{-1}of each component of diet at a ratio of 45 cells·µl^{-1} (1:1:1) for PTC and 60 cells·µl^{-1}(1:1:1:1) for PTCR. Tremblay et al. (2007), showed that larvae fed with adjusted PTC diet (on the basis of algal dry weight), to obtain biomasses similar to the PTCR diet did not show superior growth or higher biochemical content than larvae fed PTC diet at 45 cells·µl^{-1}. The post-larvae were fed continuously with the same nutritional treatment (PTC and PTCR) at 10X10^{9} cell day^{-1} for *P. lutheri*, *I. galbana* and *C. calcitrans*. As fouling in downweller was increased by the length of *R. salina* around 12 µm, concentration of this species in flow through system has been adjusted to 2.5X10^{9} cell day^{-1} for. Scallops were sampled during the early veliger stage (several times before 21 dpf), the pre-metamorphic stage (between 21 to 27 dpf) and the post-metamorphic stage (over 32 dpf), for growth, survival and lipid analyses (triacylglycerol content [TAG], fatty acid composition of neutral and polar lipids and sterols).

2.3 Growth, survival and settlement success

Growth of larvae was calculated following measurements of the shell length (anterior-posterior distance) with image analysis software, NIH (National Institut of Health, USA) for Macintosh. After metamorphosis growth of post-larvae was analysed with Nikon (10x) profile projector. Fifty scallops were measured at each sampling time and dietary treatment.
Survival was expressed as total number of individual less the cumulative amount of empty shells based on initial number of scallops on sub-samples. Metamorphosis success was determined by the difference of the number of post-larvae settled at 41 dpf in 2005 and 45 dpf in 2006 and the number of larvae transferred in downwellers.
Competent larvae were estimated by the appearance of the “double-rings” at the margin of the shell, corresponding to a peripheral groove on which the dissoconch shell will attach (Doroudi et al., 1999). This criterion was used to assess amount of larvae that began metamorphosis (Gerard et al., 1989). Samples ~300 larvae were taken every two days at the first apparition of “double-rings” and percentage was evaluated by microscopy.

2.4 Biochemical analysis

Thirty to fifty thousands pre-metamorphic scallops were filtered onto Whatman GF/F 47 mm filters pre-combusted at 450°C. For post-metamorphic scallops, only ~10 000 animals were used. Samples were stored in glass tube with 6 ml chloroform:methanol (2:1,v/v), closed under nitrogen, further sonicated for 10 minutes and kept at -20°C until extraction. Subsamples were also realized to estimated dry mass, where scallops were grinded and dried at 80°C during 24h. For triglycerides analysis, lipids were extracted following the procedure described by Folch et al. (1957). Triacylglycerol were determined by high performance thin layer chromatography (HPTLC) on silicagel plates (MERCK silicagel #60F254). Lipids were deposited on the plates with an automatic thin layer chromatography (TLC) sampler 4 CAMAG. Samples were first developed in a mixture of hexane-diethyl ether-acetic acid (20:5:0.5, v/v/v) followed by a mixture of hexane-diethyl ether (97:3, v/v). The standard utilised was oil Menhaden from fish 1,64 %. The revelation of TAG in the samples was obtained by heated 20 minutes at 160 °C with copper sulphate 3% phosphoric acid 8% and analysed with a TLC scanner 3 CAMAG at 370 nm and with the software Wincats.
To obtain fatty acids characterization, the lipids extracts were evaporated to dryness and recovered with three washings of 500 µl of chloroform:methanol (98:2, v/v). Neutral and polar lipids were separated by column chromatography on silica gel micro-columns (30x5mm I.D. Kieselgel 70-230 mesh Merck) using chloroform:methanol (98:2, v/v) to elute neutral lipids, followed by neat methanol to elute polar lipids (Marty et *al*. 1992). A known amount of 23:0 (internal standard) was added in each lipid fraction. Lipids were trans-esterified under nitrogen using BF_3/CH_3OH (12%) for 10 minutes at 100^{o}C. Fatty acid methyl esters (FAME) were analysed using a gas chromatograph (HP 6890) equipped with an on-column injector, a DB-Wax (30 m x 0.25 mm; 0.25 µm film thickness) capillary column and a flame ionization detector. Hydrogen was used as the carrier gas at 2 $ml \cdot min^{-1}$. The fatty acids were identified by comparing their retention time with known standards (37 component FAME Mix, PUFA-3 and menhaden oil) and quantified with tricosanoic acid (23:0).
Finally sterols profiles were analysed on an aliquot of the neutral lipids transesterified using sodium methoxyde (0.5M in methanol) at ambient temperature for 90 min (Soudant et al., 1998a). Sterols were analysed using gas chromatograph (Chrompak 9002, Middelburg, The Netherlands) equipped with an on-column injector followed by a Restek Rtx65 capillary column (15 m x 0.25 mm; 0.25 µm film thickness). Hydrogen was used as the gas carrier. Sterols were identified by comparing their retention time with standards and quantified with cholestane as an internal standard.

2.5 Statistical analysis

Statistical analyses were applied for each separated experiment, one for larvae (n=3) and other for post-larvae (n=2). The unit of replication was the tank in which the diet was applied. For each experimental year, differences in shell length, survival, double-rings, and biochemical composition of scallops were investigated using two-way repeated analyses of variance (ANOVAs) as a function of day and diet. Biochemical composition includes TAG content, relative proportions of selected fatty acids (namely 20:4n-6, 20:5n-3, 22:6n-3, the sum of saturated, monounsaturated and polyunsaturated fatty acids) and the total fatty acids (pg individual$^{-1)}$ in neutral and polar lipids and sterol composition (cholesterol, brassicasterol, β-sitosterol and stigmasterol). Here we used a mixed linear model and the repeated option was applied to the term "day" because repeated measurements were taken on the same experimental unit (tank). Residuals were screened for normality using the expected normal probability plot and further tested using Kolmogorov-Smirnov test. Homogeneity of variance-covariance matrices was graphically assessed and further tested using Levene test. If necessary, data were arcsine square-root transformed to achieve homogeneity of variances. Analyses were carried out using SAS 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Scallop performance

Scallops fed PTC and PTCR showed similar survival (Fig. 1A, B) at each experimental year with a significant effect of rearing time (2005: $F_{6,22}$= 114.3, p<0.001; 2006: $F_{12,42}$= 31.4, p<0.001). Time affected significantly growth (Fig. 1C, D) at each experimental year (2005: $F_{5,18}$= 454, p<0.001; 2006: $F_{12,42}$= 1436, p<0.001), but diet affected shell length (Fig. 1D) only in post-larvae over around 40 dpf, as observed in 2006 ($F_{1,4}$= 31,9, p=0.005). At each year, diet and day interacted on their effects on the occurrence of double-rings (Fig. 2; 2005: $F_{3,12}$= 86.4, p<0.001; 2006: $F_{3,10}$= 45.5, p<0.001). For both diets and year the first double-rings appeared before 19 days post-fertilization. Scallops fed PTCR showed more than 50% of double-rings at day 27 in 2005 (Fig. 2A) and at day 22 in 2006 (Fig. 2B) compared to less than 25% in scallops fed PTC at same dpf. However, the metamorphosis success was similar between diets, as no differences in survival were observed at the end of post-larval rearing (Fig. 1A, B).

3.2 Triacylglycerol

At each year, diet and day interacted on their effects on TAG concentration (Fig. 3; 2005: $F_{4,14}$= 6.1, p=0.005; 2006: $F_{4,10}$= 20.2, p<0.001). For both diets, the concentrations of TAG increased as a function of time. During larval stages, the accumulation of TAG was more rapid in larvae fed PTCR (33 to 50% more ng TAG larvae^{-1}) compared to that of to larvae fed PTC. In post-larvae, higher level of TAG was generally observed in scallops fed PTC (25 to 35% more TAG) compared to that occurring in scallops fed PTCR.

3.3 Fatty acids

A significant interaction of diet and day was obtained on total fatty acid (TFA) accumulation (2005, neutral: $F_{4,14}$= 112.2, p<0.001, polar: $F_{4,14}$= 46.1, p<0.001; 2006, neutral: $F_{4,10}$= 206.8, p<0.001, polar: $F_{4,10}$= 39.7, p<0.001). During the larval development until competence, scallops fed PTCR showed higher level of TFA compared to those fed PTCR, but in post-larvae the effect of diet was inversed with higher accumulation of TFA in scallop fed with PTC (Table 1, 2). Fatty acids were divided into three broad categories; saturated (SFA), monosaturated (MUFA) and polyunsaturated fatty acids (PUFA). No significant changes

have been observed for sum of SFA comparatively to sum of MUFA and PUFA, where significant diet and day interaction were found. Same results were obtained at each year and for neutral and polar lipids fraction with higher values of MUFA (2005, neutral: $F_{4,14}$= 12.4, p<0.001, polar: $F_{4,14}$= 16.8, p<0.001; 2006, neutral: $F_{4,10}$= 26.1, p<0.001, polar: $F_{4,10}$= 19.7, p<0.001) in larvae and post-larvae fed with PTC (Table 1 and 2), contrary to PUFA where higher values were obtained with PTCR diet (2005, neutral: $F_{4,14}$= 25.6, p<0.001, polar: $F_{4,14}$= 31.2, p<0.001; 2006, neutral: $F_{4,10}$= 16.5, p<0.001, polar: $F_{4,10}$= 21.2, p<0.001).
Particular emphasis is given in this study to the long chain PUFA 20:4n-6 (AA), 20:5n-3 (EPA) and 22:6n-3 (DHA) as presented in Fig. 4 and 5. These three fatty acids contributed for 40-56% of the total PUFA levels found in scallop larvae for neutral lipids and for 63-70% for the polar lipids at each experimental year. Diet and day showed interactions on their effects on AA, EPA and DHA in both years. Level of AA (Fig. 4A, B and 5A, B) was higher in scallops fed PTCR than in those fed PTC (2005, neutral: $F_{4,14}$= 8.5, p=0.001, polar: $F_{4,14}$= 10.7, p<0.001; 2006, neutral: $F_{4,10}$= 78.7, p=0.001, polar: $F_{4,10}$= 9.7, p=0.002). During the early larval development, scallops fed PTCR show higher level of EPA than those fed PTC (Fig. 4C, D and 5C, D). However, the inverse was observed after metamorphosis: scallops fed PTC showed higher level of EPA than those fed PTCR (neutral: $F_{4,14}$= 21.0, p<0.001, polar: $F_{4,14}$= 13.4, p=0.001; 2006, neutral: $F_{4,10}$= 804, p=0.001, polar: $F_{4,10}$= 12.5, p<0.001). During the larval development, scallops fed PTC showed higher level of DHA compared to those fed PTCR (Fig. 4E, F and 5E, F) (neutral: $F_{4,14}$= 81.84, p<0.001, polar: $F_{4,14}$= 4.39, p=0.017; 2006, neutral: $F_{4,10}$= 20.6, p<0.001, polar: $F_{4,10}$= 52.3, p<0.001). However, after the metamorphic period, there was generally no effect of diet on level of DHA in scallops. Levels of DHA in scallops fed both diet decreased during ontogeny. It is noteworthy that levels of AA and DHA were generally higher in the polar lipids compared to that in the neutral lipids, whereas levels of EPA in the polar lipids were lower than or equal to that observed in the neutral lipids.

3.4 Sterols

Particular emphasis is put on the four main sterols; cholesterol, brassicasterol, stigmasterol and β-sitosterol; which collectively contributed to 68-75% of the total sterol content in scallops (Fig. 6 and 7). Total sterol concentration was almost twice higher in animals fed PTC with total mean of 18.7±1.9 ng scallop^{-1} for all dpf and year than in those fed PTCR, where it was 9.8±4.9 ng scallop^{-1}. In 2005 experiment, diet and day interacted on their effects on brassicasterol (Fig. 6A, 7A), with increasing level during larval ontogeny in larvae fed PTCR than in larvae fed PTC where it remained low ($F_{4,13}$= 11.1, p<0.001). In 2006, only diet effect have been observed with higher level in scallop fed PTCR.006: neutral ($F_{1,4}$= 88.1, p<0.001). Diet and day interacted on level of cholesterol (Fig. 6B, 7B), with higher levels in scallops fed PTC (2005: $F_{4,13}$= 9.4., p<0.001; 2006: $F_{4,10}$= 12,9., p<0.001). Similar results were obtained on β-sitosterol (Fig. 6C, 7C) with higher level in PTC fed scallops (2005: $F_{4,13}$=4.0, p=0.026; 2006: $F_{4,10}$=3.9, p=0.035) compared to those fed PTCR. Overall, levels of cholesterol increased after metamorphosis at the expense of β-sitosterol and stigmasterol. Level of stigmasterol remained unaffected by dietary treatment (Fig. 6D, 7D) but it varied as a function of time (2005: $F_{4,13}$= 13.5, p<0.001; 2005: $F_{4,10}$= 7.8, p=0.004) with lower values in post-larvae.

4. Discussion

4.1 Effect of microalgal diets on larval performance

Although scallops fed with the two experimental diets showed generally similar growth and survival, our results suggest that animals fed PTCR undergo metamorphosis more rapidly

than those fed the standard hatchery diet, PTC. Indeed, scallops fed PTCR showed more that a twice higher level of double-rings at 27 dpf compared to larvae fed PTC as previously reported (Tremblay et al., 2007). These differences could be related to diet content. Addition of *R. Salina* seem advantageous at nutritional level to attain metamorphosis more rapidly by the higher total fatty acids content in *R. Salina* (2.5 higher than those observed in *C. gracilis*; see Tremblay et al., 2007) and the high starch content, around 40% for this species comparatively to under 15% for *C. gracilis* and *I. galbana* (Brown et al., 1997). Despite the fact that the occurrence of double-rings is generally considered as a criteria for assessing larval competency to metamorphosis in *P. maximus* (Gerard et al., 1989), the metamorphosis success (survival rate measured at 41 dpf in 2005 and at 45 dpf in 2006) were similar between the two tested diets. The more detailed sampling level in post-larval rearing in 2006 showed that the more rapid settlement and metamorphosis of scallop fed PTCR was related to higher growth rate in post-larvae over 41 dpf. This more rapid metamorphosis was related to higher accumulation of lipids during larval stages until competence, with higher levels of total fatty acid concentrations in neutral and polar fractions and higher level of triacyglycerol with PTCR diet.

It was previously reported that the addition of *R. salina* to a standard hatchery diet for *P. maximus* significantly increases the organic content of pre-metamorphic larvae due to higher levels of protein and lipid in the animals (Tremblay et al., 2007). Indeed, the starch content in the *R. salina* seemed beneficial to *P. maximus* larvae compared to other algal species (Brown et al. 1997, Tremblay et al. 2007). These carbohydrates could provide an energy source for metabolic demand during larval development or converted to and accumulated as lipids. Holland (1978) reviewed the literature on energy metabolism in many invertebrate larvae and concluded that neutral lipids, especially triacylglycerols, were most important as a storage medium for use during nutritional or environmental stress (Gallager *et al.*, 1986). In study of Pernet et al. (2003a and 2006), top-performing scallops were characterized by the accumulation of TAG reserves up to 10.4-24.4 ng larvae^{-1} during the pre-metamorphic period, comparatively to lesser 1.2 to 2.8 ng larvae^{-1} for less-performing scallops. Therefore, this accumulation appears to provide a good indicator of future growth, survival and metamorphic success, with higher values indicative of cohort success (Pernet et al., 2003b). In post-larvae raring, we observed however higher concentrations of TAG and total fatty acids in neutral and polar fraction with the use of PTC diet. However, as post-larvae fed on PTCR showed similar survival at each year and higher growth rate over 41 dpf in 2006 experiment, accumulation of TAG in post-larvae seems not be related to higher performance.

4.2 Effect of diet and ontogeny: fatty acid composition of scallops

The addition of *R. salina* in diet enhanced the accumulation of AA in neutral and polar lipids of scallops. Previous analyses have demonstrated that AA level in *R. salina* is higher than in the three other species with level near 3% comparatively to values under 0.5% for PTC diet (Tremblay et al. 2007; Soudant et al. 1998b). It is generally accepted that AA acts as a precursor of eicosanoids in several invertebrate species, including bivalves and present a group of highly biologically active hormones such as prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids (Howard and Stanley, 1999; Smith and Murphy, 2003). Several studies in bivalves suggest that deficiencies in AA may be detrimental for survival and resistance to pathogen. For example, haemocyte membrane lipids in oysters *Crassostrea gigas* also contain elevated amounts of AA presumably to regulate immune response (Delaporte et al., 2003; 2006). High mortalities and low growth rates of sea scallop, *Placopecten magellanicus*, larvae were associated with low level of AA in the diet (<1%, Pernet & Tremblay, 2004). Finally, an increase in AA levels during metamorphosis of sea scallops *Placopecten magellanicus* was attributed to an increasing demand for AA rich immune cells to degrade larval tissues and to fight surface-associated bacteria (Pernet et al. 2005).

Scallops fed PTCR showed a higher level of EPA compared to those fed PTC until end of larval stages when this pattern was inversed. This pattern may reflect the effect of a long term ingestion of *R. salina*, a microalgal species normally characterized by low level of EPA (Tremblay et al., 2007). Alternatively, it was previously reported that *P. maximus* larvae gradually eliminate EPA in their polar lipids during their early ontogeny (Delaunay et al. 1993; Marty et al. 1992). Since 20:5n-3 and 20:4n-6 compete for the same enzymes for eicosanoids synthesis, the gradual lesser accumulation of 20:5n-3 in polar lipids of scallops fed PTCR comparatively to scallops fed PTC may have been explained by higher level of 20:4n-6 in PTCR diet. Also, as the eicosanoids produced from 20:5n-3 are generally less active than those produced from 20:4n-6 (Bell and Sargent, 2003), PTCR diet could be advantageous in stressfull conditions. In most animals, including invertebrate species, 20:4n-6 is involved in the stress response (Howard and Stanley, 1999; Pernet and Tremblay, 2004).
Scallops fed PTCR showed a lower level of DHA in polar lipids compared to those fed PTC until competence (27 dpf) when it became similar. It is also noteworthy that the quantity of DHA in polar lipids of scallop larvae (20-25%) largely exceeded the values (7-14%) found in neutral lipids, which reflect dietary levels (Delaunay et al. 1993). The level of DHA in all of these microalgae (*R. salina*, *C. calcitrans*, *P. lutheri* and *I. galbana*) have been demonstrated to be lower that 10% (Soudant et al. 1998b, Tremblay et al. 2007) and reflect values obtained in neutral lipids. This observation supports the fact that DHA was the most strongly bioconcentrated fatty acid in polar lipids of scallop larvae (Marty et al., 1992; Delaunay et al., 1993; Soudant et al., 1998b). The proportion of DHA in the neutral and polar lipids of larvae decreased with ontogeny, suggesting that incorporation of DHA into membrane phospholipids could be realized at the expense of reserve lipids, most likely in response to the low dietary proportions (Plante et al., 2007). Maintenance of high level of DHA in membrane lipids was attributed to the fact that DHA plays a major role in maintaining the structural and functional integrity of cell membranes (Delaunay et al., 1993).

4.3 Effect of diet and ontogeny: sterol composition of scallops

Although bivalves are capable of selectively incorporating certain sterols, it is generally accepted that they have a negligible capacity for the biosynthesis or conversion of sterols (Holden and Patterson, 1991). Here we showed that scallops fed PTCR exhibited a marked enrichment in brassicasterol compared to those fed PTC. A pioneer study showed that brassicasterol is important for bivalves (Voogt, 1975) and it was suggested that brassicasterol can replace cholesterol for some functions (Pazos et al., 2003). Palacios et al. (2007) showed a similar and constant level of free sterols found in different tissues were in accordance to their role in cellular membranes. Cholesterol was the major sterol, in free and esterified forms, underlying the specific importance of that sterol in cell metabolism (Palacios et al., 2007). Nevertheless, the presence of phytosterols in all tissues implies that these sterols could substitute for cholesterol in the cellular membrane (Palacios et al., 2007, Milke et al., 2008). The presence of several phytosterols in substantial concentrations, mainly brassicasterol and 24-methylenecholesterol in gonad and somatic tissues, further supports a specific physiological role of these sterols in bivalves, like scallops (Palacios et al., 2007) and oyster (Wilkfors et al., 1996). PTCR diet seems to promote accumulation of brassicasterol in larvae, but our results did not allow evaluating if this increase is beneficial to the larvae.
It is also noteworthy that level of cholesterol increased markedly during metamorphosis from 12-15 to 20-25% at the expense of β-sitosterol and stigmasterol. Preferential incorporation of cholesterol over other sterols was previously observed in scallops *P. maximus* (Soudant et al., 1998a), thus emphasizing the importance of this sterol. These results suggest a specific nutritional requirement for cholesterol during metamorphosis of *P. maximus*. This requirement may be due to the fact that cholesterol is an essential component in animal membranes, with multiple effects on their physical properties including membrane fluidity, phase behaviour, thickness, and permeability (Crockett, 1998). A widely accepted notion is

that cholesterol stabilizes membranes, i.e. it increases the order of the surrounding acyl chains in membranes in the fluid phase (Crockett, 1998). It is noteworthy that other phytosterols have less of an ordering effect on membranes than cholesterol (Suckling et al., 1979). Therefore, the observed increase in cholesterol during the metamorphosis in *P. maximus* may reflect a role for cholesterol in the membrane response. However, the role of cholesterol in metamorphosing scallops requires further investigation.

5. Conclusion

In conclusion, our results suggest that scallop larvae *P. maximus* showed a more rapid metamorphosis when adding *R. salina* to a traditional hatchery diet. A more rapid accumulation of TAG until competence could indicate an advantage of using PTCR during larval development to obtain a more rapid metamorphosis process, but this trend seems to be inversed during post-larval ontogeny. We also observed a higher accumulation of AA in scallops fed PTCR, which may present an advantage for survival in pathogenic conditions. Furthermore, PTCR clearly enhanced the level of brassicasterol in scallops compared to that of animals fed PTC. However, the functional significance of this result remains unclear. Nevertheless, further experiment needs to evaluate the impact of the addition of *R. salina* during the post-larval development.

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Figures

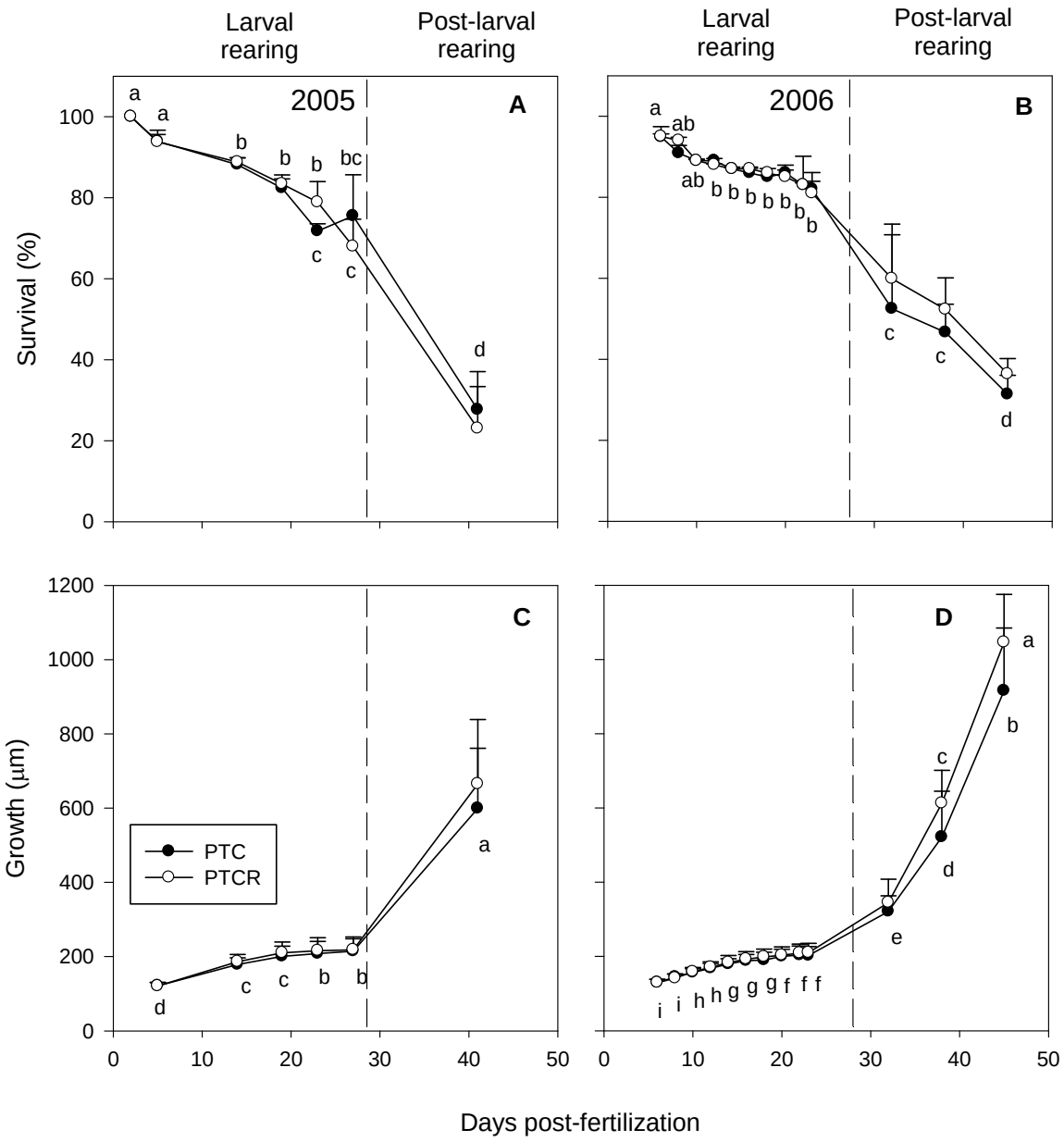

Fig. 1. Survival (A, B) and shell length (C, D) of scallop larvae and post-larvae fed PTC and PTCR as a function of the day post-fertilization (mean ± SD) in 2005 and 2006 experiments. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

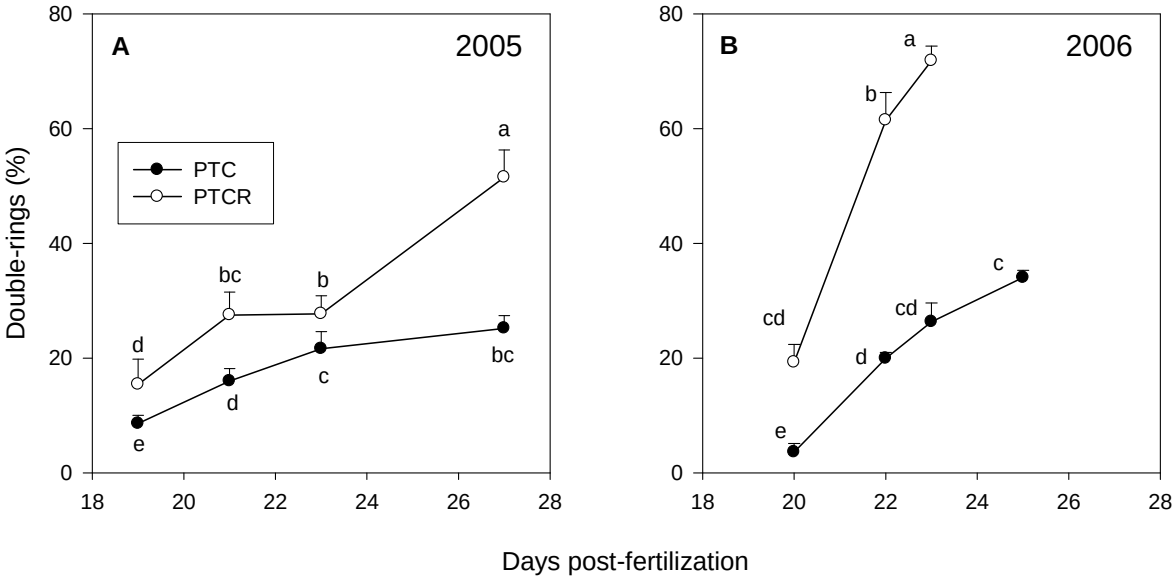

Fig 2. Occurrence of double-rings of larvae fed PTC and PTCR as a function of the day post-fertilization (mean ± SD) in 2005 (A) and 2006 (B) experiments. Different letters indicate significant differences.

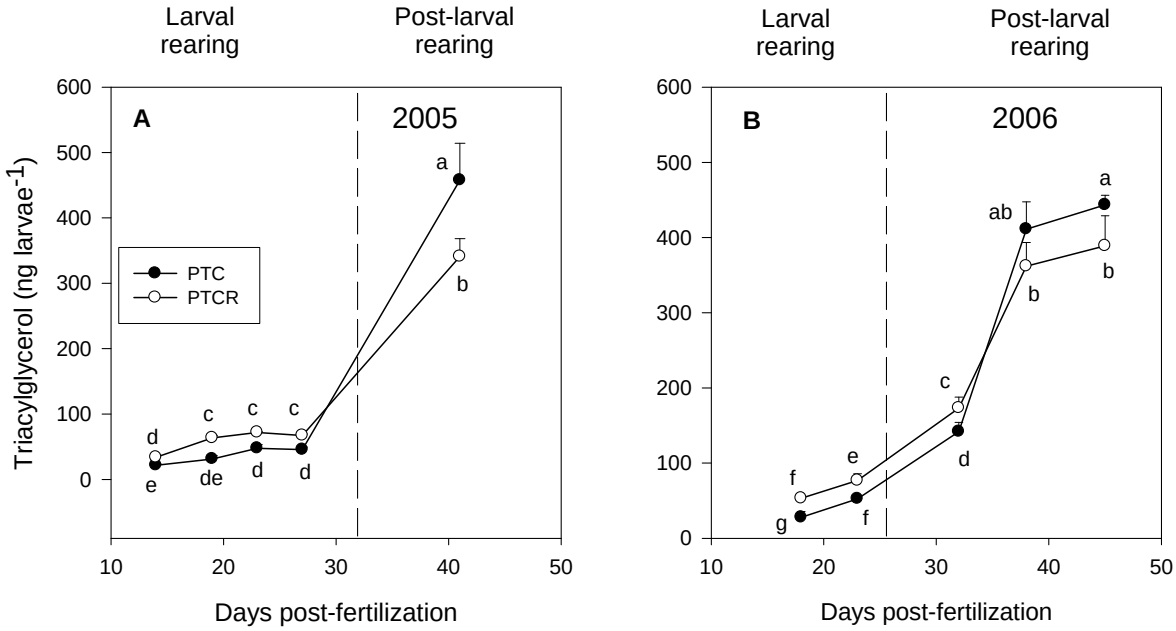

Fig. 3. Triacylglycerol concentration in scallop larvae and post-larvae fed PTC and PTCR as a function of the day post-fertilization (mean ± SD) in 2005 (A) and 2006 (B) experiments. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

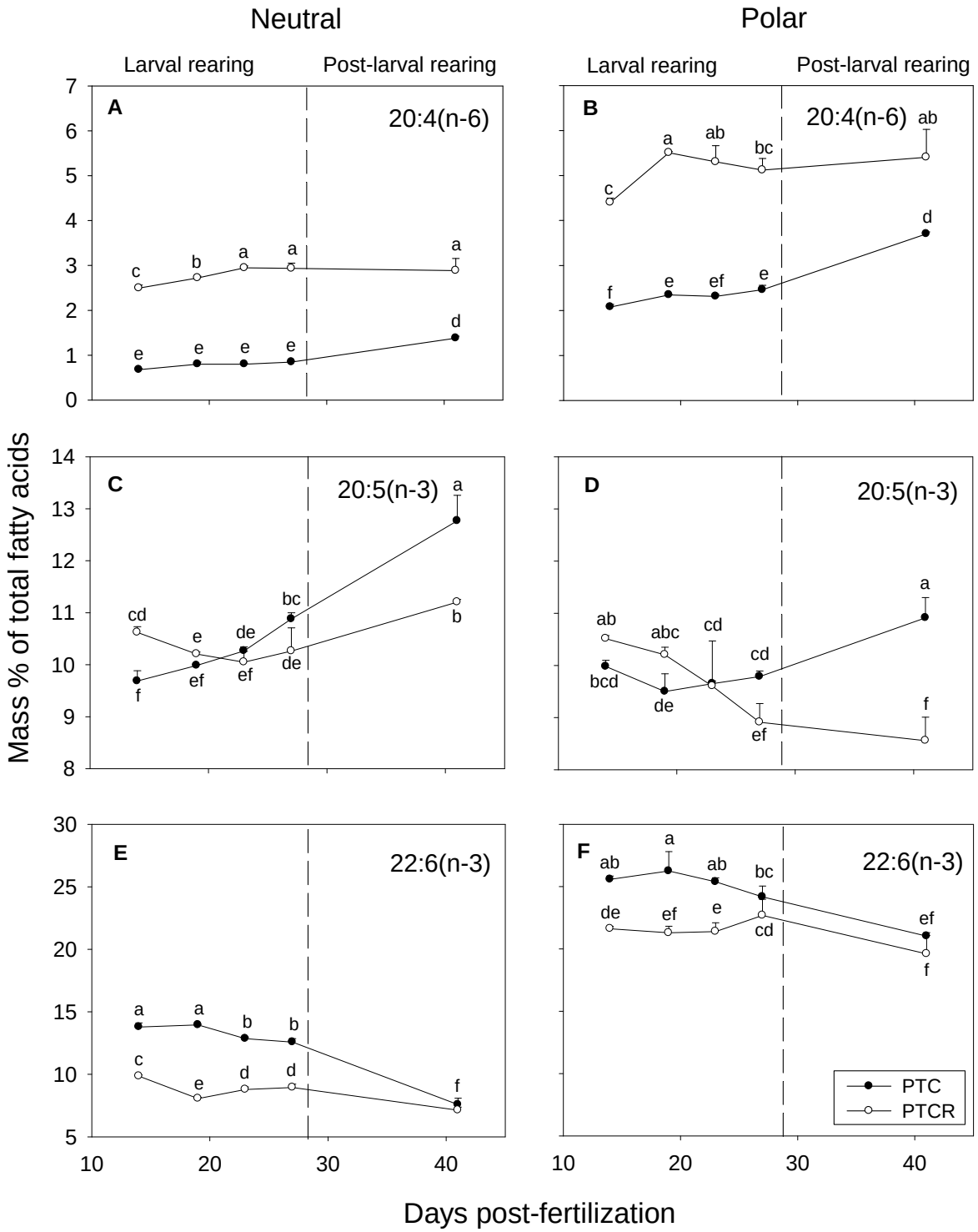

Fig. 4. Levels of AA (20:4n-6; A, B), EPA (20:5n-3; C, D) and DHA (22:6n-3; E, F) for neutral (A, C, E) and polar lipids (B, D, F) in scallop larvae and post-larvae fed PTCR and PTC as a function of the day post-fertilization (mean ± SD) in 2005 experiment. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

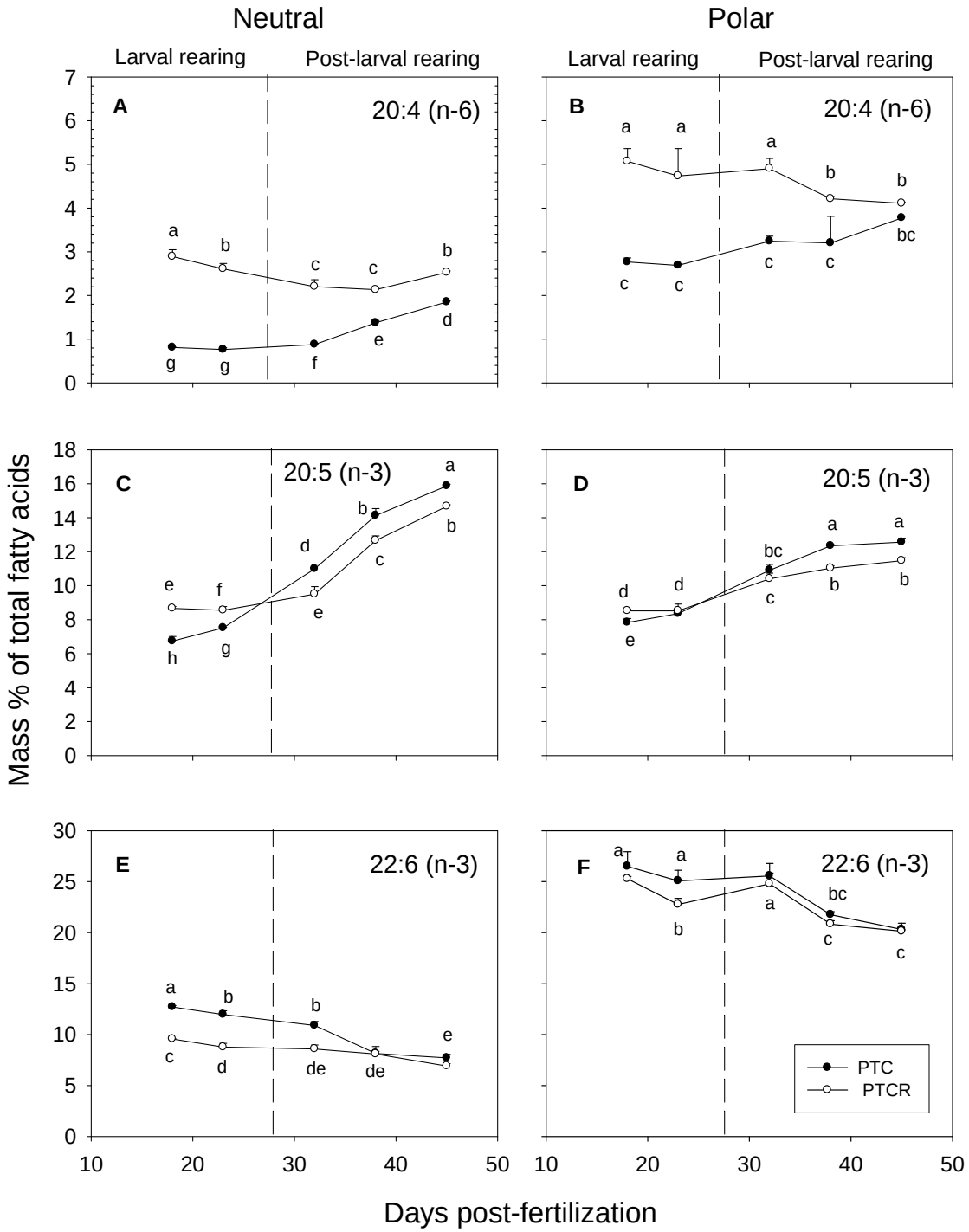

Fig. 5. Levels of AA (20:4n-6; A, B), EPA (20:5n-3; C, D) and DHA (22:6n-3; E, F) for neutral (A, C, E) and polar lipids (B, D, F) in scallop larvae and post-larvae fed PTCR and PTC as a

function of the day post-fertilization (mean ± SD) in 2006 experiment. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

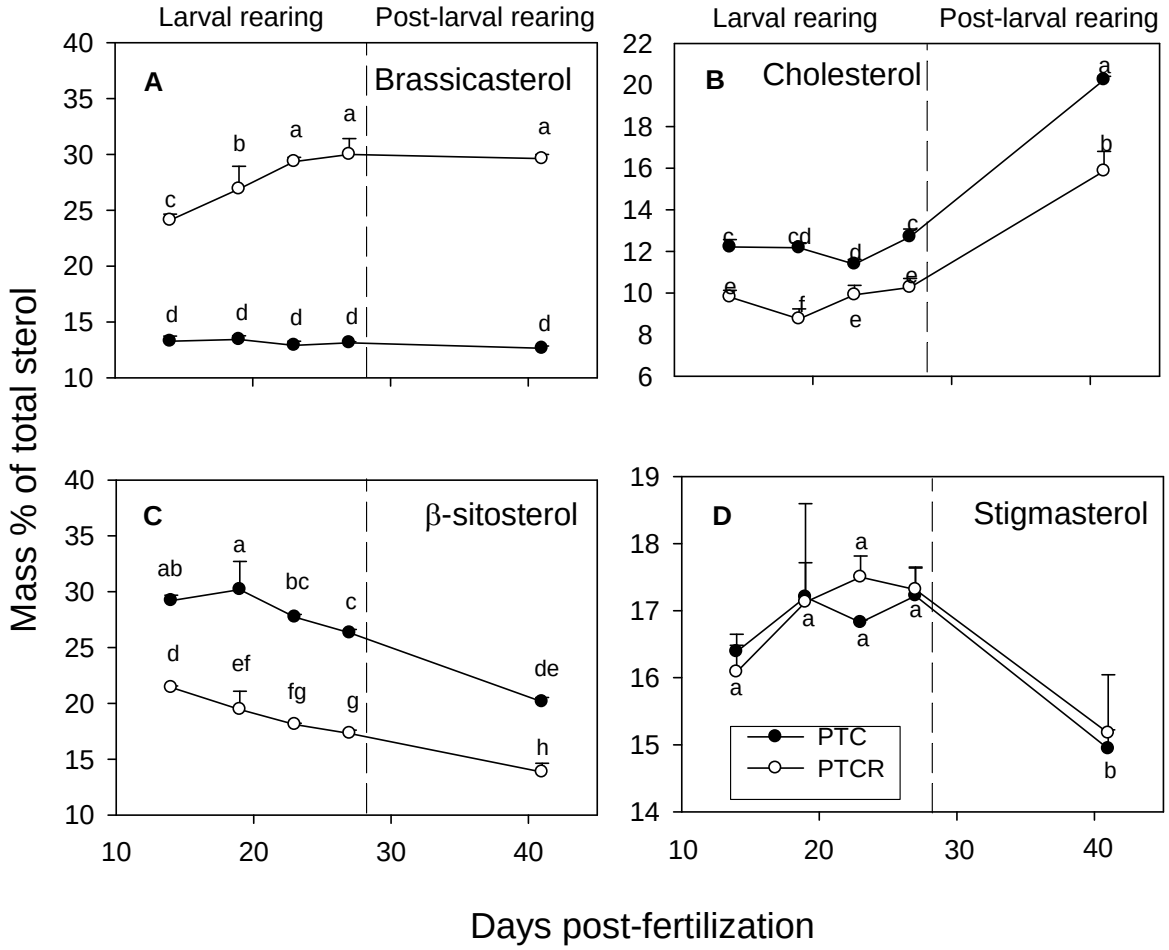

Fig. 6. Levels of brassicasterol (A), cholesterol (B), β-sitosterol(C), stigmaterol (D) in scallop larvae and post-larvae fed PTCR and PTC as a function of the day post-fertilization (mean ± SD) in 2005 experiment. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

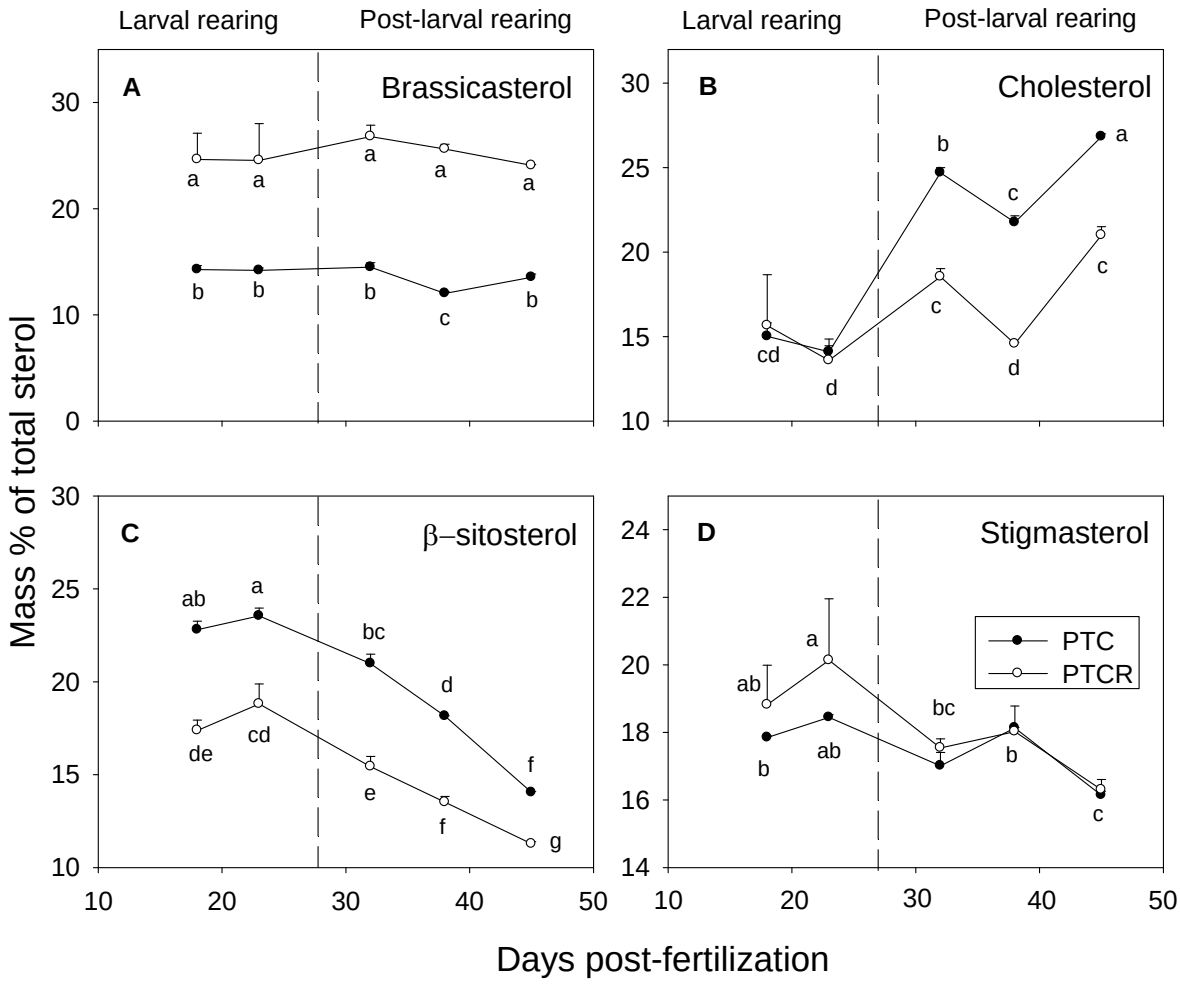

Fig. 7. Levels of brassicasterol (A), cholesterol (B), β-sitosterol(C), stigmaterol (D) in scallop larvae and post-larvae fed PTCR and PTC as a function of the day post-fertilization (mean ± SD) in 2006 experiment. Different letters indicate significant differences. Dashed line indicated switch from larval to post-larval rearing.

Tables

Table 1. Fatty acid composition for neutral lipids in competent larvae (27 dpf in 2005 and 23 dpf in 2006) and post-larvae (41 dpf in 2005 and 45 dpf in 2006) fed PTCR and PTC diets expressed as % of total fatty acid (mean ± SD) for experiments conducted in 2005 and 2006. Also shown is the total fatty acids (TFA; pg individual^{-1}). Different letters indicate significant differences.

Table 2. Fatty acid composition for polar lipids in competent larvae (27 dpf in 2005 and 23 dpf in 2006) and post-larvae (41 dpf in 2005 and 45 dpf in 2006) fed PTCR and PTC diets expressed as % of total fatty acid (mean ± SD) for experiments conducted in 2005 and 2006. Also shown is the total fatty acids (TFA; pg individual^{-1}). Different letters indicate significant differences.

Table 1.

	2005				2006			
	PTC		PTCR		PTC		PTCR	
	Larvae	Post-larvae	Larvae	Post-larvae	Larvae	Post-larvae	Larvae	Post-larvae
14:0	9.1 ± 0.8	11.9 ± 0.4	8.1 ± 0.2	8.8 ± 0.7	10.4 ± 0.3	11.4 ± 0.1	8.8 ± 0.3	9.1 ± 0.1
16:0	16.0 ± 0.8	14.8 ± 0.5	15.3 ± 0.1	14.2 ± 0.5	14.5 ± 0.1	11.6 ± 0.2	14.0 ± 0.3	11.3 ± 0.1
18:0	2.2 ± 0.1	1.7 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	2.3 ± 0.2	2.4 ± 0.1	2.6 ± 0.1
∑SFA	28.2 ± 0.9	26.4 ± 0.5	26.6 ± 0.2	26.1 ± 0.8	27.9 ± 0.3	26.2 ± 0.9	26.2 ± 0.4	24.0 ± 0.1
16:1(n-7)	9.5 ± 0.9	15.3 ± 0.2	5.1 ± 0.2	8.5 ± 0.1	9.9 ± 0.4	11.6 ±0.1	6.1 ± 0.2	8.0 ± 0.2
18:1(n-9)	8.3 ± 2.2	4.3 ± 0.2	9.5 ± 3.7	5.1 ± 0.1	9.5 ± 0.5	3.1 ± 0.1	6.6 ± 0.2	3.1 ± 0.2
18:1(n-7)	8.1 ± 0.2	5.5 ± 0.4	4.4 ± 3.8	5.0 ± 0.2	8.2 ± 0,2	6.0 ± 0.2	6.4 ± 0.2	5.6 ± 0.2
∑MUFA	28.3 ± 2.2^{a}	26.6 ± 1.0^{a}	21.4 ± 0.3^{b}	20.7 ± 0.4^{b}	30.0 ± 0.3^{a}	22.5 ± 0.3^{b}	21.4 ± 0.6^{b}	19.1 ± 0.3^{c}
18:2(n-6)	2.9 ± 0	6.1 ± 0.1	9.4 ± 0.1	11.7 ± 0.4^{a}	7.0 ± 0.1	8.1 ± 0.1	9.4 ± 0.3	11.0 ± 0.1
18:3(n-6)	0.4 ± 0.1	1.7 ± 0.6	1.6 ± 0	2.1 ± 0.4	0.6 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.6 ± 0.1
18:3(n-3)	2.6 ± 0	3.0 ± 0	5.8 ± 0.1	5.7 ± 0.3	3.8 ± 0.1	3.1 ± 0.1	5.7 ± 0.4	6.7 ± 0.1
18:4(n-3)	6.9 ± 0.1	4.8 ± 0	7.7 ± 0.3	6.0 ± 0.4	3.6 ± 0.1	5.0 ± 0.1	5.7 ± 0.3	6.6 ± 0.1
20:4(n-6) (AA)	0.8 ± 0^{c}	1.4 ± 0.1^{b}	2.9 ± 0.1^{a}	2.9 ± 0.3^{a}	0.8 ± 0.1^{c}	1.8 ± 0.1^{b}	2.6 ± 0.1^{a}	2.5 ± 0.1^{a}
20:5(n-3) (EPA)	10.3 ± 0.1^{c}	12.8 ± 0.5^{a}	10.0 ± 0.2^{c}	11.2 ± 0.1^{b}	7.5 ± 0.1^{d}	15.9 ± 0.1^{a}	8.6 ± 0.2^{c}	14.7 ± 0.2^{b}
22:5(n-6)	2.6 ± 0	1.1 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	2.3 ± 0.4	1.0 ± 0.1	1.5 ± 0.1	0.7 ± 0.3
22:6(n-3) (DHA)	12.9 ± 0.1^{a}	7.6 ± 0.5^{c}	8.8 ± 0.2^{b}	7.1 ± 0.3^{c}	12.0 ± 0.3^{a}	7.7 ± 0.6^{c}	8.8 ± 0.4^{b}	6.9 ± 0.3^{c}
∑PUFA	42.8 ± 0.2^{b}	43.2 ± 0.8^{b}	51.3 ± 0.5^{a}	52.4 ± 0.1^{a}	41.6 ± 0.2^{c}	50.9 ± 0.9^{b}	57.1 ± 0.6^{a}	56.6 ± 0.3^{a}
TFA (pg individual^{-1})	45.9 ± 1.9^{d}	207.1 ± 72.5^{a}	65.0 ± 7.9^{c}	130.0 ± 35.8^{b}	40.4 ± 2.3^{d}	504 ± 69.2^{b}	69.4 ± 13^{c}	371.6 ± 79.8^{a}

Table 2.

	2005				2006			
	PTC		PTCR		PTC		PTCR	
	Larvae	Post-larvae	Larvae	Post-larvae	Larvae	Post-larvae	Larvae	Post-larvae
14:0	1.4 ± 0.1	2.4 ± 1.0	1.3 ± 0.3	1.2 ± 0.4	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.1	1.2 ± 0.1
16:0	11.1 ± 1.3	12.0 ± 0.2	11.3 ± 1.0	10.5 ± 0.4	10.5 ± 0.3	10.9 ± 0.5	10.2 ± 0.2	10.6 ± 0.1
18:0	6.8 ± 0.7	7.6 ± 0	7.4 ± 0.2	7.4 ± 0.6	6.3 ± 0.3	7.8 ± 0.2	6.8 ± 0.3	7.3 ± 0.1
∑SFA	20.6 ± 0.5	21.3 ± 0.7	21.4 ± 1.1	20.5 ± 0.3	19.2 ± 0.9	21.8 ± 1.1	19.6 ± 0.5	20.3 ± 0.7
16:1(n-7)	2.5 ± 0.3	4.4 ± 0.3	2.1 ± 0.3	2.2 ± 0.6	3.0 ± 0.4	2.8 ± 0.4	2.5 ± 0.3	2.3 ± 0.1
18:1(n-9)	3.9 ± 0.2	3.3 ± 0.1	3.4 ± 0.3	2.9 ± 0	4.7 ± 0.2	2.2 ± 0.1	3.1 ± 0.1	2.0 ± 0.1
18:1(n-7)	5.3 ± 0.1	6.1 ± 0.2	4.4 ± 0.2	4.9 ± 0	5.5 ± 0.1	6.0 ± 0.1	3.8 ± 0.1	5.2 ± 0.1
∑MUFA	17.3 ± 0.7^{a}	17.9 ± 0.5^{a}	14.2 ± 0.2^{b}	14.1 ± 0.5^{b}	17.1 ± 0.2^{a}	17.5 ± 0.7^{a}	13.6 ± 0.6^{b}	13.8 ± 0.9^{b}
18:2(n-6)	1.0 ± 0.1	2.9 ± 0.2	3.5 ± 0.3	5.0 ± 0.5	2.8 ± 0.7	3.5 ± 0.1	4.6 ± 0.4	4.6 ± 0.1
18:3(n-6)	0.1 ± 0	0.5 ± 0	0.6 ± 0	0.7 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
18:3(n-3)	0.9 ± 0.1	1.2 ± 0.1	2.2 ± 0.3	1.8 ± 0.3	1.5 ± 0.1	1.3 ± 0.1	2.9 ± 0.2	2.3 ± 0.1
18:4(n-3)	1.9 ± 0.1	2.3 ± 0.3	2.5 ± 0.2	1.9 ± 0.3	1.9 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
20:4(n-6) (AA)	2.3 ± 0^{c}	3.7 ± 0^{b}	5.3 ± 0.4^{a}	5.4 ± 0.6^{a}	2.7 ± 0.1	3.8 ± 0.1	3.4 ± 0.3	4.1 ± 0.1
20:5(n-3) (EPA)	9.6 ± 0.1^{b}	10.9 ± 0.4^{a}	9.6 ± 0.9^{ab}	8.6 ± 0.3^{c}	8.4 ± 0.1	12.6 ± 0.3	8.5 ± 0.5	11.5 ± 0.2
22:5(n-6)	6.3 ± 0.1	5.2 ± 0.1	3.8 ± 0.4	3.3 ± 0.4	6.4 ± 0.2	3.4 ± 0.3	4.8 ± 0.5	2.6 ± 0.1
22:6(n-3) (DHA)	25.4 ± 0.3^{a}	21.0 ± 0^{b}	21.4 ± 0.7^{b}	19.6 ± 1.7^{c}	25.1 ± 1.0	20.3 ± 1.2	22.8 ± 0.7	20.1 ± 0.1
∑PUFA	51.3 ± 0.6^{b}	51.9 ± 1.0^{b}	57.2 ± 1.7^{a}	56.9 ± 0.6^{a}	55.1 ± 1.1^{b}	55.4 ± 0.3^{b}	58.1 ± 0.6^{a}	58.3 ± 0.7^{a}
TFA (pg individual^{-1})	14.1 ± 2.1^{d}	85.7 ± 2.3^{a}	19.6 ± 2.0^{c}	48.4 ± 2.7^{b}	14.3 ± 0.8^{d}	290.6 ± 60.8^{a}	19.8 ± 1.6^{c}	142 ± 29.1^{b}