Aquaculture Research
November 2017, Volume 48, Issue 11, Pages 5463-5471
http://dx.doi.org/10.1111/are.13361
http://archimer.ifremer.fr/doc/00384/49508/

Effects of hydrodynamic factors on *Pecten maximus* larval development

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Abstract :

Hatchery production of great scallop, Pecten maximus, remains unpredictable, notably due to poor larval survival. Large-scale flow-through systems up to 3500 L have been developed to avoid the use of antibiotics in static systems. Alternatively, small-scale flow-through systems have been successfully applied for oysters but they proved to be unsuitable to rear scallop larvae. By focusing on physical factors presumed to limit P. maximus larval development, this study aimed to optimize great scallop larvae rearing parameters under controlled conditions. First, the influence of aeration on larval performances, energetic metabolism and antioxidant defences were studied both in static and flow-through systems. Aeration depressed larval food intake, regardless of the intensities of flow tested (100 ml/min, 155 ml/min and 270 ml/min). On the other hand, antioxidant enzyme activities remained constant or decreased, suggesting that antioxidant defences were inactivated. The increase in citrate synthase activity suggested an increase in metabolic rate possibly due to a turbulent stressful environment. All larvae exposed to such turbulence died before reaching metamorphosis, whereas those reared without aeration survived well (≈ 95%). The effects of water renewal were thereafter studied in 50-L flow-through flat-bottomed tanks. No differences in survival (20.4 ± 0.5%), growth (3.8 ± 0.2 µm/d), competence (5.6 ± 0.2%), energetic metabolism level and antioxidant enzyme activities were observed when comparing 12.5 and 25 L/hr water renewal. Whereas air bubbling leads to detrimental effects, flow-through in small flat-bottomed tanks appears to be a suitable technique for scallop larvae rearing.

Keywords : aeration, flow-through, larval physiology, Pecten maximus

Introduction

Since the 1960's, worldwide scallop production rose from 100 000 to 2.6 million tones, mostly related to aquaculture industry responsible for 71.4% of the total production (FAO 2013). First attempts at scallop aquaculture production were realised in Japan with the use of wild juveniles obtained on artificial collectors for population enhancement and sea-ranching. However, development of hatchery-based production of juveniles was rapidly needed to avoid seasonal fluctuations (Buestel 1979). After first laboratory trials in the early 1970's (Gruffydd & Beaumont 1972), hatchery production of scallops using static systems (with a complete seawater renewal every 2 days) was rapidly established in several countries, including France (Robert & Gérard 1999), Norway (Bergh & Strand 2001), USA (Widman, Choromanski, Robohm, Stiles, Wikfors & Calabrese 2001) and Chile (Brokordt, Núñez & Gaymer 2011), but success of hatchery production remained unpredictable due to important larval survival variability (see Andersen, Christophersen & Magnesen (2011) for review). Use of preventive antibiotics stabilize the productivity, but large-scale flow-through systems up to 3500 L have been previously developed to prevent the unsustainable use of chemical products. In such systems, both algae and water are continuously added, thus leading to a more stable environment. Results are promising but this system needs to be optimized, as relatively low larval yield (6.9%) and densities (3 larvae ml^{-1}) still limit commercial extension of such systems (Andersen, Christophersen & Magnesen 2012). A newly designed small-scale flow-through system maintaining a high larval density of up to 300 larvae ml^{-1} supports survival rates >70% of oyster larvae (*Crassostrea gigas* and *Ostrea edulis*) until metamorphosis (da Costa, Petton, Mingant, Bougaran, Rouxel, Quéré, Wikfors, Soudant & Robert 2015; Robert, Vignier & Petton 2017;). Unfortunately these were unsuccessul for *Pecten maximus*, as larvae did not survive beyond 6 days post-fertilization (dpf) of rearing with a maximum density of only 10 larvae ml^{-1} (Holbach 2015). In these 5-L cylindrical shaped

tanks (104 cm height and 9 cm diameter) seawater recirculation (100% renewal h^{-1}) maintain the quality of larval environment; while, aeration allowed homogenization of food and larvae in the water column. Tolerance of bivalve larvae to stressful factors is species specific (Calabrese & Davis 1970) and pectinid larvae are known to be highly sensitive compared to oysters or clams (Robert & Gérard 1999; Helm, Bourne & Lovatelli 2004). Understanding the effects of physical parameters on pectinid larval development is therefore a prerequisite to determine optimal rearing parameters leading to reliable production of juvenile great scallops.

In the present study, we hypothesized that turbulence related to the high water aeration or high water renewal might be detrimental for the development of scallop larvae. The first objective was to determine the effects of aeration on growth, survival, microalgae consumption and physiological status of *P. maximus* larvae reared, in batch, in low volume flat-bottom tank. *Vibrio* development was concomitantly surveyed. The second objective examined the potential of flat-bottom tanks for *P. maximus* larval rearing by the use of a 50 L prototype flow-through system in which the combined effects of water renewal and aeration on growth, survival, competence to metamorphosis and physiological status of scallop larvae were performed. *Vibrio* load evolution was similarly assessed. Activities of several enzymes have been shown to be good indicators of the physiological status of bivalve larvae (Genard, Larouche, Nicolas, Miner, Beaudin & Tremblay 2014; Genard, Pernet, Lemarchand, Boudry, Moraga & Tremblay 2011). Some of those associated with larval performances results were accordingly used to estimate the effects of physical rearing conditions on *P. maximus* larval condition and stress level. Citrate synthase (CS) is a key enzyme in the formation of adenosine triphosphate (ATP) and a good indicator of energetic metabolism (Moran & Manahan 2003) while catalase (CAT) and superoxide dismutase (SOD), two antioxidant enzymes, were recorded as indicators of oxidative stress (Genard *et al.* 2011 ; Philipp, Schmidt, Gsottbauer, Sanger & Abele 2008).

Materials and Methods

Common variables between experiments

To understand the effects of hydrodynamic factors on pectinid larval development, some common general variables were measured: mortality, growth, physiological status estimated by the total protein amount and enzymatic activity of CAT, SOD and CS and the percentage of *Vibrio* in total larval flora. The first experiment determined the effects of aeration and was realized in batch. The second experiment on the impact of the water flow was based on flow-through system with continuous addition of food. Algal consumption was not determined because it was technically difficult to access in such prototype and considered for a first assay as not crucial informative. In contrast, another variable was taken into account, the competence to metamorphosis to achieve a better knowledge of *P. maximus* development and the potentiality of 50 L rectangular flat bottom flow-through tanks.

Aeration as a potential source of stress

Two dpf old D-larvae, originating from a commercial scallop hatchery (Tinduff, Finistère, France), were transferred to Ifremer's experimental facilities (Argenton, Finistère, France). Up to 11 dpf, larvae were reared in 150-L cylinder-cone shape tanks. During this period, a single 2-day preventive chloramphenicol treatment, at 8 ppm, was applied from 2 to 4 dpf. At 11 dpf, they were retained on a 100-µm mesh sieve and transferred to 5-L flat-bottomed beakers. Thus, the largest and strongest individuals known to be more resistant (Kesarcodi-Watson, Miner, Nicolas, Asmani & Robert 2014) were selected while smaller larvae were discarded. During the larval cycle, larvae were collected each two days on a 45 µm mesh, suspended and homogenized in 1 µm-filtered and UV-treated seawater at 19°C, as described by Helm, Bourne & Lovatelli (2004), for estimation of mortality and growth. Larvae were reared at a density of 10 ml^{-1} and fed two flagellates, *Pavlova lutheri* and *Tisochrysis lutea* plus one diatom, *Skeletonema marinoi* to ensure

60 cells μl^{-1} available each day (20/20/20 equivalent cell volume) over the whole experiment. This diet has been demonstrated to support optimal growth and over 80% survival on complete larval rearing of *P. maximus* (Tremblay, Cartier, Miner, Pernet, Quéré, Moal, Muzellec, Mazuret, Samain 2007). To avoid algae deposition, each beaker was gently hand mixed twice a day. On 13 dpf, four aeration conditions were applied (n = 3): no aeration (control: 0), low aeration (100 ml min^{-1}), medium aeration (155 ml min^{-1}) and high aeration (270 ml min^{-1}). Aeration was provided by a 100 kPa blower coupled to a dehumidifier and 0.1 µm filtered before delivering in each tank with one glass pipette of 1 ml having an aperture of 0.9 mm. Larvae were reared without any antibiotic until the end of the experiment when they reached the pediveliger stage (25 dpf). The lowest aeration supplied was based on previous studies (Robert, Miner & Nicolas 1996); whereas, the highest intensity corresponded to that used for flat oyster larvae (Gonzalez Araya *et al.* 2012).

Larval mortality and growth were recorded every 2 days. Shell length was measured using image analysis (software Image SXM by the National Institutes of Health). Mortality corresponded to the total number of empty shells (translucent larval shell) in samples of 300 larvae. Phytoplankton concentration was determined in μm^3 μl^{-1}, 24 (12 dpf) and 72 (14 dpf) hours following aeration exposure, in all tanks, by means of an electronic particle counter (Multisizer 3 – Beckman) equipped with a 100 µm aperture and a size range of 2-20 µm. Larval feeding was expressed as clearance rate (% day^{-1}) corresponding to the percentage of total algal ration daily consumed. Positive results will represent efficient microalgae consumption; whereas negative values will denote low food intake, lower than microalgal growth.

Scallop larval mortality has been often associated to the development of *Vibrios* such as *V. pectenicida* in *Pecten maximus* (Lambert, Nicolas, Cilia & Corre 1998), *V. splendidus* in

Argopecten purpuratus (Rojas, Miranda, Opazod & Romerod 2015), *V. alginolyticus* in *Argopecten ventricosus* (Sainz, Maeda-Martinez & Ascencio 1998) and we accordingly paid attention to its potential development. The percentage of *Vibrio* in total bacteria flora of larvae was thus recorded at 19 dpf using plate counting method on previously crushed larvae, with thiosulfate-citrate-bile salts-sucrose medium for *Vibrio* culture and marine agar for total flora (Azandegbe 2010).

At 17 dpf, 50 000 larvae (≈ 50 mg wet weight minimum regardless to ontogenic stage) were collected on a 20-µm mesh and stored at -80°C. Samples were weighted and homogenised in a Tris HCl buffer (pH 7.4) as described in Guévélou, Huvet, Galindo-Sánchez, Milan, Quillien, Daniel, Quéré, Boudry & Corporeau (2013). Total protein extraction was performed using an Ultra-Turax (Kinematica Kriens-Lu) in an ice-bath followed by a centrifugation at 15000 g during 20 min at 4°C. Total protein amount was obtained using a DC protein assay (BIO RAD) using bovine serum albumin as standard in a microplate reader (Bio-Tek Synergy) as well as KC4 version 3 software (Bio-Tek Instrument Inc.). The activity of the three enzymes were analysed with the same microplate reader and software as protein content analyses, by applying a suitable program. Briefly, CAT (EC 1.11.1.6) activity was determined using the AmplexrRed Catalase Assay Kit (Invitrogen, USA) according protocol of the manufacturer measuring the decrease of absorbance at 240 nm, due to H_2O_2 consumption. Total SOD (EC 1.15.1.1) activity was assessed with a SOD determination kit (Sigma-Aldrich) according to the manufacturer protocol using the absorbance at 450 nm after 30 min incubation at 37°C. CS (EC 4.1.3.7) activity was obtained using the method of Childress and Somero (1979) by measuring the absorbance at 412 nm during 10 min.

Impact of water flow

In this experiment, larvae underwent the same treatment as that described above until 10 dpf. After sieving, larvae were distributed, at 10 larvae ml^{-1}, in four triplicate treatments, in 50 L flow-through tanks. Rectangular flat bottom units with low surface on volume ratio (= 0.196) were used with banjo mesh screens water outlet (Andersen, Christophersen & Magnesen 2011) to prevent loss of larvae. The screen size was adjusted as the larvae grew, with an 80 µm screen replaced to a 100 µm screen on day 15. In such tanks, larval survival at pediveliger stage reached 75.7 ± 5.4% with 29.2 ± 0.8% of competent larvae (unpublished results of a previous trial). Treatment "0" corresponded to an absence of water inflow (water being totally renewed each second day at once), "¼" corresponded to water flow of 25% of the tank volume per hour (12.5 l h^{-1}) "½" to 50% (25 l h^{-1}) and "¼+air" to a water flow of 25% of the tank volume per hour plus aeration at 300 ml min^{-1}. Induced by the same system set up in the first experiment, a similar high aeration condition was applied.

On 17 and 22 dpf, mortality and shell length were recorded as described above. At 22 dpf, the criterion of double ring appearance at the margin of the shell was used to estimate metamorphosis competence (number of larvae ready for metamorphosis: Gérard, Salaun & Tritar 1989). The percentage of *Vibrio* in total bacteria flora was recorded during the whole experiment, as already described. At 17 dpf, larvae were sampled to quantify their total protein content and enzymatic activities of CAT, SOD and CS.

Statistical analyses

Permutational Analyses of Variances (PERMANOVA - PRIMER-E 6.0 PERMANOVA plus; PRIMER-E Ltd, Plymouth, UK) were preferred to ANOVAs, as it can be used on non-normal data generally recorded in larval rearing trials. If significant, posteriori comparisons were done using a PERMANOVA pairwise test to identify differences among treatment. When t-Tests were

necessary, they were performed using the software R (2012) following Shapiro-Wilk normality test except for not normal data for which Wilcoxon-Mann-Whitney tests were used.

In aeration experiment, larval mortality, growth, and microalgal consumption were analyzed with a univariate one-way PERMANOVAs (9999 permutations), based on Euclidean resemblances. The independent variable in the PERMANOVAs was "aeration" with 4 fixed levels 0, 100, 150 and 270 ml mn^{-1}. Larval mortality was detailed on 21 dpf; whereas, growth was and bacterial load studied two days preceeding mortalities, on 19 dpf. Food consumption was examined at 12 and 15 dpf (24 and 72h after aeration application) separately in an one-way PERMANOVA as homoscedasticity was not achieved for the factor "time" in the two-way crossed PERMANOVA ($p_{(PERMDISP)} = 0.032$). CAT, SOD and CS activities were compared using one-way PERMANOVAs, with "aeration" as a source of variation.

In water flow experiment univariate one-way PERMANOVAs (9999 permutations) were used to evaluate the impact of flow rate on larval survival, growth, and bacterial contamination at 17 dpf. The independent variable was "flow rate" with three fixed levels (0, ¼, ½). The same PERMANOVAs were used for each of the three enzyme activities measurements. As all larvae, reared in treatment without flow rate (0), died before 22 dpf, t-tests were only applied to larval mortality, growth, competency, and bacterial contamination for ¼ and ½ treatments. T-tests were also used to compare ¼ and ¼+air treatments for larval mortality, growth, *Vibrio* contamination, and enzymatic activities.

Results

Impact of aeration

On 21 dpf, aeration significantly impacted larval survival ($p = 0.0007$, Pseudo-$F_{(3-11)} = 37.7$). Larvae reared at the higher aeration intensity (270 ml min^{-1}) exhibited severe mortality, 74.1 ± 2.9% (Fig. 1), significantly different from others treatments with values ≤ 40% (270 *vs* 0: $p_{(MC)} = 0.0001$, $t = 24.02$; 270 *vs* 100: $p_{(MC)} = 0.012$, $t = 4.44$; 270 *vs* 155: $p_{(MC)} = 0.0018$, $t = 7.53$). The two other aeration treatments (155 ml min^{-1} and 100 ml min^{-1}) led to statistically similar mortalities ($p_{(MC)} = 0.7$, $t = 0.40$), with an overall mean of 37.5 ± 1.8% (Fig. 1). Control treatments displayed the lowest mortalities with 3.7 ± 0.5% (Fig. 1) that remained as low until 25 dpf. In contrast, all larvae exposed to the highest aeration (270 ml min^{-1}) died on 23 dpf; whereas, those exposed to intermediate aerations (100 and 155 ml min^{-1}) collapsed on 25 dpf. At 19 dpf, no *Vibrio*s were detected over the whole experiment and no significant differences in bacterial load were observed ($p = 0.84$, Pseudo-$F_{(3-32)} = 0.41$) with an overall mean of 336.4 ± 95.7 CFU $larva^{-1}$.

Larvae reared in still water (*i.e.* control condition) exhibited positive microalgae consumption (between 8 to 25% of microalgae consumption per day equivalent to 37811 ± 9390 to 157283 ± 13432 cells eaten per larvae); whereas, larvae exposed to aeration registered negative consumption (Fig. 2). That means that in aerated treatments larval food consumption was lower than microalgal growth. Thus, aeration significantly depressed larval food intake, regardless to its intensity (24h: $p = 0.0001$, Pseudo-$F_{(3-35)} = 51.1$; 72h: $p = 0.0001$, Pseudo-$F_{(3-23)} = 98.3$).

When exposed to aeration, larval growth was depressed (Fig. 3) but no significant differences were detected as regard to aeration levels (100 *vs* 155: $p = 0.12$, $t = 1.63$; 100 *vs* 270: $p = 0.20$, $t = 1.36$; 155 *vs* 270: $p = 0.91$, $t = 0.12$). On 19 dpf, larval lengths ranged from 169.7 µm ± 0.5 to 173.5 ± 0.7 µm, corresponding to a daily growth of ≈ 1 µm d^{-1}. In contrast, larvae reared in still

water were statistically larger than those submitted to aeration (0 *vs* 100: $p = 0.0001$, $t = 8.35$; 0 *vs* 155: $p = 0.0001$, $t = 11.69$; 0 *vs* 270: $p = 0.0002$, $t = 9.68$) and exhibited a mean length of 185.7 µm ± 1.0, corresponding to a daily growth of about 3.3 µm d^{-1}.

On the other hand, when considering antioxidant defenses and specifically CAT activity, larvae reared in still water or exposed to the lowest aeration intensity (100 ml mn^{-1}) exhibited similar results ($p_{(MC)} = 0.41$, $t = 0.9$) with an overall mean of 88.5 ± 2.3 U mg of $protein^{-1}$ (Table 1). For the two highest aeration exposures, larval CAT activities were similarly depressed ($p_{(MC)} = 0.52$, $t = 0.68$) with an overall mean of 63.5 ± 1.2 U mg of $protein^{-1}$ (Table 1). Likewise, SOD activity of larvae, exposed to low and intermediate aerations (100 and 155 ml mn^{-1}), were close to control (0 *vs* 100: $p_{(MC)} = 0.06$, $t = 2.7$; 0 *vs* 155: $p_{(MC)} = 0.26$, $t = 1.3$; 0 *vs* 270: $p_{(MC)} = 0.09$, $t = 2.3$) with an overall mean of 3.8 ± 0.4 U mg of $protein^{-1}$ (Table 1). In contrast, larvae submitted to the highest aeration value (270 ml mn^{-1}) showed significant lower SOD activities compared to the two other aeration intensities (270 *vs* 100: $p = 0.0063$, $t = 5.6$ and 270 *vs* 155: $p = 0.0098$, $t = 4.3$ - Table 1).

Finally, on 17 dpf, CS activities were different between all larvae reared with aeration (100 *vs* 155: $p_{(MC)} = 0.0006$, $t = 9.4$; 100 *vs* 270: $p_{(MC)} = 0.004$, $t = 6.05$; 155 *vs* 270: $p_{(MC)} = 0.02$, $t = 4.1$ - Table 1). The highest value was recorded in larvae exposed to 155 ml mn^{-1} aeration with 10.8 ± 0.5 mU mg of $protein^{-1}$.

Impact of water flow and aeration in flow through-system

After seven days of flow through rearing, the intensity of water renewal led to weak effects on larval performance (Table 2). On 17 dpf, larvae from all treatments (0, ¼, ½) showed similar survival ($p = 0.57$, Pseudo-$F_{(2-8)} = 0.60$), with a mean of 58.03 ± 2.43%. Growth was slightly lower at higher water flow renewal ($p_{(MC)} = 0.008$, Pseudo-$F_{(2-8)} = 11.1$), with larvae growing 1.3 times slower (½ vs 0: $p_{(MC)} = 0.015$, $t = 4.0$; ½ vs ¼: $p_{(MC)} = 0.04$, $t = 2.9$). The ratio of *Vibrio* to

total bacteria was 6.4 times higher in this treatment (½ vs 0: $p_{(MC)}$ = 0.0004, t = 13.26; ½ vs ¼: $p_{(MC)}$ = 0.003, t = 6.86) with a total bacterial load of 1048 ± 296 CFU. In contrast, larval growth and percentages of *Vibrio* were similar between 0 and ¼ water renewal (growth: $p_{(MC)}$ = 0.13, t = 1.9; Vibrio: $p_{(MC)}$ = 1, t = 5.47 x 10^{-4}), with respective means of 5.9 ± 0.2 µm d^{-1} and 1.98 ± 0.68%, despite total bacteria loads at ½ and 0 conditions reaching 952 ± 521 CFU and 7001 ± 6002 CFU respectively. Considering larval enzyme activities measured on 17 dpf (Fig. 4), no significant differences between treatments were registered (CAT: p = 0.07, $PF_{(2-8)}$ = 3.49; SOD: p = 0.13, $PF_{(2-8)}$ = 3.59; CS: p = 0.08, $PF_{(2-8)}$ = 4.68).

On 22 dpf, only larvae reared in flow-through system survived with no significant differences in larval performances due to the intensity of water renewal (Table 2). Performances of larvae reared in flow-through (½ and ¼) were similar for survival (p = 0.85, t = 0.2; mean = 20.4 ± 0.5%), growth (p = 0.93, t = 0.096; mean = 3.8 ± 0.2 µm d^{-1}), competence (p = 0.93, t = 0.098; mean = 5.6 ± 0.2%) and relative *Vibrio* concentration with a mean of 0.6 ± 0.2% and a total bacterial load of 800 ± 256 CFU (p = 0.45, t = 8.84).

The impact of aeration on performances of larvae reared in flow through (¼ *vs* ¼+air) is reported in Table 3. On 17 dpf, survival was similar regardless of experimental conditions (p = 0.41, t = 0.92). However, larvae reared without aeration grew 1.4 times faster (p = 0.002, t = 6.83) and *Vibrio* were 3.4 times less abundant (p = 0.047, t = 2.84). On the other hand, SOD activities were similar (p = 0.36, t = 1.02) between treatments, with a mean of 1.7 ± 0.2 U mg $protein^{-1}$, but CAT activity showed significant differences, with values 2.7 times higher (p = 0.008, t = 4.8) in larvae reared without aeration (Fig. 4). Lastly, CS activity was 1.7 times higher (p = 4.8 × 10^{-5}, t = 18.7) in larvae reared with aeration (Fig. 4), which all died before the end of the experiment. In flow-through system and in presence of aeration, all larvae died

before reaching the pediveliger stage. Without aeration survival and competence reached 21.0 ± 8.5% and 5.8 ± 2.6% respectively.

Discussion

The objective of this study was to document how physical factors may impact *Pecten maximus* larval development in order to improve pectinid hatchery production systems. Our results clearly showed that aeration, even at low flow, decreases rearing performances and physiological larval conditions regardless of the rearing system, static or flow-through. However, water renewal (without any aeration) in rectangular flat-bottomed tank do not impact larval development and limit *Vibrio* load. This system demonstrated, thus, its ability to produce pectinid larvae until metamorphosis in small tanks of 50 L.

Impact of aeration

In contrast to still water, microalgal intake of larvae exposed to aeration was lower than microalgal growth 24 h after the onset of the experiment regardless of bubbling intensity. Aeration swiftly impeded larvae to feed normally. The absence of microalgal consumption had a rapid and direct impact on larval growth. Gregg & Bergersen (1980) reported that turbulence increased the mortality of the shrimp *Mysis relicta*. They suggested that abrasion and exhaustion due to continuous swimming and inability to feed could be the principal causes of the mortality observed. Scanning electron microscopy examinations of eventual mechanical abrasive impact on scallop larvae remain to be done to explore this hypothesis. On the other hand, zebra mussel larvae withstand greater aeration because 40% survival was recorded at 5 L ml^{-1} airflow (Rehmann, Stoeckel & Schneider 2003). Moreover, Helm & Spencer (1972) showed that aeration improve growth and development of eyespot in European flat oyster larvae. Similarly, settlement of *Potamocorbula amurensis* (Crimaldi, Thompson, Rosman, Lowe & Koseff 2002), *Mytilus*

edulis (Pernet, Tremblay & Bourget 2003b) and *Ilyanassa obsolete* (Fuchs, Mullineaux & Solow 2004) was also improved with aeration. This pattern has been proposed as an adaptation to disperse and settle more successfully in highly turbulent and energetic coastal regions (Fuchs & DiBacco 2011). *P. maximus* appears to react differently, as in the present study, larvae died in presence of aeration, regardless to rearing system, sequential or flow-through system. Oysters and mussels mainly live in intertidal zones exposed to substantial short-term environmental variations (Gosling 2008); whereas, pectinids live in subtidal areas, subject to more stable environment (Brand 2006 and references therein). Great scallop are usually found in extreme low tide down to 250 m and are known to be more abundant far away of high water streams (Brand 2006). This could be a partial explanation of the sensitivity of pectinid larvae (Helm, Bourne & Lovatolli 2004) and confirm the necessity to adapt zootechnical procedure for scallop larval rearing.

The rise of CS activity in larvae exposed to aeration in the present experiments (static and flow-through systems) could be a sign of an increased metabolic rate in a turbulent stressful environment. Positive correlation between respiration and CS activity has been previously demonstrated in bivalves (Moran & Manahan 2004 ; Meyer, Green, Moore & Manahan 2007). This correlation as well as the high energetic metabolism recorded here suggest that larvae swim endlessly until lethal exhaustion. Thus, an increase in ATP production could result in a higher energy demand to support additional efforts to swim due to a turbulent environment (Gregg & Bergersen 1980). Since larval size was lower in all treatments including aeration, the increased metabolic rate did not seem to be sufficient to provide more energy to swim without impacting larval growth. In contrast, Genard *et al.* (2011) and Genard, Miner, Nicolas, Moraga, Boudry, Pernet & Tremblay (2013) reported a decrease of CS activity in oyster larvae during a stresfull event. However, in these studies focused on exposition of larvae to bacterial pathogen, the CS

decrease corresponded to a different behavior consisting in larval shell closure followed by sinking.

In both our conditions with aeration, SOD activity remained constant; whereas, CAT activity was either stable or decreased, suggesting that antioxidant defences were inactivated. In our flow-through system, this phenomenon coupled to the high proportion of *Vibrio* detected in larvae exposed to aeration, is likely to be a consequence rather than the cause of the observed high number of moribund larvae.

Impact of water flow

In flat-bottomed flow-through tank without any aeration, enzyme activities, used as indicators of physiological stress, did not suggested stressful responses of scallop larvae, as no clear trend in the three enzymes activities studied was observed.

Rearing scallop larvae without any antibiotic is generally performed in large volume tanks (>500 L) to avoid mass mortality (Campa-Cørdova, Luna-GonzaLez, Zarain-Herzberg & Caceres-Martinez 2005 ; Magnesen, Bergh & Christophersen 2006 ; Merino, Uribe, Soria & von Brand 2009 ; Pernet, Tremblay & Bourget 2003a). Volumes tested varied from 500 to 4700 L with densities ranging from 1.5 to 13 larvae ml^{-1} (Magnesen *et al.* 2006 ; Torkildsen & Magnesen 2004). In the present study, the use of flat-bottomed tanks led to acceptable scallop larval development performances with 20.4 ± 0.5% survival and 5.6 ± 0.2% competence in small tank volume of only 50 L. The ratio surface to volume has been suggested to be important for *P. maximus* larval rearing and need to be as low as possible to allow successful larval development (Helm, Bourne & Lovatolli 2004). The 50 L flat-bottomed tanks used in our study had a surface on volume ratio of 0.196 which might explain this relative rearing achievement compared to the 5 L cylindrical tanks that exhibited a surface on volume ratio of 0.585 and previously led to unsuccessful results (Holbach 2015).

No significant variations were observed in CS enzyme activities between the three conditions tested (0, ¼, ½) suggesting that ATP demand was similar until mortality occurred (Moran & Manahan 2003). Indeed, no clear establishment of antioxidant defences was noted at 17 dpf, as SOD and CAT activities did not increase (Genard *et al.* 2013). On 17 dpf, the high proportion of *Vibrio* observed in ½ flow-through seawater renewal was surprising because it reached similar values than in the ¼ condition, five days later (22 dpf) without any of mortality. This might result in external contamination of the samples. On the other hand, the lower growth recorded in those larvae could be related to high water movement and the inability of larvae to feed efficiently as discussed above. However, consumption was difficult to measure in this system and we could not validate this hypothesis.

Conclusion

In our study, we showed how hydrodynamic factors (aeration and flow through) impact scallop larval development. Aeration was clearly detrimental for scallop larvae in contrast to the intensity of water renewal. The water flow needs to be adjusted in order to optimize environment homogenization without disturbing larval natural behavior. The results that seemed to be related to the physiological needs of this species and ecological niche, bring useful information for future application in aquaculture production. The flat-bottomed tanks of 50 L led to encouraging larval development performances but further investigations are needed to confirm such results, in particular with higher larval densities. The optimization of this rearing system needs to be settled, with compromise between production costs (microalgal production and water warming) and yield of competent larvae.

Acknowledgements

The authors would like to thank the Tinduff hatchery for providing scallop larvae as well as the technical team of Ifremer facilities in Argenton, - Isabelle Quéau and Luc Lebrun for algal production; and Claudie Quéré and Catherine Séguineau for their assistance for enzymatic analyses. We are also grateful to the "Ministère du Développement Economique, de l'Innovation et de l'Exportation du Québec" (MDEIE), which contributed to the funding of a Ph.D. scholarship, to "Ressource Aquatiques Québec" (RAQ), and to "Fonds de Recherches du Québec – Nature et technologies" (FRQNT), which provided travel grant to Marine Holbach. This work was carried out during the EU funded project ReproSeed (FP7-KBBE-2009-3), which partly supported functional outlays.

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Table 1: Activities of CAT (catalase), SOD (superoxide dismutase) and CS (citrate synthase), in *Pecten maximus* larvae exposed to four aeration levels (0, 100, 155 and 270 ml min^{-1}), 17 days post-fertilization (dpf). Data are expressed as units per mg of proteins. Data are mean ± standard error. Different letters indicate significant differences for each enzyme studied.

dpf	Aeration intensity (ml min^{-1})	CAT (U mg^{-1})	SOD (U mg^{-1})	CS (mU mg^{-1})
17	**0**	84.3 ± 4.2^{a}	3.8 ± 0.4^{ab}	6.9 ± 0.6^{bc}
	100	88.8 ± 2.5^{a}	4.2 ± 0.2^{a}	5.5 ± 0.3^{c}
	155	64.8 ± 3.3^{b}	4.5 ± 0.4^{a}	10.8 ± 0.5^{a}
	270	62.3 ± 1.4^{b}	2.8 ± 0.2^{b}	8.3 ± 0.4^{b}

Table 2: Survival, growth, competence and *Vibrio* on total flora load (V/FT) of *Pecten maximus* larvae reared in 50-L rectangular tanks, in flow through, at three different water flow renewal: 0 (no inflow), 12.5 L h^{-1} (¼), 25 L h^{-1} (½), 17 and 22 days post-fertilization (dpf). Data are mean ± standard error; different letters indicate significant differences among treatments.

dpf	Volume of renewed tank water h^{-1}	Survival (%)	Growth (µm day^{-1})	Size (µm)	V/FT (%)	Competence (%)
17	**0**	60.9 ± 69^{a}	6.1 ± 0.2^{a}	178.7 ± 1.2^{a}	2.0 ± 0.5^{b}	–
	¼	60.0 ± 4.2^{a}	5.6 ± 0.2^{ab}	175.5 ± 1.2^{ab}	2.0 ± 1.4^{b}	–
	½	53.2 ± 4.8^{a}	4.4 ± 0.4^{b}	166.7 ± 2.8^{b}	12.7 ± 1.0^{a}	–
22	**0**	0	0	0	0	0
	¼	21.0 ± 8.5^{a}	3.8 ± 0.2^{a}	175.5 ± 1.2^{a}	0.8 ± 0.4^{a}	5.8 ± 2.6^{a}
	½	19.9 ± 3.7^{a}	3.8 ± 0.2^{a}	181.3 ± 1.9^{a}	0.4 ± 0.2^{a}	5.4 ± 2.2^{a}

Table 3: Survival, growth, competence and *Vibrio* as a percentage of total bacteria flora (V/FT) of *Pecten maximus* larvae reared in 50-L rectangular tanks, in flow through, at 12.5 L h^{-1} (¼) water flow renewal and with or without aeration, 17 and 22 days post-fertilization (dpf). Data are mean ± standard error; different letters indicate significant differences between treatments. No statistical analyses were done at day 22 since only one condition survived.

dpf	Volume of renewed tank water h^{-1}	Aeration (ml min^{-1})	Survival (%)	Growth (µm day^{-1})	Size (µm)	V/FT (%)	Competence (%)
17	¼	**0**	60.0 ± 4.2^{a}	5.6 ± 0.2^{a}	175.5 ± 1.2^{a}	2.0 ± 1.4^{b}	–
		300	53.0 ± 6.4^{a}	4.1 ± 0.2^{b}	165.0 ± 1.0^{b}	6.3 ± 0.4^{a}	–
22	¼	**0**	21.0 ± 8.5	3.8 ± 0.2	175.5 ± 1.2	0.8 ± 0.4	5.8 ± 2.6
		300	0	0	0	0	0

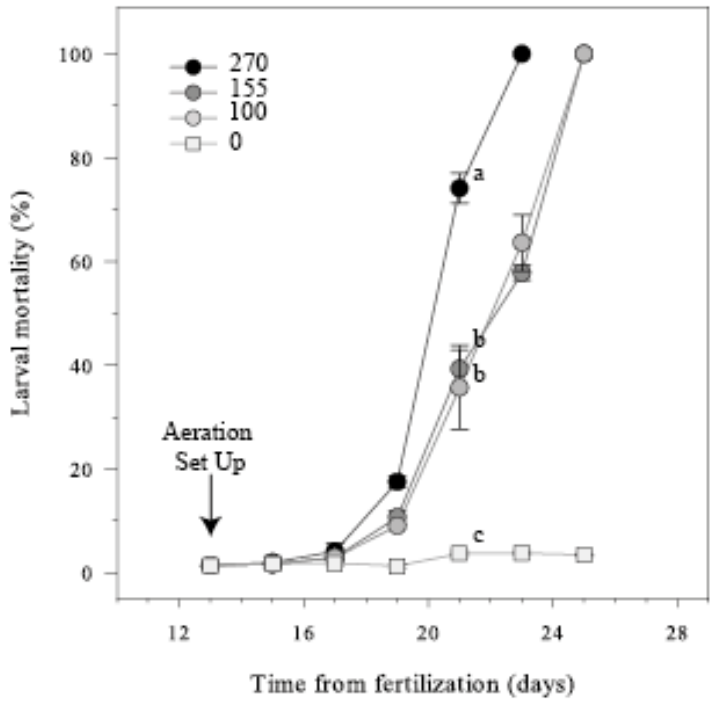

Figure 1: Evolution of mortalities in *Pecten maximus* larvae, exposed, from 13 days post-fertilization (dpf), to four different aeration levels, 0, 100, 155 and 270 ml min^{-1}. Data are mean ± standard error and are expressed in percentage. Different letters indicate significant differences measured on 21 dpf.

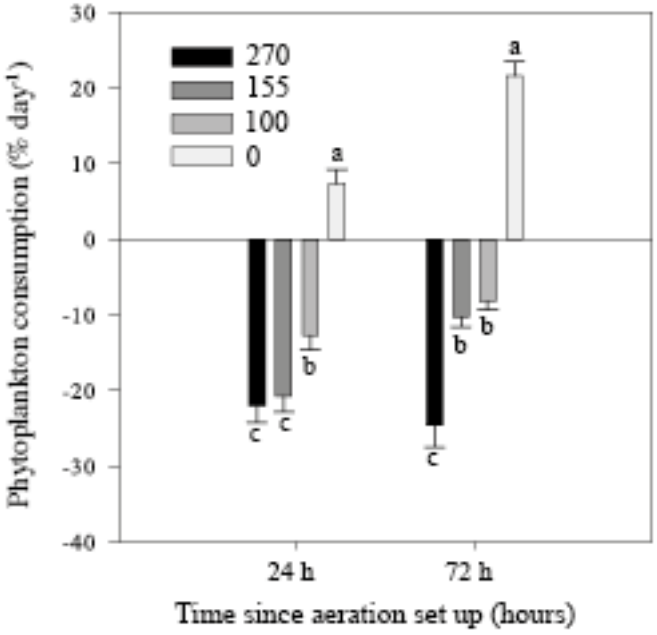

Figure 2: Food uptake of *Pecten maximus* larvae, exposed to four different aeration levels, 0, 100, 155 and 270 ml min^{-1}, one and three days after aeration set up on 13 days post-fertilization (dpf). Data are mean ± standard error and are expressed as percentages of food uptake per day. Different letters indicate significant differences.

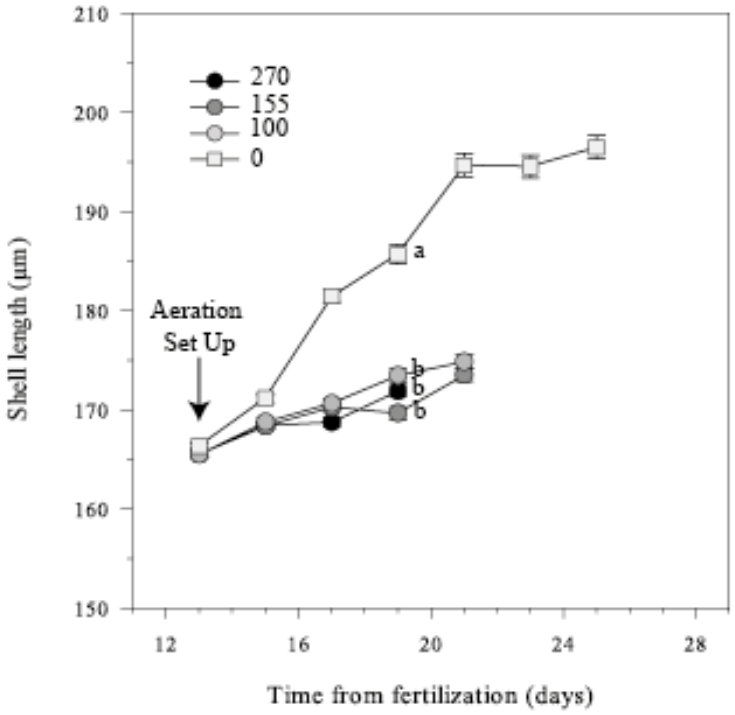

Figure 3: Evolution of shell length (µm) in *Pecten maximus* larvae, exposed, from 13 days post-fertilization (dpf), to four different aeration levels, 0, 100, 155 and 270 ml min^{-1}. Data are mean ± standard error. Different letters indicate significant differences measured on 19 dpf.

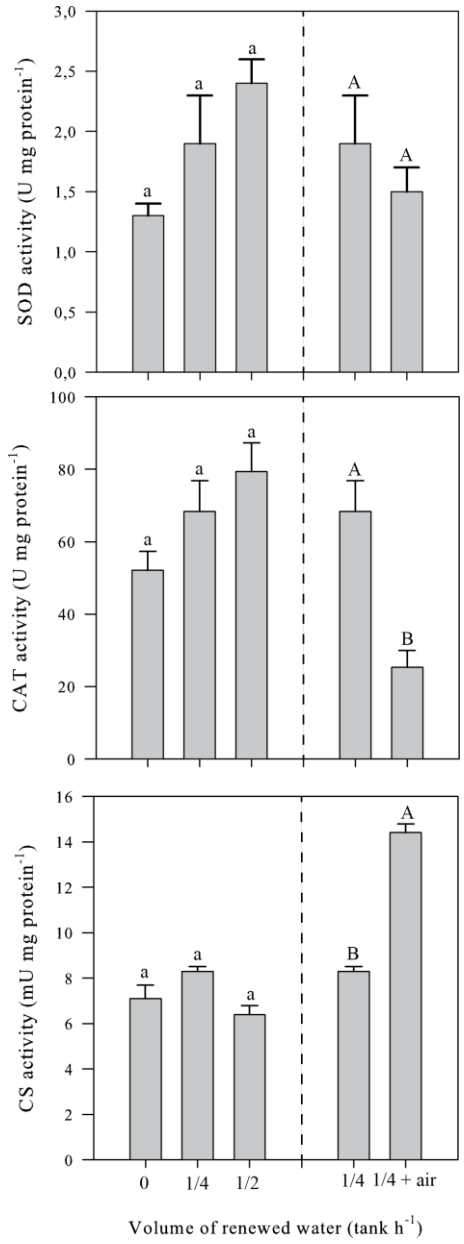

Figure 4: Enzymatic activities of superoxide dismutase (SOD - U mg protein^{-1}), catalase (CAT - U mg protein^{-1}), and citrate synthase (CS - mU mg protein^{-1}) in *Pecten maximus* larvae reared in 50-L rectangular tanks, in flow through at three different levels of water flow renewal: 0 (no inflow), 12.5 L h^{-1} (¼), 25 L h^{-1} (½) on 27 days post-fertilization (dpf). At 12.5 L h^{-1} seawater renewal an aeration of 300 ml h^{-1} was also implemented (¼ + air). Data are mean ± standard error

and different letters indicate significant differences between flow-through treatments and between aeration treatments.