Improving scallop (*Pecten maximus* and *Placopecten magellanicus*) spat production by initial larvae size and hydrodynamic cues used in nursery system

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ABSTRACT

There are several factors affecting scallops during the metamorphosis process that could explain the relatively low post-larvae yield observed in hatcheries. Competent bivalve larvae respond to different settlement cues to undergo metamorphosis and without adequate cues, larvae delay their metamorphosis. The objective of this study is to improve the settlement ratio of the two scallop species, *Placopecten magellanicus* and *Pecten maximus* by physical cues associated with hydrodynamic conditions, stocking density in settlement units and larval size at time of transfer to settling units. For each treatment, physiological condition was determined by fatty acid analysis to determine the energetic reserves and structural lipids. We observed similar results for the two important commercial pectinid species and validate the hypothesis on the positive effect of increased flow rate and larval size after transfer to settlement systems on settlement success. Increasing flow rate also affects positively the physiological condition of settled post-larvae by a higher accumulation of total fatty acids in neutral lipid fractions. Furthermore, no effect of larval stocking density until 90 larvae cm^{-2} in the downwelling sieves was observed. To our knowledge this study is the first to characterize the effect of seawater flow rate on settlement success of different pectinid species cultured under similar conditions. The experiments were performed in a close to commercial scale and thus are relevant to industry situations.

1. Introduction

Worldwide aquaculture scallop production has increased from around 0.5 million tonnes in the mid-seventies to 2.6 million tonnes in 2013–2015 (FAO, Online information, http://www.fao.org/fishery/statistics/global-production/en), and in the North Atlantic Ocean the sea scallop (*Placopecten magellanicus*) and the great scallop (*Pecten maximus*) are the main scallop species commercially exploited. The total production of these two species reached a maximum of 0.37 million tonnes in 2004, but was reduced to 0.25 million tonnes in 2015 according to FAO. Spat supply is still one of the major limiting factors for the development of these aquaculture industries, and the collection of spat from nature has been unreliable or highly variable (Dao et al., 1999; Cyr et al., 2007; Shumway and Parson, 2016), thus stimulating intensive hatchery production (Bergh and Strand, 2001; Andersen et al., 2011). Moreover, hatchery production of scallop spat has until recent years been characterized by variable yields (Karney, 1991; Couturier et al., 1995; Robert and Gérard, 1999; Andersen et al., 2011). After several years of optimization, increased survival and lower variability in larval production have been achieved in *P. maximus* and *P. magellanicus* hatcheries (Pernet and Tremblay, 2004; Pernet et al., 2005; Magnesen et al., 2006; Tremblay et al., 2007; Magnesen and Jacobsen, 2012). However, only around 20% of the competent larvae normally result in viable metamorphosed and settled post-larvae (Andersen et al., 2011, 2013). According to Galley et al. (2017), the difficulties in the culture of *P. maximus* are associated to the transition from larval to juvenile stage, showing low development synchronicity and variable survival. To achieve successful hatchery development of *P. maximus* and *P. magellanicus*, settlement and metamorphosis rates need to be increased and stabilized.

There are several factors affecting scallops during the metamorphosis process that could explain the relatively low post-larvae yield observed in hatcheries. Competent bivalve larvae respond to different settlement cues to undergo metamorphosis and without adequate cues, larvae delay their metamorphosis (Pechenik, 1990; Martel et al., 2014; Lagarde et al., 2018). In scallops, some chemical cues have been demonstrated to increase settlement (Yvin et al., 1985; Chevolot et al., 1991; Mesías-Gansbiller et al., 2008; Galley et al., 2017), but generally

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scallops are less sensitive to chemical inducers than oysters (Nicolas et al., 1998). Settlement may also depend on physical factors such as turbulence and seawater flow, as demonstrated with mussels in high turbulence conditions (Pernet et al., 2003c). Pearce et al. (1998) showed that in 9.5 m deep polyethylene tube mesocosms most *P. magellanicus* settled above a strong thermocline from 5°C to 17°C in absence of turbulence. When the thermocline was weak or absent, the number of settled spat increased with depth in the mesocosms. This study suggests that *P. magellanicus* could respond positively to turbulence. In a downwelling flow-through system continuously supplied with seawater and algae, no effect of seawater flow at the relatively low ranges between 2.7 and $8.3 \, \text{L h}^{-1}$ were observed for *P. maximus* settlement during the first 5 weeks (Robert and Nicholas, 2000). However, no data at high flow rates in relation to measure of turbulence are available, as for the size of larvae and their density in the settlement systems.

An important factor acting on metamorphosis success is the ability of post-larvae to meet energetic demands during intense morphogenesis (Beninger et al., 1994). Their metabolic requirement in intensive cultures is entirely dependent on acquired microalgal energy reserves accumulated. The primary endogenous energy reserve fuelling basal metabolism is the neutral lipid, triacylglycerol (Baker and Mann, 1993; Tremblay et al., 2007). Studies on *P. magellanicus* and *P. maximus* established the link between accumulation of neutral lipids during larval development and metamorphosis success, but with maximum levels generally less than 30% (Pernet et al. 2005, 2006; Gagné et al., 2010). The top-performing scallop larvae were characterized by a pronounced increase of neutral lipids accumulation during the pre-metamorphic period, followed by utilization during metamorphosis (Pernet et al., 2006). During metamorphosis, the pronounced morphogenesis of the feeding structures from a velum to full developed gills in *P. maximus* (Beninger et al., 1994) and *P. magellanicus* (Veniot et al., 2003) is relatively protracted and punctuated by critical transitional stages important for the survival and growth capacity of post-larvae. During early stages of the metamorphosis process, the gill may not be very effective in suspension feeding and accumulation of energetic reserve is essential.

The primary objective of this study is to improve the settlement ratio of the two scallop species, *Placopecten magellanicus* and *Pecten maximus* by physical cues associated with hydrodynamic conditions, stocking density in settlement units and larval size at time of transfer to settling units (transfer day). For each treatment, physiological condition was determined by fatty acid analysis to determine the total contents of neutral (energetic reserves) and polar (structural) lipids and their compositions. We tested the hypothesis that larger sized larvae stocked at low density will settle more intensively in high hydrodynamic conditions than smaller larvae in low hydrodynamic conditions independent of the post-larvae physiological condition.

2. Materials and methods

2.1. Rearing procedures

For each species, two batches of larvae were obtained with the larval rearing procedure described below, one batch to test size and stocking density and the other batch to test the hydrodynamic condition.

P. maximus larvae were obtained from the commercial hatchery Scalpro AS (60°31′N, 4°54′E) near Bergen in Norway. According to the standard hatchery procedures, broodstock had been collected from local stocks and conditioned until the gonads had reach maturity. The scallops (30) were induced to spawn by thermal shock (12–18°C) based on methods described by Gruffydd and Beaumont (1972) and Andersen et al. (2011). Three days post-fertilization larvae were transferred from the hatchery to Austevoll Research Station (Institute of Marine Research, 60°09′N; 5°26′E, Austevoll, Norway) for completion of the larval

planktonic phase before start of settlement studies. Competent (ready-to-settle) larvae were harvested 22–23 days post-fertilization (dpf) from a 8000 L flow-to-waste rearing system (Andersen et al., 2000) with continuous supply of algae diet (mixture of *Isochrysis galbana*, *Pavlova lutheri* and *Chaetoceros mulleri* at a ratio of 1:1:2 based on cell volume estimated by an electronic particle counter). During larval rearing the temperature was 16.5°C and the algal concentration fed to the larval tanks increased from 7.5 to 28.5 cells μL^{-1} following the growth of the larvae. Larval development was monitored two-three times a week until settlement experiments when 20% of the larvae were collected on 150 μm mesh and > 50% of the larval population contained foot and eyespots.

P. magellanicus ready to spawn (30) were obtained from the commercial hatchery Fermes Marines du Québec (48°15′N, 65°07′E) in Québec Canada and transported to the Station aquicole de Pointe-au-Père (UQAR, 48°27′N; 68°32′W, Québec, Canada). Spawning and larval rearing were realized based on methods described in Pernet et al. (2003a, b). We obtained immediate spawning by thermal shock from 10 to 16°C. The larvae were reared in a batch system using four 1000 L tanks filled with 1 μm filtered and UV treated seawater at a stocking density of 4 larvae mL^{-1} and temperature of 13.5°C. Larvae were fed at 30 cells μL^{-1} with a mixture of *Isochrysis galbana*, *Pavlova lutheri* and *Chaetoceros mulleri* at a ratio of 1:1:2 based on cell volume estimated by an electronic particle counter. Water and food were renewed every 2–3 days and larval development estimated. At 30 dpf, > 50% of the larvae retained on 210 μm mesh showed foot and eyespots, and were used for the settlement experiment.

2.2. Settlement experiments

The settlement system was similar for both species and consisted of 2 (in Canada) or 4 (in Norway) of 1000 L square (1 m x 1 m x 1 m) holding tanks, each with up to 16 partly submerged circular growth units (height 14 cm, diameter 24.4 cm, area = 467.4 cm², 150 μm mesh bottom) termed sieves. Four sieves were used for each factor studied and distributed in equal numbers between tanks. Here, the sieves represented the replication unit used for statistical analyzed. The tanks were used to maintain homogeneity of temperature and food concentration between sieves. The water depth in the sieves was 12 cm representing a volume of 6.3 L. An airlift system supplied the sieves with a downwelling flow of 20% new seawater mixed with algae and reused seawater with algae from the holding tank (80%) as described in Christophersen et al. (2006). Temperature and tank flow were monitored daily, the sieve flow rates were monitored twice a week (n = 8–9), algal flow and algal concentration in the tanks were monitored daily Monday to Friday. An algal diet was added to a calculated concentration of 15 cells μL^{-1} in the new seawater running into the tanks. Post-larvae were fed a mixture of three species, Tahitian *Isochrysis galbana*, *Pavlova lutheri* and *Chaetoceros mulleri* at a ratio of 1:1:2, respectively, based on cell volume. The feed was distributed from mixing tanks to each 1000 L holding tank by dosage pumps. The feed mixing tanks were filled with 30–90 L algae of concentrations 8–12 million cells mL^{-1} and topped with seawater to 400–600 L two-three times a week.

Shell height (perpendicular to the hinge) of 50 individuals per sieve was measured at the start and finish of the settlement experiments. The samples were stored in alcohol (35% in seawater) 1–4 weeks prior to measurements under a dissecting microscope. Lipid samples were also obtained at the start and the end of experiments with 1500–2000 individuals filtered onto a pre-combusted GF/C Whatman glass fibre filter (25 mm), kept on ice while carrying out the sampling and stored at −80°C before being extracted. Samples were transported from Norway to Canada on ultrafreeze ice-pack to maintain the samples around −40°C (Pellican BioThermal' Plymouth, MN, USA).

In Norway, two settlement experiments with two larval batches were carried out at 15°C with four replicate sieves per treatment. The

first experiment aimed to measure the effect of flow rate on settlement and larval condition with 6 flow rate treatments, 185 ± 6, 380 ± 6, 565 ± 4, 750 ± 1, 1125 ± 17 and $1297 \pm 26\,\mathrm{mL\,min^{-1}\,sieve^{-1}}$ representing a flow rate of 0.4, 0.8, 1.2, 1.6, 2.4 and $2.8\,\mathrm{mL\,min^{-1}}$ $\mathrm{cm^{-2}}$. Larvae of 18 dpf that retained on $150\,\mu\mathrm{m}$ mesh with a shell height of $166 \pm 20\,\mu\mathrm{m}$ (mean $\pm$ sd, n = 50) were stocked at a density of 60 larvae $\mathrm{cm^{-2}}$. The second experiment measured the effect of larval size and stocking density on settlement success at four size categories (> 150, 150–160, > 160 and $> 170\,\mu\mathrm{m}$) and three mean stocking densities (30, 60 and 90 larvae $\mathrm{cm^{-2}}$) at an intermediary flow rate of $1.2\,\mathrm{mL\,min^{-1}\,cm^{-2}}$. Due to lower number of larvae sized $> 170\,\mu\mathrm{m}$, the stocking density of 90 larvae $\mathrm{cm^{-1}}$ was not tested for this size category. Larvae of 20 dpf having a shell height of $175 \pm 28\,\mu\mathrm{m}$ (mean $\pm$ sd, n = 50) were categorized with the use of different mesh sizes. The $> 150\,\mu\mathrm{m}$ category represented all larvae collected on $150\,\mu\mathrm{m}$ mesh, the > 160 and $> 170\,\mu\mathrm{m}$ categories represented larvae collected on 160 and $170\,\mu\mathrm{m}$ mesh, respectively. Thus, this treatment measured the impact to eliminate gradual lower size of larvae on settlement success. Collecting larvae on $150\,\mu\mathrm{m}$ mesh for settlement was the standard procedure in several hatcheries at the time (Andersen et al., 2011). Another treatment containing the smaller larvae (150–$160\,\mu\mathrm{m}$) was obtained by collecting the larvae on $150\,\mu\mathrm{m}$ mesh but passed through $160\,\mu\mathrm{m}$ mesh to validate the impact of small larvae.

In Canada, the effects of the flow rate and size were also carried out on two different larval batches at $13.5\,^{\circ}\mathrm{C}$ with four replicate sieves per treatment. The first larval batch had 32 dpf a shell height of $233 \pm 20\,\mu\mathrm{m}$ (mean $\pm$ sd, n = 50) and was used to estimate size effect (> 210, > 220, > 230 and $> 240\,\mu\mathrm{m}$) on the settlement at the stocking density of 60 larvae $\mathrm{cm^{-2}}$. Due to lower larval availability than *P. maximus* experiments, stocking density was not tested. The second larval batch had 31 dpf and a shell height of $232 \pm 11\,\mu\mathrm{m}$ (mean $\pm$ sd, n = 50) and was used to estimate the effect of the flow rate (0.4, 0.8, 1.2 and $2.8\,\mathrm{mL\,min^{-1}\,cm^{-2}}$) on larvae $> 210\,\mu\mathrm{m}$ at the stocking density of 60 larvae $\mathrm{cm^{-2}}$.

Cumulative larval settlement was measured weekly for 4 weeks as estimated number of settled larvae in the sieves. Detached or loose larvae were gently removed by rinsing with seawater and stored in seawater until after the counting of settled individuals. They were then returned to the sieves. Settlement was examined by positioning the sieve bottom on a premade plastic counting slide marked to facilitate an exact position of the sieve and counting the number of settled larvae in 28 randomly distributed 0.5, 1.0 or $2.0\,\mathrm{cm^2}$ squares, depending on larval stocking density. The number of larvae per sieve was estimated by multiplying the average number of larvae $\mathrm{cm^{-2}}$ by the total sieve area. Settlement success was given as the % of attached larvae based on the initial number transferred to the sieves. By the end of the experiments, the sieves were emptied and the number of settled and detached larvae were estimated by volume. Samples of settled (metamorphosed) larvae were collected for subsequent measurement of shell height and lipid content.

2.3. Flow characterisation in downwelling sieves

The water flow characteristics were determined as described in Pernet et al. (2003c) using a 3-axis Acoustic Doppler Velocimeter, ADV, (Vectrino, NortekUSA, Annapolis, MD, USA) at a data rate of 25 Hz. 7500 data points were averaged for each measure. The x-axis corresponded to the diameter of the sieve with the coordinate 0 as the central point of the sieve and the coordinates 1 to 3 (or -1 to -3) at each three cm (Fig. 1). Coordinates -2 and 2 corresponded to the two-water input at the surface of the sieve. Thus, turbulence fluctuations (Δ) were measured in the x- (ΔU), y- (ΔV) and z- (ΔW) axes during 3 min on seven different points in the sieves. Four replicate sieves were investigated in both Norway and Canada (n = 8 as both location data was pooled). Velocity measurements were carried out 1 mm above the bottom screen and were determined in flow rates of 0.4, 1.2 and

$2.8\,\mathrm{mL\,min^{-1}\,cm^{-2}}$.

2.4. Fatty acid analysis

Lipids were extracted using the Folch method (Folch et al., 1957), separated into neutral and polar lipid fractions using silica gel ($30 \times 5\,\mathrm{mm}$ i.d., packed with Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) hydrated with 6% water, and eluted with 10 mL of chloroform:methanol (98:2 v/v) for neutral lipids followed by 20 mL of methanol for polar lipids (Marty et al., 1992). The neutral lipid fraction was further eluted on an activated silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid methyl esters (FAME) were prepared as described by Lepage and Roy (1984) and analyzed in MSMS scan mode (ionic range: 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan, Mississauga, ON, CA) equipped with a Valcobond VB-5 capillary column (VICI Valco Instruments Co. Inc., Broakville, ON, CA). FAME were identified by comparison of retention times with known standards (37 component FAME Mix, PUFA-3, BAME, and menhaden oil; Supelco Bellefonte, PA, USA) and quantified with tricosanoic acid (23:0) and nonadecanoic acid (19:0) as internal standards. Chromatograms were analyzed using the Xcalibur 1.3 integration software (Thermo Scientific, Mississauga, ON, CA).

2.5. Statistical analyzes

With the absence of normal distribution for settlement of *P. maximus* in relation to larval size and density, Kruskal-Wallis analyzes were used for each factor (four levels of size category, three levels of stocking density and four levels of week). As no stocking density effect was determined, data was pooled to measure the size category effect. Effects of larvae size category (four levels) and weeks (four levels) were tested with two-way ANOVA for each species separately. Settlement success were analyzed for each species separately using two-way ANOVA for temporal effect of flow with four levels for each of the factors flow and week for *P. magellanicus*, and six flow levels and four weeks levels for *P. maximus*. Where differences were detected, least-square means multiple comparison tests were used to determine which of the means were significantly different. Normality and heteroscedasticity were tested by using the Kolmogorov–Smirnov and Levene's tests, respectively. When necessary, data was $\log + 1$ transformed to achieve normality of residuals and homogeneity of variances. one-way ANOVA was performed for each species separately for accumulation of total fatty acids and specific fatty acids at the beginning and at the end of the four weeks settlement experiments with four flow levels or four size category levels for *P. magellanicus*, and six flow levels and three density levels for *P. maximus*. When absence of normal distribution was observed, Kruskal-Wallis analyzes were used. These statistical analyzes were done using Systat V12.02 software.

Permutational multivariate analysis of variance (PERMANOVA with 9999 permutations) based on Euclidean dissimilarities, including a posteriori pair-wise comparisons, was performed with PRIMER 6 (v. 7.0.13) and PERMANOVA $+1$ on fatty acids profiles from polar and neutral lipid fractions. For *P. maximus*, each PERMANOVA was tested with one factor for flow experiments and two factors (size larvae and density) for the second experiment. For *P. magellanicus*, one-way PERMANOVA was used for flow rate and size experiment results. Assumptions of homoscedasticity were verified with a PERMDISP test, and data was arcsine square root transformed when necessary. The similarity percentages (SIMPER) procedure was performed on untransformed data to identify specific fatty acids explaining the most important dissimilarity between significant levels of factors.
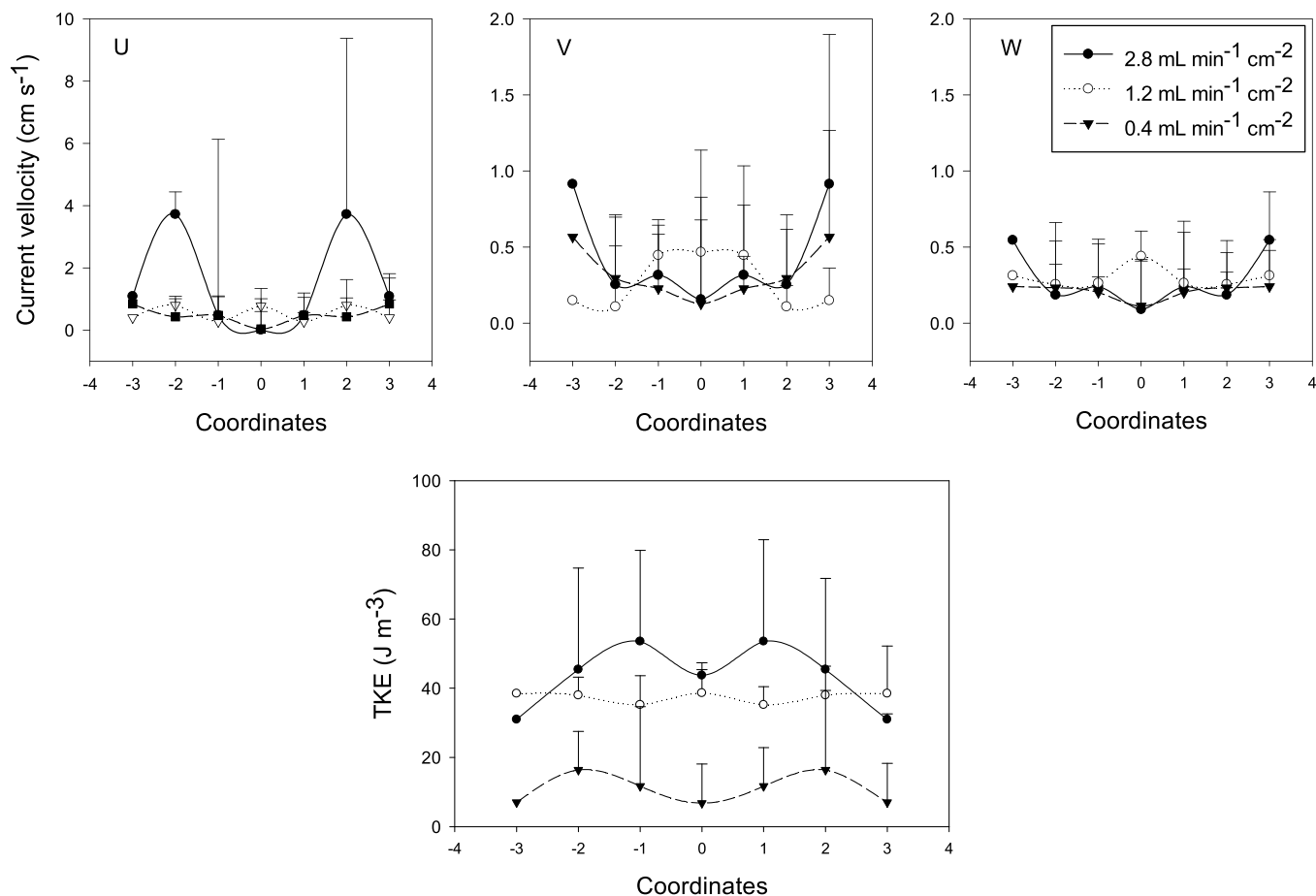
Fig. 1. Velocity in cm s^{-1} obtained on the x-, y and z spatial components (termed respectively U, V and W) and turbulence kinetic energy (TKE) in J m^{-3}. TKE were measured along the *x*-, *y*- and *z*-axes. Measurements in circular downwellers (sieves) were carried out at three different seawater flow rates (mL min^{-1} cm^{-2}) for two species. Mean ± SE, n = 8 (results from Norway and Canada pooled) replicate sieves.

3. Results

3.1. Hydrodynamic characterization of flow in a downwelling sieve

Flow and turbulence are presented as means of measures obtained in each sieve with flow rates of 0.4, 1.2 and 2.8 mL min^{-1} from both Norway and Canada (Fig. 1, n = 8), since similar downwelling sieves and flow rates were used. There was a clear effect of flow rate increase on flow velocity and turbulence measured in the sieves, and this varied with the position of the sampling cell along the transect. Specifically, ΔU was higher with a maximum value of 3.72 cm s^{-1} at the coordinates −2 and 2, corresponding to water input position at the surface of the sieve. The effect was predominant in the highest flow regime of 2.8 mL min^{-1} cm^{-2}. The ΔV and ΔW components of the flow showed the highest values close to the edge of the sieve with a maximum value of 0.92 cm s^{-1} for the v components and 0.55 cm s^{-1} for the component w. Exception was noted for the flow of 1.2 mL min^{-1} cm^{-2} where higher values were registered in the middle of the sieve for v and w components. Turbulence also varied with the position of the sampling cell along the transect for flow rates 0.4 and 2.8 mL min^{-1} cm^{-2}, with higher values around the position of water input (coordinates −2 and 2). Turbulence increased progressively with flow rate, with the highest value of 53.5 J m^{-3} for 2.8 mL min^{-1} cm^{-2} (Fig. 1B).

3.2. Settlement success

Stocking density of larvae in the sieves was tested only for *P. maximus*. Densities of 30, 60 and 90 larvae cm^{-1} presented no significant difference in settlement success between treatments ($KW = 0.793$, $p = 0.673$). However, the effects of size category (KW = 14.26, $p = 0.003$) and week (KW = 121.5, $p < 0.001$) were significant for this species, with the highest value registered after four weeks for larvae > 170 μm after transfer to the sieves (Fig. 2). Similar effects of size category ($F = 78.2$, $p < 0.001$) and week ($F = 343.7$, $p < 0.001$) were observed in *P. magellanicus* without significant interaction between factors ($F = 2.17$, $p = 0.061$). Maximum value of 40.1% settlement was observed after four weeks for *P. magellanicus* larvae from the largest size category (> 240 μm) (Fig. 2).

For both *P. maximus* (flow: $F = 8.95$, $p < 0.001$; week: $F = 163.7$, $p < 0.001$) and *P. magellanicus* (flow: $F = 119.3$, $p < 0.001$; week: $F = 205.3$, $p < 0.001$), flow and weeks had a significant effect on settlement success (% settled) without interaction between factors (*P. maximus*: $F = 0.47$, $p = 0.948$; *P. magellanicus*: $F = 1.17$, $p = 0.09$). For both species, higher settlement success was observed at flow rates > 2 mL min^{-1} cm^{-2} after four weeks in the sieves (Fig. 3). The maximum settlement percentages registered was 40.7% and 40.1% for *P. maximus* and *P. magellanicus*, respectively, both at 2.8 mL min^{-1} cm^{-2}. The minimum level was 11.6% and 9.1% for *P. maximus* and *P. magellanicus*, respectively. For *P. maximus* the minimum value was observed at 1.2 mL min^{-1} cm^{-2} and for *P. magellanicus* at 0.4 mL min^{-1} cm^{-2}.

3.3. Physiological condition of settled post-larvae

Physiological condition of larvae distributed in each sieve was characterized at the beginning of each experiment by the measure of
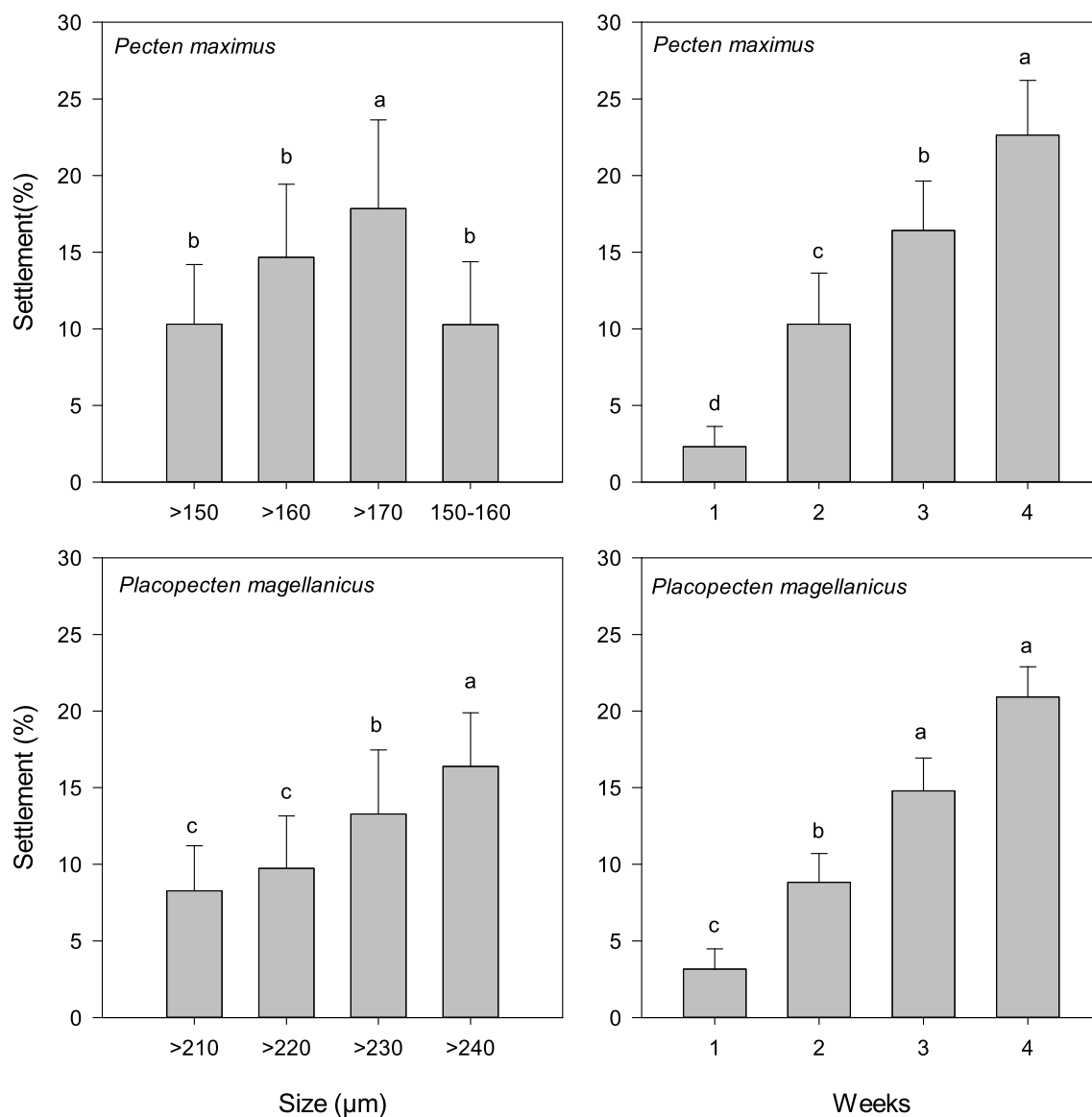
Fig. 2. Settlement success (% of settled larvae in the sieves) of *Pecten maximus* and *Placopecten magellanicus* in a circular downwellers as a function of larvae size category (right; data from different weeks were pooled as no interaction was observed) and week (left, data from different larval size were pooled). Different Anova was applied for each species. Different letters indicate significant differences following least-square means multiple comparison tests. Mean ± SE, n = 4.

total fatty acids in the neutral lipids fraction corresponding to the accumulation of energetic reserves. For each experiment, the physiological condition of larvae were similar before being exposed to different treatments (*P. maximus* experiments on flow rates $F = 2.12$, $p = 0.151$ and larval size x stocking density $F = 0.615$, $p = 0.204$; *P. magellanicus* experiments on flow rate $F = 0.47$, $p = 0.948$ and size $F = 1.51$, $p = 0.401$) with a mean (± sd) of 22.5 ± 8.1 pg larvae^{-1} and 31.9 ± 10.7 pg larvae^{-1} for *P. maximus* and *P. magellenicus*, respectively. Flow rate had a significant effect on the accumulation of total fatty acids in settled post-larvae in the neutral lipid fraction, but not in polar lipids in both species (*P. maximus* Neutral: $F = 10.61$, $p = 0.014$; Polar: $KW = 2.58$, $p = 0.461$ and *P. magellanicus* Neutral: $KW = 8.98$, $p = 0.01$; Polar: $KW = 3.75$, $p = 0.178$). In both species, accumulation of total fatty acids in the neutral lipids at the highest flow rate of 2.8 mL min^{-1} cm^{-2} was nearly twice as high as at the other flow rates (Fig. 4). In relation to the size category of larvae when transferred to sieves, we observed a significant difference in total fatty acids of settled post-larvae of both species after four weeks in the sieves, but only in the polar lipid fraction (*P. maximus* Neutral: $KS = 2.5$, $p = 0.283$; Polar: $KW = 9.99$, $p = 0.007$ and *P. magellanicus* Neutral: $F = 3.88$, $p = 3.88$;

Polar: $F = 12.77$, $p = 0.009$). The highest values were measured in the highest sizes categories > 170 μm (*P. maximus*) and > 230 and 240 μm (*P. magellanicus*) (Fig. 5).

Flow rate also impacted the composition of fatty acids in both lipid fractions of each species as demonstrated by PERMANOVA analyzes (*P. maximus* Neutral: *Pseudo-F* = 24.5, *P-perm* < 0.001; Polar: *Pseudo-F* = 9.47, *P-perm* < 0.001 and *P. magellanicus* Neutral: *Pseudo-F* = 14.5, *P-perm* < 0.001; Polar: *Pseudo-F* = 11.2, *P-perm* < 0.001). Simper analyzes showed that the difference in the fatty acid composition was explained mainly by the variation of docosahexaenoic acid, DHA (22:6n3) contributing to between 28 and 56% of the differences observed. Post-larvae that settled at higher flow rates accumulated more DHA after 4 weeks in the sieves than post-larvae exposed to low flow rates (Fig. 6). This applied to the neutral and polar fraction in *P. maximus* (Neutral: $F = 21.4$, $p < 0.001$; Polar: $KW = 5.51$, $p = 0.013$) and *P. magellanicus* (Neutral: $KS = 7.566$, $p = 0.016$; Polar: $F = 17.7$, $p < 0.001$). The size category of larvae had no effect on the fatty acid composition of polar and neutral lipids in neither species.
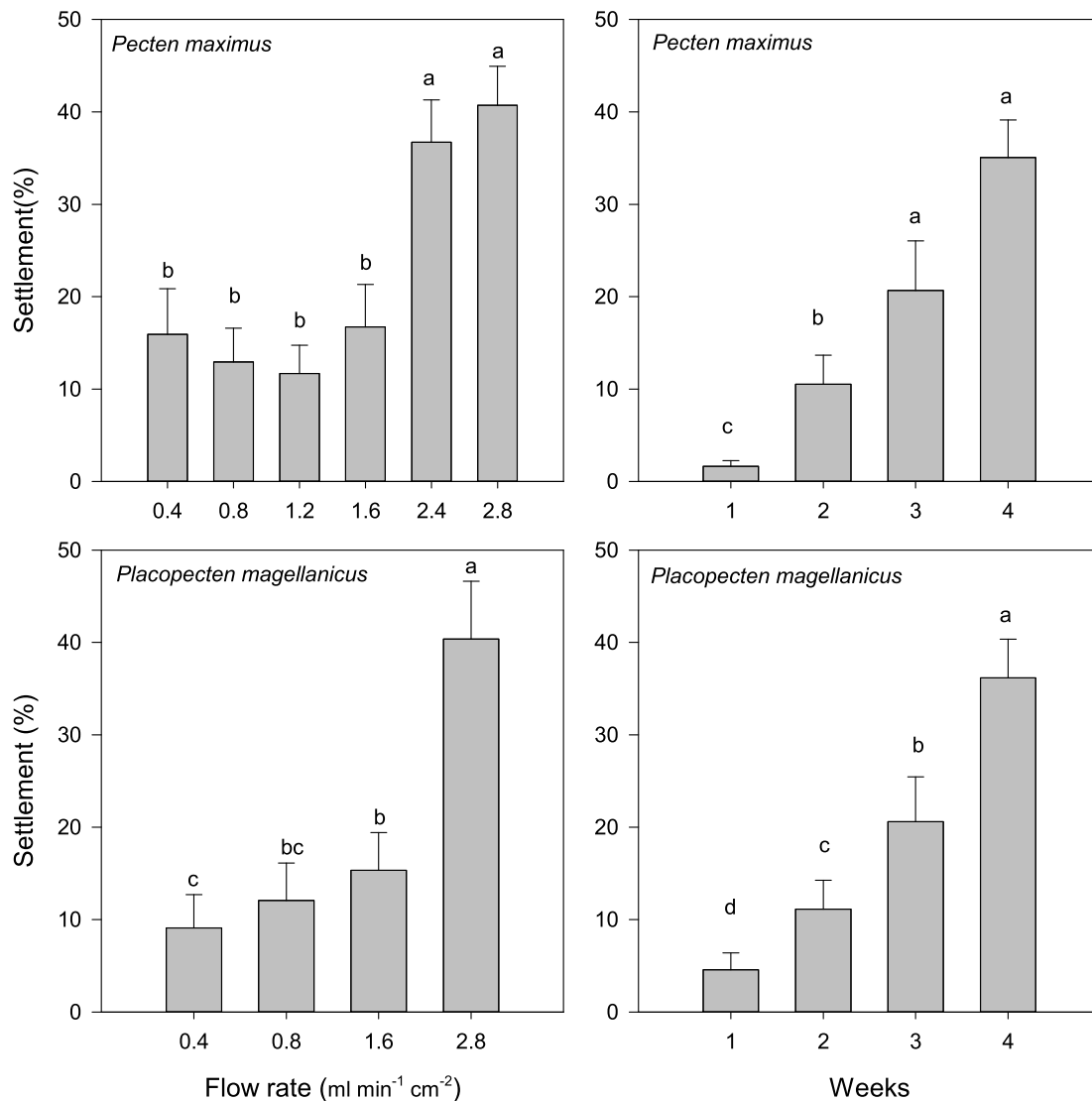
Fig. 3. Settlement success (% of settled larvae in the sieves) of *Pecten maximus* and *Placopecten magellanicus* in a circular downwellers as a function of flow rate (right; data from different weeks were pooled as no interaction was observed) and week (left, data from different flow rate were pooled). Different Anova was applied for each species. Different letters indicate significant differences following least-square means multiple comparison tests. Mean ± SE, n = 4.

4. Discussion

Our study showed that increasing the flow rate from 0.4 to 2.8 mL min^{-1} cm^{-2} in settlement sieves of 24.4 cm in diameter improved largely the settlement success and affected positively the physiological condition of settled post-larvae by a higher accumulation of total fatty acids in neutral lipid fractions and higher accumulation of DHA. This study demonstrated also for *P. maximus* an absence of effect of larval stocking density between 30 and 90 larvae cm^{-2} in the downwelling sieves on the settlement success (% of initial number in settlement sieves) for each larval size tested. Furthermore, we validated the hypothesis of the positive effect of larval size and increased flow after transfer to settlement systems on settlement success for two important commercial pectinid species, *P. maximus* and *P. magellanicus*. Results show that the larger size class; > 170 μm for *P. maximus* and > 240 μm for *P. magellanicus,* is the best to increase the relative settlement success. However, these large larvae tended to represent a very small fraction of the total larval batch, thus the total outcome from settlement in terms of number is very low for this size class, decreasing the interest at the industrial level.

The behavior of settling bivalve larvae in response to water flow in a

downwelling system (sieves) usually used in hatcheries for settlement, have rarely been investigated. The only studies we could find on the effect of flow rate on settlement in down-welling sieves was by Robert and Nicolas (2000) in *P. maximus* and Pernet et al. (2003) on mussel larvae. However, in flume or pipe systems several investigations are reported (Butman et al., 1988; Mullineaux and Butman, 1991; Mullineaux and Garland, 1993; Olivier et al., 1996; Eckman and Duggins, 1998; Finelli and Wethey, 2003; DiBacco et al., 2011; Koehl et al., 2013). The advantageous of flume or pipe is the possibility to isolate particular hydrodynamic variables. In downwelling sieves, each hydrodynamic variable (U, V, W) varies with all others. However, as demonstrated by Pernet et al. (2003c), this system allows the quantitative measurement of survival and settlements success, not possible in flume or pipe as the area studied there represents only a fraction of the experimental unit. For aquaculture studies, a downwelling sieve is advantageous because of the possibility to apply a wide range of treatments and replicates simultaneously with post-larvae originating from the same larval batch. Thus, results can be directly transferred to commercial hatcheries. Our results showed that the major hydrodynamic component related to flow regime in downwelling sieves was the turbulence which increased three times when flow rate increased
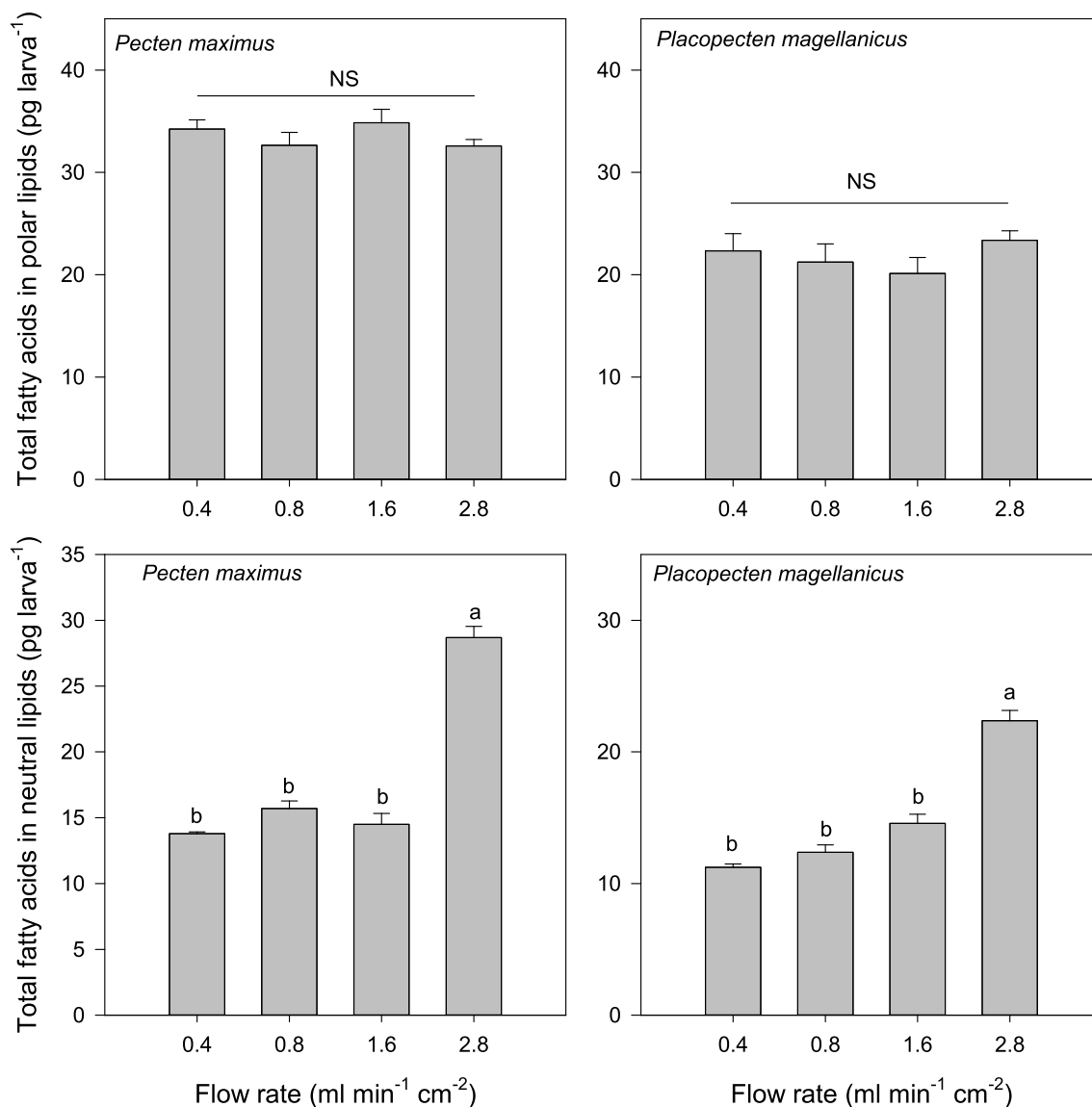
Fig. 4. Effect of flow rate on total fatty acid accumulation in polar and neutral lipid fraction in settled post-larvae of each species. Different Anova was applied for each species. Different letters indicate significant differences. NS – not significant. Mean ± SE, n = 4.

from 0.4 to $2.8 \, \text{mL} \, \text{min}^{-1} \, \text{cm}^{-2}$. The higher turbulence at $2.8 \, \text{mL} \, \text{min}^{-1} \, \text{cm}^{-2}$ was related to a settlement percentage that was more than doubled compared to a flow rate of $0.4 \, \text{mL} \, \text{min}^{-1} \, \text{cm}^{-2}$. These results are in accordance with previous studies on mussels (Pernet et al., 2003c) and clams (Crimaldi et al., 2002). Water motion affected substrate exploration of competent larvae, like pectinid pediveliger larvae, by stimulating or altering the exploratory behavior (Butman, 1990; Mullineaux and Butman, 1991; Kobak, 2005; Dobretsov and Wahl, 2008). Turbulence can increase the larval fluxes to substratum and some mollusk veliger larvae respond to turbulence by sinking or diving more frequently (Fuchs et al., 2013), increasing their contact and subsequent settlement. The basic decision of larvae regarding swimming or sinking in response to turbulence cues have a dramatic effect on settlement rates (Hubbard et al., 2015). However, peaks in hydrodynamic force on settling larvae can also decrease their probability of anchoring (Crimaldi et al., 2002; Koehl et al., 2013). The maximum flow rate studied in our downwelling sieve seemed not to develop hydrodynamic forces washing settled larvae off the surface. Thus, the generated turbulence seemed to increase contact of pediveliger larvae to settling surface explaining, at least partially, the higher settlement rate observed. The positive effect we found of flow rate and

turbulence on pediveliger larvae is in contrast to the negative effect of hydrodynamic forces reported by Holbach et al. (2017) on early D-veliger larvae of *P. maximus* exposed to aeration. However, their larval system was very different from our settlement system.

Turbulence can also enhance the contact of food with pediveliger larvae (Fréchette et al., 1989; Gallager, 1993) and thus increase their feeding efficiency stimulating settlement success. Furthermore, as algae concentration was the same at all flow rates, the level of particles offered to the larvae per unit time therefore increased with flow rates. Settlement and metamorphosis of post-larvae and early juveniles are highly energetic processes (Holland and Spencer, 1973) and competent pediveliger larvae have a limited ability to feed on phytoplankton during the degradation of the velum and development of gills (Baker and Mann, 1993; Veniot et al., 2003). However, a limitation to increased turbulence in settlement system is expected and further works is needed to identify this limitation. Recent work on American oyster larvae, *Crassostrea americanus*, demonstrated that pediveliger larvae swim harder when they are tumbling in strong currents (Fuchs et al., 2017). The ciliary swimming uses a small fraction of metabolic energy in still water (Crawford, 1992), but in high turbulent environment oyster larvae are unable to gain energy in strong turbulence partly by
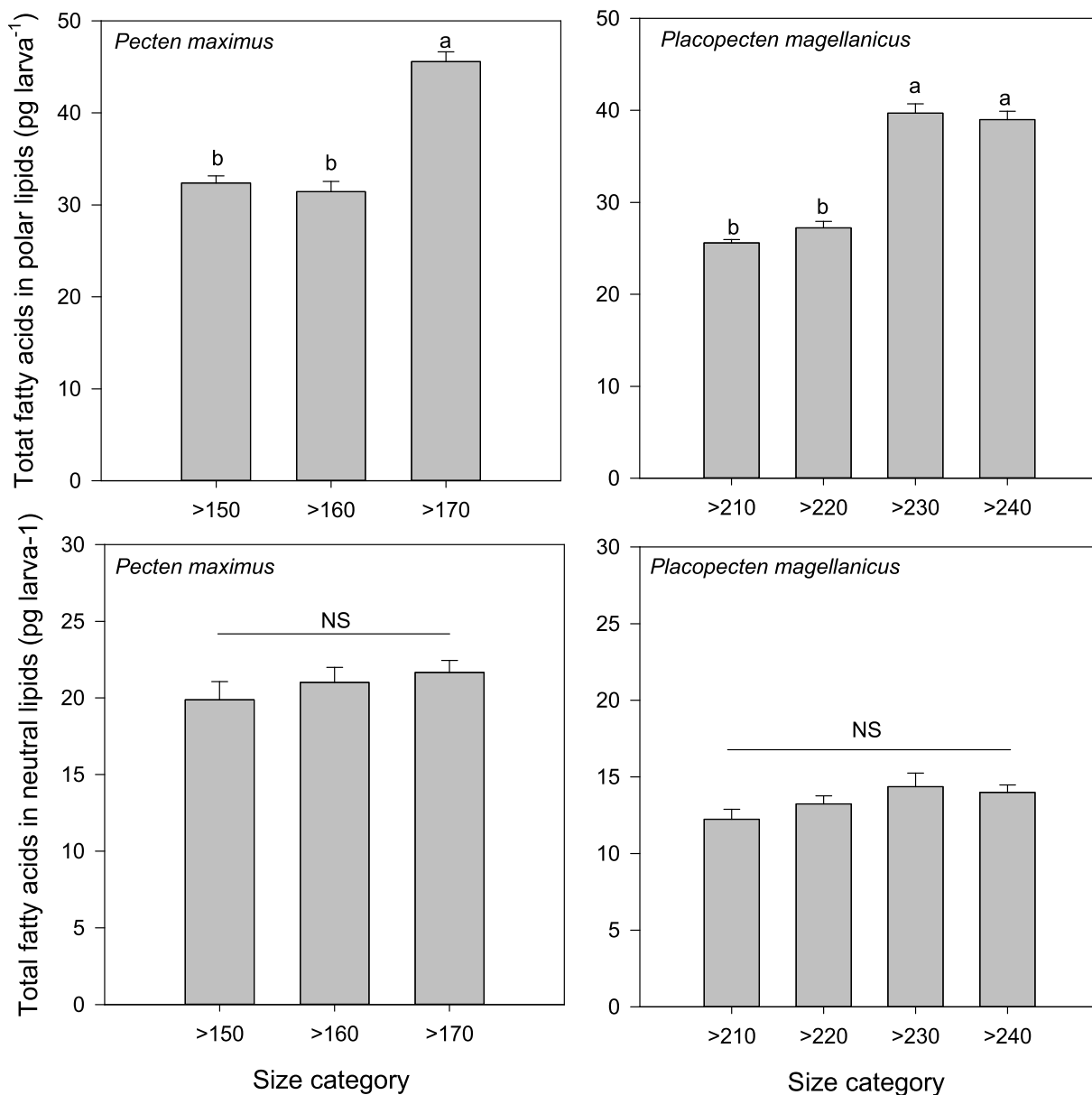
Fig. 5. Effect of larvae size category on total fatty acid accumulation in settled post-larvae of each species. Different Anova was applied for each species. Different letters indicate significant differences. NS – not significant. Mean ± SE, n = 4.

inhibited food capture (Fuchs et al., 2017). As larvae swim and feed at the same time using the same appendage, it seems that at too high turbulent conditions, they cannot do both at full capacity simultaneously. In your experiments, it seems that the higher flow rate used does not seem sufficient to generate turbulence impact on food ingestion by pediveliger larvae, as their accumulation of fatty acid content in neutral lipid fraction (energetic lipid reserve) were higher.

P. maximus and *P. magellanicus* larvae characterized by the greater accumulation of neutral fatty acids during the pre-metamorphic period have been demonstrated to have better settlement and metamorphosis success (Pernet et al., 2006; Gagné et al., 2010). These results are in accordance with our study where the larvae in higher turbulent conditions showed higher accumulation of total fatty acids in the neutral lipid fraction. Neutral lipids are mainly constituted by triacylglycerol, the main energetic lipids in bivalve larvae and polar lipids by the phospholipids, the main constituent of membrane lipids (Delaunay et al. 1992, 1993; Genard et al., 2011). As no difference was observed in polar lipids, the differences in physiological conditions seem not related to the difference in shell height of settled larvae related to flow rate

condition, but only to energetic lipid accumulation. Thus, keeping pectinid pediveliger larvae in downwelling sieves for four weeks in turbulent conditions will stimulate higher feeding efficiency resulting in higher settlement success. These larvae will also accumulate higher proportion of DHA (22:6n3), probably related to higher feeding efficiency resulting in higher retention of this essential fatty acids. DHA has been demonstrated to be a growth-limiting factor for pectinid larvae (Delaunay et al., 1993; Soudant et al., 1998; Nevejan et al., 2003; Pernet and Tremblay, 2004; Pernet et al., 2005). DHA are incorporated in membrane phospholipids and is involved in the maintenance of the structural and functional integrity of biological membranes (Hazel, 1995). DHA comes from the dinoflagellates in the diet, as *Isochrysis galbana* and *Pavlova lutheri* are richer in DHA compared to *Chaetoceros mulleri*. The results could suggest a higher feeding efficiency for the two dinoflagellates compared to *C. mulleri* under turbulent conditions.

To our knowledge this study is the first to characterize the effect of seawater flow rate on settlement success of different pectinid species cultured under similar conditions. Previous studies have focused mainly on the substratum (Harvey et al. 1993, 1997; Heasman et al., 2002; De
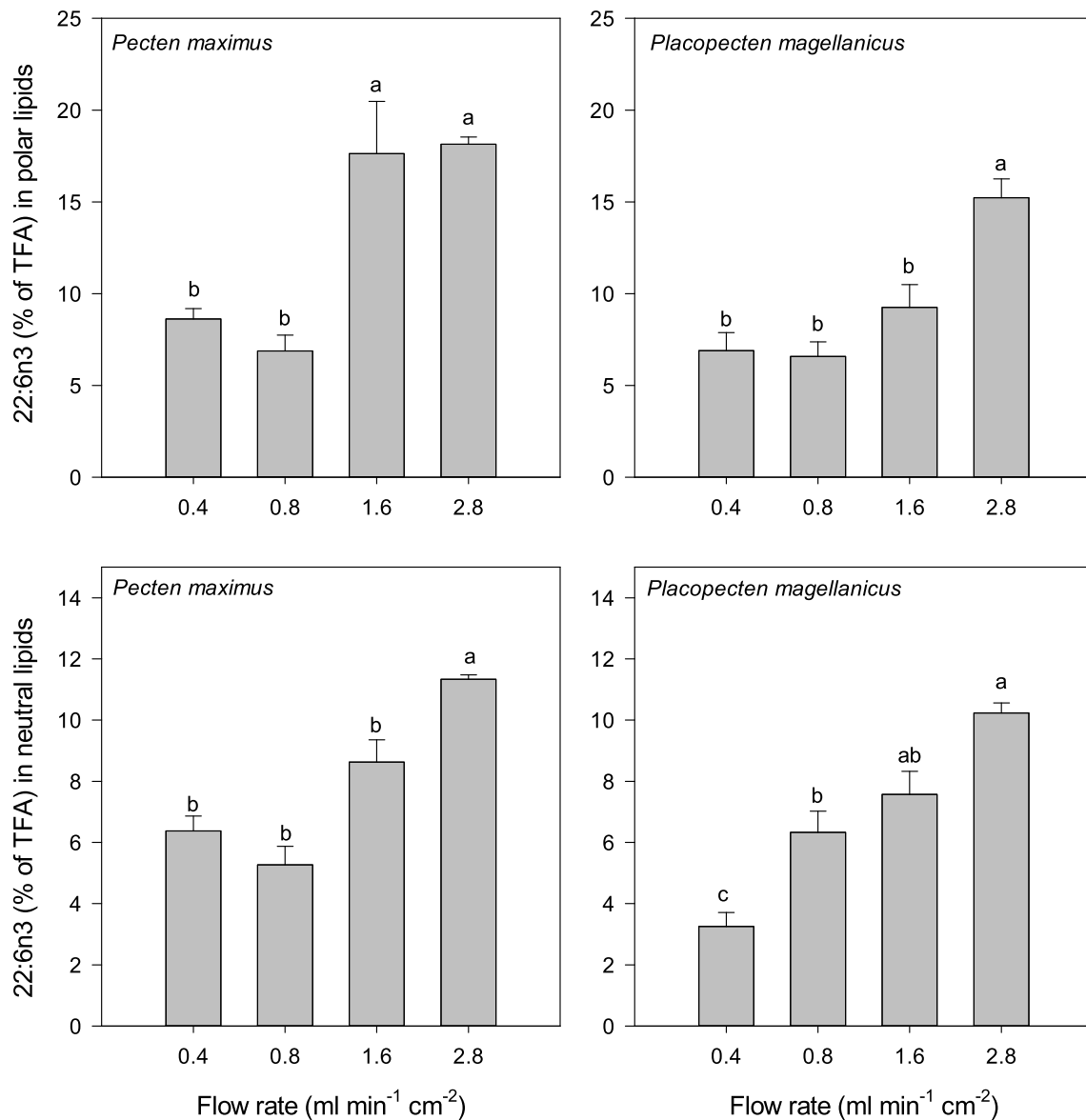
Fig. 6. Effect of flow rate (mL min^{-1} cm^{-2}) on mass % of docosahexaenoic acid, DHA (22:6n3), related to total fatty acids (TFA) in settling post-larvae of each species. Different Anova was applied for each species. Different letters indicate significant differences. Mean ± SE, n = 4.

La Roche et al., 2005), temperature and salinity (Pearce et al., 1996; Christophersen and Magnesen, 2009), trophic condition (Nicolas and Robert, 2001; Pernet et al., 2006; Gagné et al., 2010), biofilm (Tritar et al., 1992; Parsons et al., 1993; Leyton and Riquelme, 2008) and chemical cues (Martinez et al., 1999; Mesías-Gansbiller et al., 2008). We demonstrated the importance of controlling water flow parameters in downwelling settlement system. We used an experimental design close to the commercial scale and thus the results obtained are relevant to industry situations. The possibility of improving and stabilizing scallop spat production by using correct larval size, time and hydrodynamic cues was shown. Clearly, we demonstrated that the hydrodynamics of settlement growth systems are important for maximizing the output of viable scallop post-larvae and juveniles (spat). However, to further improve the settlement success, interaction between size and high flow rates could be tested in the future. In the literature, the number of studies aiming at increasing settlement success of *P. maximus* and *P. magellanicus* are limited (Pernet et al., 2005: 2006; Andersen et al., 2011, 2013; Galley et al., 2017), but with values rarely over 20%, the transition from larval to juvenile stage is a limiting factor. Our results are promising and shows that the larvae size before transfer to settlement sieves and the sieve flow conditions increase synchronicity and settlement success to over 40%. We observed positive effects of flow rate and size of larvae but no effect of larval stocking density. However, we suggest that these positive results may be related to the levels of flow used in the experiments – they may be too low to find negative impacts. Thus, it is necessary to test increasing levels until observation of negative results is made to determine the optimum level of turbulence generated by the flow rate for the settlement success of these scallop species.

Declaration of competing interest

We have no conflict of interest.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.734650.

References

Andersen, S., Burnell, G., Bergh, Ø., 2000. Flow-through systems for culturing great scallop larvae. Aquacult. Int. 8, 249–257.

Andersen, S., Christophersen, G., Magnesen, T., 2011. Spat production of the great scallop (*Pecten maximus*): a roller coaster. Can. J. Zool. 89, 579–598.

Andersen, S., Christophersen, G., Magnesen, T., 2013. Implications of larval diet concentration on post-larval yield in a production scale flow-through system for scallops (Pecten maximus Lamarck) in Norway. Aquacult. Int. 21, 435–452.

Baker, S., Mann, R., 1993. Description of metamorphic phases in the oyster *Crassostrea virginica* and effects of hypoxia on metamorphosis. Mar. Ecol. Prog. Ser. 104, 91–99.

Beninger, P.G., Dwiono, S.A.P., Le Pennec, M., 1994. Early development of the gill and implications for feeding in *Pecten maximus* (Bivalvia: Pectinidae). Mar. Biol. 119, 405–412.

Bergh, Ø., Strand, Ø., 2001. Great scallop, *Pecten maximus*, research and culture strategies in Norway: a review. Aquacult. Int. 9, 305–318.

Butman, C., Grassle, J.P., Webb, C.M., 1988. Substrate choices made by marine larvae settling in still water and in a flume flow. Nature 333, 771–773.

Butman, C.A., 1990. Sediment-trap experiments on the importance of hydrodynamical processes in distributing settling invertebrate larvae in near-bottom waters. J. Exp. Mar. Biol. Ecol. 134, 37–88.

Christophersen, G., Magnesen, T., 2009. Settlement of *Pecten maximus* L. - effects of the postlarval rearing environment. Bull. Aquacult. Assoc. Can. 107–1,2, 55–60.

Christophersen, G., Torkildsen, L., van der Meeren, T., 2006. Effect of increased water recirculation rate on algal supply and post-larval performance of scallop (*Pecten maximus*) reared in a partial open and continuous feeding system. Aquacult. Eng. 35, 271–282.

Chevolot, L., Cochard, J.-C., Yvin, J.-C., 1991. Chemical induction of larval metamorphosis of *Pecten maximus* with a note on the nature of naturally occurring triggering substances. Mar. Ecol. Prog. Ser. 74, 83–89.

Couturier, C., Dabinett, P., Lanteigne, M., 1995. Scallop culture in Atlantic Canada. In: Boghen, A.D. (Ed.), Cold-Water Aquaculture in Atlantic Canada. The Canadian Institute for Research on Regional Development, Canada, pp. 297–340.

Crawford, D.W., 1992. Metabolic cost of motility in planktonic protists: theoretical considerations on size scaling and swimming speed. Microb. Ecol. 24, 1–10.

Crimaldi, J., Thompson, J., Rosman, J., Lowe, R., Koseff, J., 2002. Hydrodynamics of larval settlement: the influence of turbulent stress events at potential recruitment sites. Limnol. Oceanogr. 47, 1137–1151.

Cyr, C., Myrand, B., Cliche, G., Desrosiers, G., 2007. Weekly spat collection of sea scallop, Placopecten magellanicus, and undesirable species as a potential tool to predict an optimal deployment period of collectors. J. Shellfish Res. 26, 1045–1054.

Dao, J.C., Fleury, P.G., Barret, J., 1999. Scallop Sea Bed Culture in Europe. Stock Enhancement and Sea Ranching. Fishing News Books, Blackwell Science, Oxford, UK, pp. 423–436.

De La Roche, J.P., Louro, A., Roman, G., 2005. Settlement of *Chlamys varia* (L.) in the hatchery. J. Shelfish Res. 24, 363–368.

Delaunay, F., Marty, Y., Moal, J., Samain, J.-F., 1992. Growth and lipid class composition of *Pecten maximus* (L.) larvae grown under hatchery conditions. J. Exp. Mar. Biol. Ecol. 163, 209–219.

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.

DiBacco, C., Fuchs, H.L., Pineda, J., Helfrich, K., 2011. Swimming behavior and velocities of barnacle cyprids in a downwelling flume. Mar. Ecol. Prog. Ser. 433, 131–148.

Dobretsov, S., Wahl, M., 2008. Larval recruitment of the blue mussel *Mytilus edulis*: the effect of flow and algae. J. Exp. Mar. Biol. Ecol. 355, 137–144.

Eckman, J.E., Duggins, D.O., 1998. Larval settlement in turbulent pipe flows. J. Mar. Res. 56, 1285–1312.

Finelli, C.M., Wethey, D.S., 2003. Behavior of oyster (*Crassostrea virginica*) larvae in flume boundary layer flows. Mar. Biol. 143, 703–711.

Fuchs, H.L., Reidenbach, M.A., 2013. Biophysical constraints on optimal patch lengths for settlement of a reef-building bivalve. PLoS One 8, e71506.

Fuchs, H.L., Specht, J.A., Adams, D.K., Christman, A.J., 2017. Turbulence induces metabolically costly behaviors and inhibits food capture in oyster larvae, causing net energy loss. J. Exp. Biol. 220, 3419–3431.

Fréchette, M., Butman, C.A., Geyer, W.R., 1989. The importance of boundary-layer flows in supplying phytoplancton to the benthic suspension feeder. *Mytilus edulis* L. Limnol. Oceanogr. 34, 19–36.

Gagné, R., Tremblay, R., Pernet, F., Miner, P., Samain, J.-F., Olivier, F., 2010. Lipid requirements of the scallop *Pecten maximus* (L.) during larval and post-larval development in relation to addition of *Rhodomonas salina* in diet. Aquaculture 309, 212–221.

Galley, T.H., Beaumont, A.R., Le Vay, L., King, J.W., 2017. Influence of exogenous chemicals on larval development and survival of the king scallop *Pecten maximus* (L.). Aquaculture 474, 48–56.

Gallager, S.M., 1993. Hydrodynamic disturbances produced by small zooplankton: case study for the veliger larva of a bivalve mollusc. J. Plankton Res. 15, 1277–1296.

Genard, B., Pernet, F., Lemarchand, K., Boudry, P., Moraga, D., Tremblay, R., 2011. Physiological and biochemical changes associated with massive mortality events occurring in larvae of American oyster (*Crassostrea virginica*). Aquat. Liv. Ress. 24, 247–260.

Gruffydd, L.D., Beaumont, A.R., 1972. A method for rearing of Pecten maximus in the laboratory. Mar. Biol. 15, 350–355.

Harvey, M., Bourget, E., Gagné, N., 1997. Spat settlement of the giant scallop, *Placopecten magellanicus* (Gmelin, 1791), and other bivalve species on artificial filamentous collectors coated with chitinous material. Aquaculture 148, 277–298.

Harvey, M., Bourget, E., Miron, G., 1993. Settlement of Iceland scallop *Chlamys islandica* spat in response to hydroids and filamentous red algae: field observations ans laboratory experiments. Mar. Ecol. Prog. Ser. 99, 283–292.

Hazel, J.R., 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57, 19–42.

Heasman, M.P., O'Connor, W.A., Frazer, A.W., Languet, Y., O'Connor, S.J., 2002. Alternative means of nursery culture for commercial scallop (*Pecten fumatus* Reeve) spat. Aquaculture 213, 323–338.

Holbach, M., Robert, R., Miner, P., Mingant, C., Boudry, P., Tremblay, R., 2017. Effects of hydrodynamic factors on *Pecten maximus* larval development. Aquacult. Res. 48, 5463–5471.

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.

Hubbard, A.B., Reidenbach, M.A., 2015. Effects of larval swimming behavior on the dispersal and settlement of the eastern oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 535, 161–176.

Karney, R.C., 1991. Ten years of scallop culture on Martha's Vineyard. In: Shumway, S.E., Sandifer, P.A. (Eds.), An International Compendium of Scallop Biology and Culture. Selected Papers from the 7th International Pectinid Workshop. The World Aquaculture Society, USA, pp. 308–312.

Kobak, J., 2005. Recruitment and distribution of *Dreissena polymorpha* (Bivalvia) on substrates of different shape and orientation. Int. Rev. Hydrobiol. 90, 159–170.

Koehl, M.A., Crimaldi, R., J.P., Dombroski, D.E., 2013. Wind chop and ship wakes determine hydrodynamic stresses on larvae settling on different microhabitats in fouling communities. Mar. Ecol. Prog. Ser. 479, 47–62.

Lagarde, F., Richard, M., Bec, B., Roques, C., Mortreux, S., Bernard, I., Chiantella, C., Messiaen, G., Nadalini, J.B., Hori, M., Hamaguchi, M., Pouvreau, S., Roque d'Orbcastel, E., Tremblay, R., 2018. Trophic environments influence size at metamorphosis and recruitment performance of Pacific oysters. Mar. Ecol. Prog. Ser. 602, 135–153.

Leyton, Y.E., Riquelme, C.E., 2008. Use of specific bacterial-microalgal biofilms for improving the larval settlement of *Argopecten purpuratus* (Lamarck, 1819) on three types of artificial spat-collecting materials. Aquaculture 276, 78–82.

Magnesen, T., Bergh, O., Christophersen, G., 2006. Yields of great scallop, *Pecten maximus*, larvae in a commercial flow-through rearing system in Norway. Aquacult. Int. 14, 377–394.

Magnesen, T., Jacobsen, A., 2012. Effect of water recirculation on seawater quality and production of scallop (*Pecten maximus*) larvae. Aquacult. Eng. 47, 1–6.

Martel, A.L., Tremblay, R., Toupoint, N., Olivier, F., Myrand, B., 2014. Veliger size at metamorphosis and temporal variability in prodissoconch II morphometry in the blue mussel (*Mytilus edulis*): potential impact on recruitment. J. Shellfish Res. 33, 443–455.

Martinez, G., Aguilera, C., Campos, E.O., 1999. Induction of settlement and metamorphosis of the scallop *Argopecten purpuratus* Lamarck by excess K+ and epinephrine: energetic costs. J. Shellfish Res. 18, 41–46.

Mesías-Gansbiller, C., Bendimerad, M.E.A., Román, G., Pazos, A.J., Sánchez, J.L., Pérez-Parallé, M.L., 2008. Settlement behavior of black scallop larvae (*Chlamys varia*, L.) in response to GABA, epinephrine and IBMX. J. Shellfish Res. 27, 261–264.

Mullineaux, L.S., Butman, C.A., 1991. Initial contact, exploration and attachment of barnacle (*Balanus amphitrite*) cyprids settling in flow. Mar. Biol. 110, 93–103.

Mullineaux, L.S., Garland, E.D., 1993. Larval recruitment in response to manipulated field flows. Mar. Biol. 116, 667–683.

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, L., Robert, R., 2001. The effect of food supply on metamorphosis and post-larval development in hatchery-reared P*ecten maximus*. Aquaculture 192, 2–4.

Nicolas, L., Robert, R., Chevolot, L., 1998. Comparative effects of inducers on metamorphosis of the Japanese oyster *Crassostrea gigas* and the great scallop *Pecten maximus*. Biofouling 12, 1–3.

Olivier, F., Vallet, C., Dauvin, J.C., Retiere, C., 1996. Drifting in post-larvae and juveniles in an *Abra alba* (Wood) community of the eastern part of the Bay of Seine (English Channel). J. Exp. Mar. Biol. Ecol. 199, 89–109.

Parsons, G.J., Dadswell, M.J., Roff, J.C., 1993. Influence of biofilm on settlement of sea scallop, *Placopecten magellanicus* (Gmelin, 1791), in Passamaquoddy Bay, new Brunswick, Canada. J. Shellfish Res. 12, 279–283.

Pearce, C.M., Gallager, S.M., Manuel, J.L., Manning, D.A., O'Dor, R.K., Bourget, E., 1998. Effect of thermoclines and turbulence on depth of larval settlement and spat recruitment of the giant scallop *Placopecten magellanicus* in 9.5m deep laboratory mesocosms. Mar. Ecol. Prog. Ser. 165, 195–215.

Pearce, C.M., Gallager, S.M., Manuel, J.L., Manning, D.A., O'Dor, R.K., Bourget, E., 1996. Settlement of larvae of the giant scallop, *Placopecten magellanicus*, in 9-m deep mesocosms as a function of temperature stratification, depth, food, and substratum. Mar. Biol. 124, 693–706.

Pechenik, J.A., 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? Ophelia 32, 63–94.

Pernet, F., Bricelj, M.V., Cartier, S., 2006. Lipid class dynamics during larval ontogeny of sea scallops, *Placopecten magellanicus*, in relation to metamorphic success and

response to antibiotics. J. Exp. Mar. Biol. Ecol. 329, 265–280.

Pernet, F., Bricelj, V.M., Parrish, C.C., 2005. Effect of varying dietary levels of ω6 poly-unsaturated fatty acids during the early ontogeny of the sea scallop, *Placopecten magellanicus*. J. Exp. Mar. Biol. Ecol. 327, 115–133.

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., Bourget, E., 2003a. Biochemical indicator of sea scallop (*Placopecten magellanicus*) quality based on lipid class composition. Part I: broodstock conditioning and young larvae performance. J. Shellfish Res. 22, 365–375.

Pernet, F., Tremblay, R., Bourget, E., 2003b. Biochemical indicator of sea scallop (*Placopecten magellanicus*) quality based on lipid class composition. Part II: larval rearing, competency and settlement. J. Shellfish Res. 22, 377–388.

Pernet, F., Tremblay, R., Bourget, E., 2003c. Settlement success, spatial pattern and be-havior of mussel larvae *Mytilus* spp. in experimental "downwelling" system of varying velocity and turbulence. Mar. Ecol. Prog. Ser. 260, 125–140.

Robert, R., Gérard, A., 1999. Bivalve hatchery technology: the current situation for the Pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France. Aquat. Living Resour. 12, 121–130.

Robert, R., Nicholas, L., 2000. The effect of seawater flow and temperature on

metamorphosis and postlarval developement in great scallop. Aquacult. Int. 8, 513–530.

Shumway, S.E., Parsons, G.H., 2016. third ed. Scallops: Biology, Ecology and Aquaculture: Biology, Ecology and Aquaculture, vol. 40 Elsevier.

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.

Tremblay, R., Cartier, S., Miner, P., Pernet, F., Quéré, C., Moal, J., Muzellec, M.-L., Mazuret, M., Samain, J.-F., 2007. Effect of *Rhodomonas salina* addition to a standard hatchery diet during the early ontogeny of the scallop *Pecten maximus*. Aquaculture 262, 410–418.

Tritar, S., Prieur, D., Weiner, R., 1992. Effect of bacterial films on the settlement of the oysters, *Crassostrea gigas* (Thunberg, 1793) and *Ostrea edulis*, (Linnaeus, 1750) and the scallop *Pecten maximus* (Linnaeus, 1758). J. Shellfish Res. 11, 325–330.

Veniot, A., Bricelj, V.M., Beninger, P., 2003. Ontogenic changes in gill morphology and potential significance for food acquisition in the scallop *Placopecten magellanicus*. Mar. Biol. 142, 123–131.

Yvin, J., Chevolot, L., Chevolot-Magueur, A., Cochard, J., 1985. First isolation of jacar-anone from an alga, Delesseria sanguinea. A metamorphosis inducer of *Pecten* larvae. J. Nat. Prod. 48, 814–816.