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## RUNNING HEAD

Rearing of scallop post-larvae.

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## TITLE

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

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## ABSTRACT

In a commercial scallop hatchery the spat production depends on a culture system which ensures high survival and good growth. Reuse of water with algae may increase the food exploitation and hence reduce the costs. Post-larvae of great scallop (*Pecten maximus*) were studied in a commercial hatchery using a partial open and continuous feeding tank system. Three different water recirculation rates (67 %, 83 % and 92 %) were tried out in two experiments with post-larvae originating from 3 spawning groups of ages between 43 to 57 days post-spawn, 316-886  $\mu\text{m}$  shell-height and 1.1-9.6  $\mu\text{g}$  ash free dry weight. The post-larvae were held in sieves in tanks of 2500 litres where a downwelling flow was maintained by airlifts. New water with a mix of monocultured algae was continuously added to the tanks at algal concentrations of 10 and 15 cells  $\mu\text{l}^{-1}$  in experiment 1 (group 1 and 2) and 2 (group 3), respectively. The algal supply to each sieve was reduced along with increased recirculation rate, but was kept between 6 and 13 cells  $\mu\text{l}^{-1}$ . No significant differences in survival or growth were found between the recirculation rates, while in terms of chemical content a higher carbohydrate level was found at 67 % than at 83 % and 92 % recirculation. Large variation in survival was found between and within groups (1-81 %). Highest survival was found in experiment 1, and where post-larvae from two settlements were used, the first settlement survived better than the second. The daily growth ranged from 15 to 62  $\mu\text{m}$  shell-height and from 0.3 to 2.6  $\mu\text{g}$  ash free dry weight. The scallop post-larvae could well be reared at all three recirculation rates studied as an increase from 67 % to 92 % did not seem to affect the post-larval performance seriously. The algal supply, however, had to be compensated by an increasing number of cells ( $>10$  cells  $\mu\text{l}^{-1}$ ) when increasing the recirculation rate.

## KEY WORDS

Algal supply, Chemical content, Growth, *Pecten maximus*, Post-larvae, Scallop, Survival, Water recirculation

## INTRODUCTION

The choice of cultivation technology and methods are fundamental to a viable production of bivalve spat. A culture system ensuring high survival and good growth at a minimum of cost is essential. The use of recirculation systems to intensify aquaculture production is developing. Purification treatment and careful control of the water quality are necessary in water recirculation systems used for fish cultivation (van Rijn, 1996; Summerfelt *et al.*, 2004). The use of closed recirculation systems is not common in bivalve rearing, but have been tried on experimental basis with nursery sized scallops, *Argopecten irradians irradians* (Widman, 1998), and clams, *Mercenaria mercenaria* (Pfeiffer and Rusch, 2000). More commonly used in intensive culture of bivalve larvae and post-larvae are batch (static) or flow-through (open) systems where a regular replacement of water avoids the build-up of organic matter and oxygen deficiency.

Seawater heating, pumping, and culture of microalgae for feed are major costs in bivalve production, and the mass production of live algal cells is both a quantitative and a qualitative constraint (de Pauw, 1981; Smith and Wikfors, 1998; Robert and Gérard, 1999; Wikfors and Ohno, 2001; Heasman *et al.*, 2002). The post-larvae are fed cultured algae in the water during intensive production, while filtered seawater may be adequate in semi-intensive systems during the natural production season. Partly recirculation of the water in post-larval rearing tanks reduces the volume of new water with algae that has to be added per unit time, thereby increasing the utilisation of algae and lowering the total production costs. The algae may get an extended residence time in the tank, which may influence the nutritional quality in a way that affects the growth progress of post-larvae. Changes in biochemical composition of algae are related to the algal growth phase (Whyte, 1987; Pernet *et al.*, 2003), and have shown to affect growth of juvenile bivalves (Flaak and Epifanio, 1978; Ryan *et al.*, 1998).

The feeding regime may affect scallop growth and metabolism. Continuously feeding is experienced to give better growth and food assimilation in *Argopecten purpuratus* larvae and early juveniles compared with feeding ones or twice a day (Martínez *et al.*, 1995). Juvenile bay scallops (*Argopecten irradians*) fed four times per day have superior growth rates to both less (1 and 2 times) and more (8 times) frequent fed scallops (Smith and Wikfors, 1998). In the hatchery, the scallops are usually fed a mixture of cultured algae rather than a single species diet to meet the required

nutritional quality for good growth (Laing and Psimopoulous, 1998). Algal concentration, ration, water flow, and velocity are other factors affecting the growth of scallop post-larvae and juveniles (Wildish and Saulnier, 1992; Lu and Blake, 1996; Laing, 2000; Robert and Nicolas, 2000; Nicolas and Robert, 2001; Rupp *et al.* 2004). As there is a rapid increase in biomass during early life stages, the demand for algae and water increases correspondingly (Le Borgne, 1981; Bourne *et al.*, 1989; Millican, 1997). By using a recirculation downwelling growth system in scallop post-larval production, a continuous algal supply and cost-effective reuse of water and algae may be provided.

Hatchery production of the great scallop (*Pecten maximus*) spat in Europe involves induced spawning and intensive larval and post-larval rearing to a size of approximately 2 mm shell-height (Millican, 1997; Robert and Gérard, 1999; Bergh and Strand, 2001). Batch culture and flow-through water systems have been the usual methods in the larval and post-larval rearing respectively, but recently flow-through systems have been tried out also during the larval phase (Robert and Gerard 1999; Andersen *et al.*, 2000, Torkildsen and Magnesen 2004). After the planktonic larval phase, “ready-to-settle” larvae are transferred to a settlement system for further growth.

Using a water recirculation rate of 67 % in post-larval rearing tanks has been a common practice at the Norwegian scallop hatchery Scalpro AS. Pilot studies of algal performance in tanks of different recirculation rates showed that up to 92 % recirculation of added water per minute maintained acceptable algal concentrations (van der Meeren *et al.*, 1997). In the present study, the effects of three different recirculation rates (67, 83 and 92 %) on great scallop (*P. maximus*) post-larvae were investigated in the commercial production system at Scalpro AS. The aim was to assess if the selected recirculation rates would affect the feeding conditions (algal supply), survival, growth, and chemical content of post-larvae.

## MATERIALS AND METHODS

The *P. maximus* post-larvae used in the experiment originated from induced spawnings in November 1996 and January 1997 at the hatchery Scalpro AS in Øygarden, western Norway. Each larval group was the product of cross-fertilisation between eggs and sperm of different parental origin. The scallop larvae were transferred to the settlement tank system between day 22 and 27 post-spawning when they reached

the “ready to settle” stage and retained on 150  $\mu\text{m}$  mesh. The eyed pediveliger larvae were ready to settle at different age, depending on fast or slow larval growth, resulting in different settlement groups from each spawning. Two experiments were carried out, using post-larvae from two (group 1 and 2) and one (group 3) spawning groups respectively. From group 2 and 3 post-larvae from two larval settlement groups (2a and 2b, 3a and 3b) were included (Table 1). Experimental start was January 15 (experiment 1) and February 19 (experiment 2), and day 0 in the studies were day 57, 50, and 43 post-spawn for the larval groups 1, 2, and 3, respectively. The larvae were allowed to stay in the settlement system 3-5 weeks before experimental start (Table 1). Initial mean shell height ranged from 316 to 886  $\mu\text{m}$ , and the ash-free dry weight from 1.1 to 9.6  $\mu\text{g}$  per post-larvae (Table 1).

The settlement system consisted of circular growth units termed sieves (height 15 cm, diameter 39.5 cm, 140  $\mu\text{m}$  mesh bottom) partly submerged in tanks (3 m x 1 m x 1 m) that held approximately 2500 litres of water. Each tank had room for 14 sieves positioned in two parallel rows. Seven and six sieves per tank, i.e. 2-3 replicate sieves per group, took part in the first and second experiment, respectively (Table 1). The experimental sieves were placed in the centre of the two rows. A downwelling flow of 3  $\text{l min}^{-1}$  sieve<sup>-1</sup> was maintained by an airlift system, of which 1, 0.5, and 0.25  $\text{l min}^{-1}$  was new water at recirculation rates of 67, 83 and 92 %, respectively (Table 2). However, a flow of 1  $\text{l min}^{-1}$  sieve<sup>-1</sup> was used for group 3 the first week due to earlier post-larval age at start. The water outlet was placed in the tank bottom. Separate tanks were used for each of three experimental recirculation rates.

The seawater was supplied from 60 m depth, filtered through 10 and 1  $\mu\text{m}$  bag-filters, and heated to a rearing temperature of 15 °C. The new seawater was mixed with cultured algae and added continuously to the post-larval rearing tanks. Tank water with unconsumed algae was recirculated and mixed with the new water, resembling a total of 42  $\text{l min}^{-1}$  tank<sup>-1</sup> (Table 2). The amount of new water with algae corresponded to 14, 7, and 3.5  $\text{l min}^{-1}$  tank<sup>-1</sup> for the recirculation rates of 67, 83 and 92 %, respectively (Table 2). Both new and recirculated water was added to the sieves through a half-pipe manifold positioned lengthwise in the middle of the tank between the two rows of sieves. Distribution to every sieve was carried out through silicone tubes terminated with T-shape polypropylene connectors.

The algal concentration in the new water introduced to the tanks was tried to be kept at 10 cells  $\mu\text{l}^{-1}$  in experiment 1 and 15 cells  $\mu\text{l}^{-1}$  in experiment 2. In experiment 1 a mixed diet of the monocultured algae *Pavlova lutheri* (Droop), *Isochrysis galbana* (Parke) and *Skeletonema costatum* in a 1:1:2 ratio was introduced to the tanks. In experiment 2 the post-larvae were in addition given *Chaetoceros mülleri* (1-2 cells  $\mu\text{l}^{-1}$ ) to a feeding ratio of 2.3:2.3:4.5:1. The algal concentrations in the new water introduced to the tanks, in the mixed water to the sieves (Table 3), and in the outlet water of the tanks were determined 2-3 times a week. From 100 to 400 algal cells were counted from each sample in a Jessen counting chamber. Total cell numbers of flagellates (*P. lutheri* and *I. galbana*) and diatoms (*S. costatum* and *C. mülleri*) were counted. Removal of algae was calculated as number of algae in the tank outlet subtracted from the numbers introduced to the sieves.

Collection of post-larvae for estimation of survival and growth was carried out initially and at day 14 and 28 for group 1, 2a and 2b, and at day 21 and 34 for group 3a and 3b. This enabled survival and growth to be inspected for two distinct periods in each experiment. Total lipid, protein and carbohydrate contents were determined at termination of the experiments. Small patches of attached post-larvae were sampled from each sieve for measurements and analyses. The byssus threads were detached and scallops removed from the mesh with the help of a piece (2 x 5 cm) of transparency film. The shell-height was measured for 100 randomly chosen specimens from each sample under a dissection microscope with a calibrated eyepiece micrometer inserted. Following the shell-height measurements the group of 100 post-larvae were washed in fresh water and immediately frozen to  $-20\text{ }^{\circ}\text{C}$  for later weight determinations. Total dry weight (DW) and ash weight were determined after drying at  $60\text{ }^{\circ}\text{C}$  for 72 hours and re-weighing after combustion at  $490\text{ }^{\circ}\text{C}$  for 6 hours using a Sartorius micro M3P balance. Total ash free dry weight (AFDW) was obtained by subtracting total ash weight from DW, and condition index (CI) was calculated as  $\text{AFDW}/\text{DW} \times 100\%$ . Growth was calculated from the parameters (P) shell-height and ash free dry weight data as specific growth rate ( $\% \text{ day}^{-1}$ ):  $\text{SGR} = (e^g - 1) \times 100\%$ , where the instantaneous daily growth coefficient  $g = (\ln P_{\text{final}} - \ln P_{\text{initial}}) \text{ days}^{-1}$  (Ricker, 1979; Claus, 1981).

Samples of post-larvae for chemical analyses were washed with 0.5 M ammonium formate and immediately frozen to  $-80\text{ }^{\circ}\text{C}$ . The material was freeze-dried

before analysing groups of whole animals with shell included. Lipids were extracted using a modified method of Bligh and Dyer (1959), and total lipids were determined gravimetrically. Total content of carbohydrates and proteins were determined colorimetrically (Dubois *et al.*, 1956; Lowry *et al.*, 1951). The chemical data were based on 5 replicate analyses per sieve and expressed as amount of organic matter ( $\mu\text{g mgAFDW}^{-1}$ ).

The number of post-larvae on each sieve was estimated by counting individuals on 40 random areas of  $1\text{ cm}^2$  and multiplying the average number with the total area of the sieve ( $1225\text{ cm}^2$ ). A circumference equal to the sieve and 20 randomly selected squares of  $1\text{ cm}^2$  were drawn on a transparent plate of Plexiglass. By placing the mesh-bottom of the sieve on the plate the number in each of the 20 squares was counted by eye. The sieve was then turned 180 degrees and 20 more areas counted. Initially density on the sieves varied (Table 1) and the variation of the post-larval number  $\text{cm}^{-2}$  was calculated as the coefficient of variation ( $\text{CV} = \text{SD}/\text{mean} * 100$ ). A larger CV in experiment 1 ( $\text{CV}=72.5$ ,  $\text{SD}=33.7$ ,  $n=21$ ) than in experiment 2 ( $\text{CV}=39.3$ ,  $\text{SD}=6.9$ ,  $n=18$ ) expressed a more patchy distribution at lower densities.

Statistical analyses of variance (1-way ANOVA) were performed on mean values by using Statistica, version 6.1. The data was tested by the Kolmogorov-Smirnov test of normality and Levene test of homogeneity of variances. The percentage survival data were arcsine transformed prior to analysis to obtain variance homogeneity (Sokal and Rohlf, 1995). Tukey HSD test was used for further comparison of groups significantly different at a probability level of 0.05, and Pearson product-moment correlation coefficients to correlate survival and growth with density, AFDW and shell height.

## RESULTS

The algal concentration in the new water introduced to the tanks fluctuated during the experimental period and averaged  $10\text{ cells }\mu\text{l}^{-1}$  ( $\text{SD} = 2.9$ ,  $n = 9$ ) in experiment 1 (groups 1, 2a and 2b) and  $15\text{ cells }\mu\text{l}^{-1}$  ( $\text{SD} = 3.5$ ,  $n = 20$ ) in experiment 2 (groups 3a and 3b) (Fig. 1). The concentration into the sieves fluctuated accordingly and varied between the recirculation rates, ranging from 4 to 11 and from 3 to 17 algal cells  $\mu\text{l}^{-1}$  in experiment 1 and 2, respectively (Fig. 1). Increased recirculation of water and

algae resulted in a decreasing amount of algae introduced to each sieve (Fig. 2, Table 3). In experiment 2 the post-larvae were fed a higher concentration of algae, and also showed a higher percentage of removed algae (Table 3). In both experiments higher removal rates of algae were observed at the intermediate recirculation rate of 83 % (Table 3). However, the removal rates (cells  $\mu\text{l}^{-1}$  and %) were not shown to be significantly different between the recirculation rates (1-way ANOVA  $p=0.477$  and  $0.373$  (exp. 1),  $p=0.309$  and  $0.083$  (exp. 2)).

No significant differences in survival were found between the three recirculation rates (ANOVA  $p=0.739$ ). Different survival was found between groups, showing better survival of the groups 1 and 2 in experiment 1 (20-81 %) than of group 3 in experiment 2 (1-64 %) (Fig. 3). The first “ready-to-settle” post-larvae (groups 2a and 3a) from each spawning showed significant higher survival (Tukey  $p=0.001$  and  $p<0.001$ ) compared to post-larvae from the second settlement groups (2b and 3b) (Fig. 3). Large variation in survival between and within the post-larval groups was observed (Fig. 3), and final mean density per sieve was reduced from 10-34 post-larvae  $\text{cm}^{-2}$  (Table 1) to a number of 2-14  $\text{cm}^{-2}$  (Table 4). Within the post-larval groups survival was not affected significantly by the water recirculation rate during the first growth period until day 14 (groups 1 and 2) and day 21 (group 3) (ANOVA,  $0.090 \leq p \leq 0.597$ ). During the second growth period the post-larvae from group 2b reared at 92 % recirculation had significant lower survival compared to post-larvae reared at 67 and 83 % (ANOVA  $p=0.008$ ). Considering the total experimental period significant difference between recirculation rates was shown for group 2a (ANOVA  $p=0.008$ , 67 % < 83 % and 92 %) and 3a (ANOVA  $p=0.018$ , 83 % > 92 %). Survival correlated significantly to initial shell-height during period 1, and to shell-height at sampling date during period 2 (Fig. 4, Table 5). There was a significant negative correlation between survival and both initial density and AFDW (Table 5).

Nor were any significant differences in post-larval growth rates found between the three recirculation rates considering all the groups together (ANOVA shell-height  $p=0.151$ , AFDW  $p=0.527$ ). Mean final shell-heights were found to be from 1.1 to 2.5 mm and mean AFDW from 20 to 71  $\mu\text{g}$  (Table 4). Absolute growth per day in terms of shell-height ranged from 15 to 62  $\mu\text{m}$  and of AFDW from 0.3 to 2.6  $\mu\text{g}$ , which corresponded to specific growth rates of 2.8-5.6 %  $\text{day}^{-1}$  and 2.2-14.4 %  $\text{day}^{-1}$



respectively (Fig. 5). Considering the total experimental period, significant differences in shell-height growth between recirculation rates were shown for group 2a (ANOVA  $p=0.002$ , 67 % > 83 % and 92 %) and 2b (ANOVA  $p=0.003$ , 67 % and 83 % > 92 %). A significant difference in AFDW growth was found between recirculation rates within the 3a post-larval group (ANOVA  $p=0.038$ , 67 % < 92 %). The 3b post-larval group showed significant higher shell-height growth rate than all the other groups (Tukey  $0.000 \leq p \leq 0.004$ ), while in view of AFDW growth group 2b was significant lower than the others (Tukey  $0.000 \leq p \leq 0.029$ ). Group 2 showed significant difference in AFDW growth between the first and second settlement groups (Tukey  $p=0.018$ ), while no difference was found between the settlement groups of group 3 (Tukey,  $p=0.124$ ). Shell-height showed significant correlations with initial density, AFDW and shell-height (Table 5). AFDW growth was significantly correlated to initial density and initial AFDW, while final biomass (AFDW) correlated significantly with all parameters (Table 5).

No significant differences were found between final CI, lipid or protein content of the post-larvae and the three recirculation rates (ANOVA CI  $p=0.528$ , lipid  $p=0.154$ , protein  $p=0.723$ ). However, carbohydrate content was found to be significant higher at 67 % recirculation than at 83 % and 92 % (ANOVA,  $p=0.002$ ). The only significant difference in chemical content between groups was found for protein where group 2a showed a higher content than group 2b (ANOVA  $p=0.009$ , Tukey  $p=0.018$ ) (Table 6). The condition index (CI) decreased over time from a range of 12-24 % initially to mean values from 7.3 to 11.4 % finally (Table 6). Protein contributed the most and carbohydrate the least to the chemical composition (Table 6). On average the protein accounted for 69.2 % (SD=7.6, n=31), lipid for 24.4 % (SD=6.8, n=31) and carbohydrate for 7.8 % (SD=1.4, n=31) of the AFDW.

## DISCUSSION

The present study showed that scallop, *Pecten maximus*, post-larvae could well be reared in a partial open and continuous feeding system at water recirculation rates of 67 %, 83 % and 92 %. Hence, the results confirmed that a more efficient reuse of water and algae than already practiced at Scalpro AS was achievable. By increasing the recirculation rate from 67 % to 92 % the amount of new water added to the rearing

tanks could be reduced by a factor of 4 per day. Consequently, it was possible to reduce the cost of producing algae and heating seawater from ambient sea temperature to culture temperatures.

The method of one-layer post-larval culture on downwelling screens, as used at the Norwegian scallop hatchery, is a common but space-consuming technique used for rearing the *P. maximus* in Europe (Millican, 1997; Robert and Gerard, 1999). Therefore, growth strategies resulting in increased utilisation of algae and reuse of water are welcomed. Compared to scallops, oysters and clams can be raised at high densities in upwelling growth systems where the water is forced up through a multi-layer of bivalves (Bayes, 1981; Manzi *et al.*, 1984; Flimlin, 2000). A passive water reuse upwelling system has, however, been developed for scallop, *Pecten fumatus*, spat from the size of 750  $\mu\text{m}$  (Heasman *et al.*, 2002). This system consisted of stacks of screens with seawater entering via the bottom screen and flowing out through the top screen. The scallops were not reared at higher density per unit surface area in the described system (Heasman *et al.*, 2002), but a better utilisation of each water volume unit was achieved. Some form of upwelling system might be useful considering space, but need to be compared with the use of downwellers in the partly open recycling system in terms of labour costs.

To maintain the algal concentration of about 10 cells  $\mu\text{l}^{-1}$  into the sieves for all recirculation rates, the results from experiment 1 showed that the algal quantity has to be increased. An additional supply of algae from 10 to 15 cells  $\mu\text{l}^{-1}$  obtained feasible food levels in tanks at all recirculation rates. Optimum algal concentrations are suggested between 10 and 20 cells  $\mu\text{l}^{-1}$  for *P. maximus* (6-63 mm) when fed a single cell diet of *Chaetoceros gracilis* (Skjæggestad *et al.*, 1999). Likewise Lu and Blake (1996) found 10 cells  $\mu\text{l}^{-1}$  of *Isochrysis galbana* optimal for 0.5-6.0 mm *Argopecten irradians concentricus*. The post-larvae offered the mean algal concentration closest to 10 cells  $\mu\text{l}^{-1}$  (83 %, experiment 2) in our study showed the highest mean removal of cells from the water. The algal removal did not differ significantly from the other recirculation rates, nor could any effect be related to better growth or increased nutritional status of the post-larvae. According to Nicolas and Robert (2001) a low food level of 7 cells  $\mu\text{l}^{-1}$  was sufficient to support post-larval *P. maximus* growth during 8 weeks. Thus, the standard hatchery procedure of keeping a food level of approximately 10 cells  $\mu\text{l}^{-1}$  for post-larvae

agrees with the findings of Nicolas and Robert (2001). Despite the reduction in algal quantity due to increased recirculation, we managed to keep the mean algal concentrations between 6 to 13 cells  $\mu\text{l}^{-1}$  during the experiments.

A successful recirculation system prevents accumulation of organic matter and toxic metabolites and/or exudates which deteriorate the water quality. The feeding regime affects the water conditions, and the quantity of algae supplied to the system must balance the filtering capacity of the scallops to prevent starving or overloading. The filtration rate of *P. maximus* spat is inversely proportional to algal concentrations between 5 and 210 cells  $\mu\text{l}^{-1}$ , and decreases at cell concentrations  $>200 \mu\text{l}^{-1}$  (Laing, 2004). Further, continuous feeding has been found to promote food utilization of *A. purpuratus* of the same size as the scallops in our study ( $<2 \text{ mm}$ ) (Martínez *et al.*, 1995). Thus, continuous feeding, which allows lower algal concentrations than batch feeding, is advantageous as feeding strategy to prevent build-up of excess food. In the partial open feeding system used in our experiments as much as 8-33 % of the tank water was replaced per minute. The water exchange of the tank volumes was 2, 4 and 8 times per day, resulting in a rather low residential time of algae compared to in a closed recirculation system. Accordingly, water quality was probably not affected by the applied recirculation rates. This assumption was supported by the results as the post-larval performance barely showed any differences between the recirculation rates.

The post-larval growth and survival were not significantly affected by increasing the recirculation rate from 67 % to 83 % or 92 %. As shown in our experiments, large variation in survival and growth within and between groups of scallop post-larvae are commonly experienced in culture (Bourne and Hodgson, 1991; Heasman *et al.*, 1994; Couturier *et al.*, 1995; Robert and Gerard, 1999). The post-larvae that originated from the second settlement groups showed substantial lower survival than those originated from faster settled larvae, while in terms of growth rates the same trend was not obvious. The relatively high growth rates shown for the post-larvae coming from the second settlement groups may be due to the fact that growth was measured on surviving post-larvae. The 2b, 3a and 3b post-larval groups initially consisted of very small specimens ( $<500 \mu\text{m}$ ). Consequently a large fraction of the larvae transferred to the sieves were of an immature “ready-to-settle” stage ( $<200 \mu\text{m}$ ) and possibly not capable of undergoing metamorphosis and survive in the settlement system.

Survival varied from 1 to 81 % in the present study, and the lowest survival was observed in the second experiment with post-larval group 3. This could be due to higher initial density on the sieves, but also due to age (i.e. size) at experimental start. The initial density was not only much higher in experiment 2 than in experiment 1 (29-34 vs. 10-17 cm<sup>-2</sup>), but also showed an even distribution (CV = 39 vs. 72) on the sieve mesh surfaces. A dense layer of post-larvae might clog the mesh and lead to a deterioration of the rearing environment inside the sieve and in turn affect the survival. Dead and detached post-larvae are regularly removed from the sieves due to the cleaning procedure in the hatchery, and the main mortality usually occurs within the first 2 weeks after transfer from larval tanks (T. Magnesen, pers. com.). The higher mortality observed in experiment 2 could therefore be the effect of an expected reduction in larval number by using younger larvae. Hence, the lower initial number in experiment 1 compared to in experiment 2 can be explained, as group 3 spent 1 week shorter time in the settlement system before experimental start.

In our study, the survival was dependent on initial shell-height, which also could explain differences in survival between the post-larval groups. During the post-larval stages scallops are vulnerable, and tolerance to changes in environmental conditions is size dependent for *P. maximus* (Christophersen and Magnesen, 2001) and *Placopecten magellanicus* (Grecian *et al.*, 2000). *P. maximus* post-larvae have restricted feeding ability up to a size of 4 mm due to undeveloped gills (Beninger *et al.*, 1994) which may as well have affected survival. A 10-30 % survival during the post-larval growth period has commonly been experienced at Scalpro AS (T. Magnesen, pers. com.), which is similar to what is reported for *P. fumatus* (Heasman *et al.*, 2002). In experimental work with *P. maximus* post-larvae higher survival rates has been reported, up to 60-90 % after 4-5 weeks (Robert and Nicolas, 2000; Nicolas and Robert, 2001). This is in the same range as achieved for several groups in our study.

Regarding post-larval condition index or chemical content, no significant differences were found between the three recirculation rates, with the exception of the carbohydrate content. The quantity and quality of protein, lipid and carbohydrate determine the nutritional status or condition of the post-larvae, but the higher carbohydrate level observed at 67 % recirculation was not reflected in the growth. Based on these results, we may assume that the algae kept the nutritional quality even if

the residential time in the tanks was extended along with the increased recirculation rate. Stored energy reserves are important during critical stages in early scallop life, and are essential for larvae to metamorphose successfully (Whyte *et al.* 1987, Delauney *et al.* 1992, Whyte *et al.*, 1992; Farias *et al.*, 1998, Lu *et al.*, 1999). During the intensive rearing phase the produced algae will ensure a balanced diet supporting growth and survival. The chemical content was not measured at the larval stage in the present study, but at the end of the experiment when the post-larvae were large enough to be transferred to a nursery. This is another critical stage in scallop spat production which require post-larvae of good condition (Grecian *et al.*, 2000; Christophersen and Magnesen, 2001; Rupp *et al.*, 2004). The level of protein (692-925  $\mu\text{g mgAFDW}^{-1}$ ) showed comparable levels to post-larvae of the same size produced in the spring 1996 at the Scalpro hatchery (656-858  $\mu\text{g mgAFDW}^{-1}$ ) and successfully transferred to nursery (Christophersen and Lie, 2003), while the lipid and carbohydrate content were higher in our study (lipid 275-334 vs. 162-254 and carbohydrate 86-94 vs. 22-31  $\mu\text{g mgAFDW}^{-1}$ ). Therefore, the post-larvae reared at all three recirculation rates should have obtained a nutritional status making them capable of surviving the next production step.

A further increase in recirculation rate beyond 92 % seems possible based on the results from the present experiments. However, it is important to ensure enough algae added with the new water to maintain a concentration into the sieves of 10 cells  $\mu\text{l}^{-1}$ . An exchange of the tank volume less than twice per day may well be recommended, but should be tried out comparatively with the recycling rates reported in this study to recognise any effects on the water and feed quality.

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## FIGURE LEGEND

**Figure 1.** The algal concentration (cells  $\mu\text{l}^{-1}$ ) in new water added to the rearing tanks and into each sieve at water recirculation rates of 67, 83 and 92 %.

**Figure 2.** The relationship between the algal concentration (cells  $\mu\text{l}^{-1}$ ) supplied to the tanks (new water) and to the sieves at water recirculation rates of 67, 83 and 92 %.

**Figure 3.** Mean survival (%) after the total experimental period of scallop post-larvae reared at water recirculation rates of 67, 83 and 92 %. The vertical bars show the minimum and maximum values of replicate sieves.

**Figure 4.** Initial shell height related to survival (%) of scallop post-larvae reared at water recirculation rates of 67, 83 and 92 % after growth period 1 (A) and 2 (B). The growth periods were day 0-14 and 14-28 for group 1, 2a and 2b and day 0-21 and 21-34 for group 3a and 3b.

**Figure 5.** Daily specific growth rates (SGR) for mean shell height (SH) A), and ash free dry weight (AFDW) B), during the total experimental period of scallop post-larvae reared at water recirculation rates of 67, 83 and 92 %. The vertical bars show the minimum and maximum values of replicate sieves.

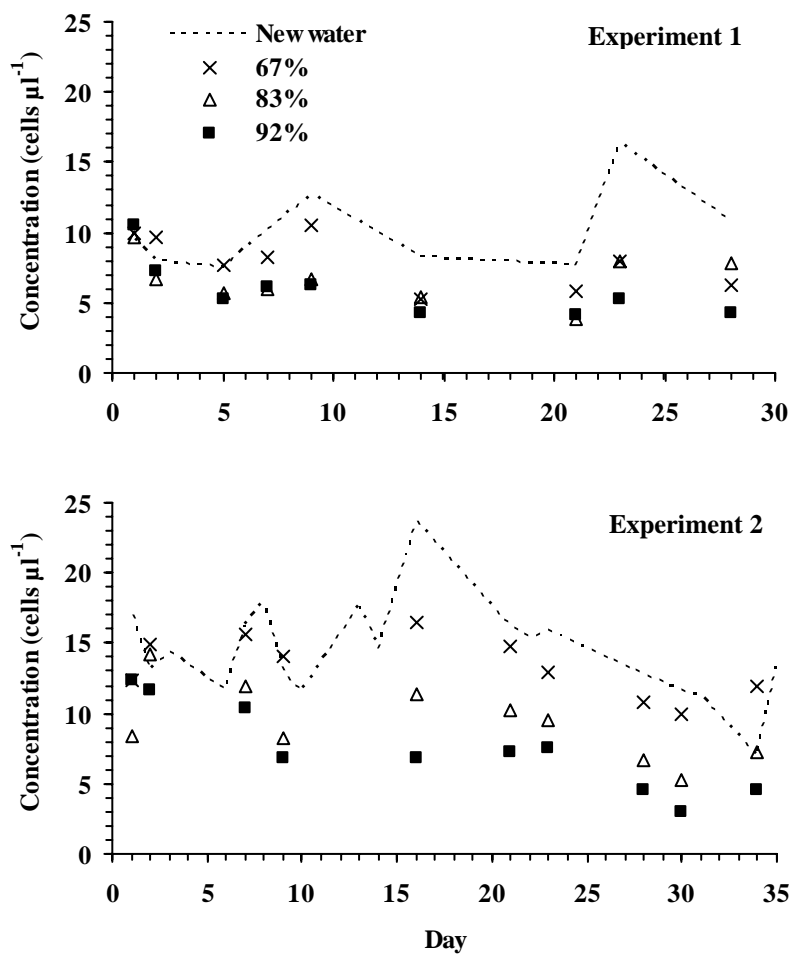


Figure 1. Christophersen, Torkildsen &amp; van der Meeren

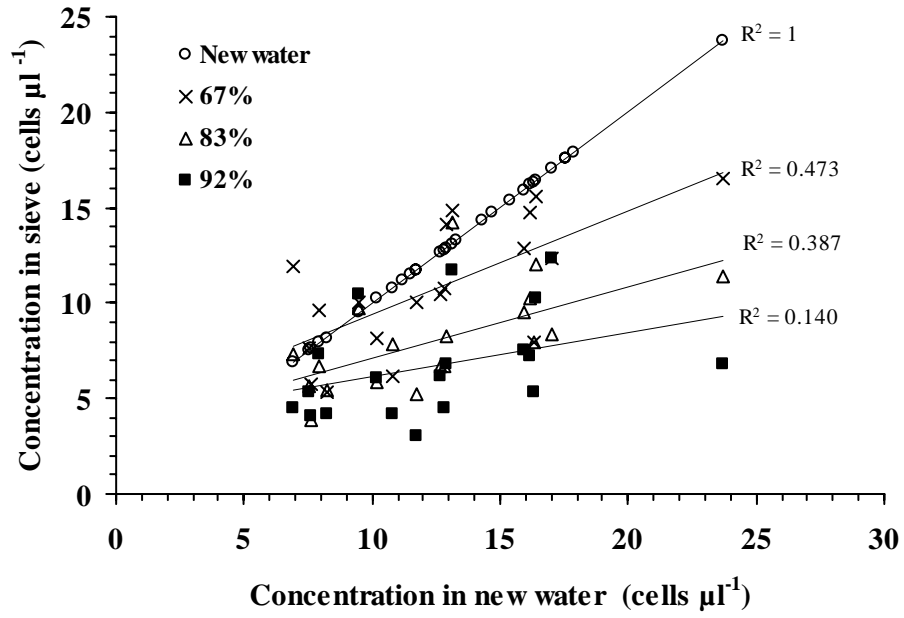


Figure 2. Christophersen, Torkildsen &amp; van der Meeren

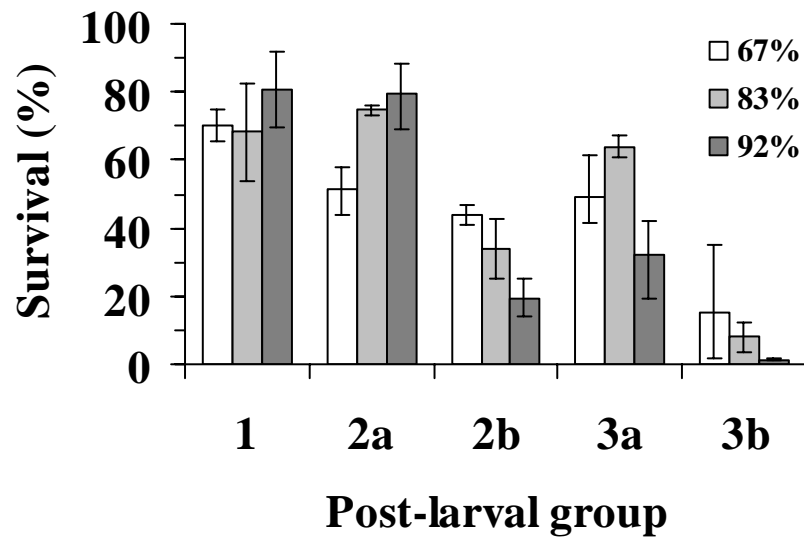


Figure 3. Christophersen, Torkildsen & van der Meeren

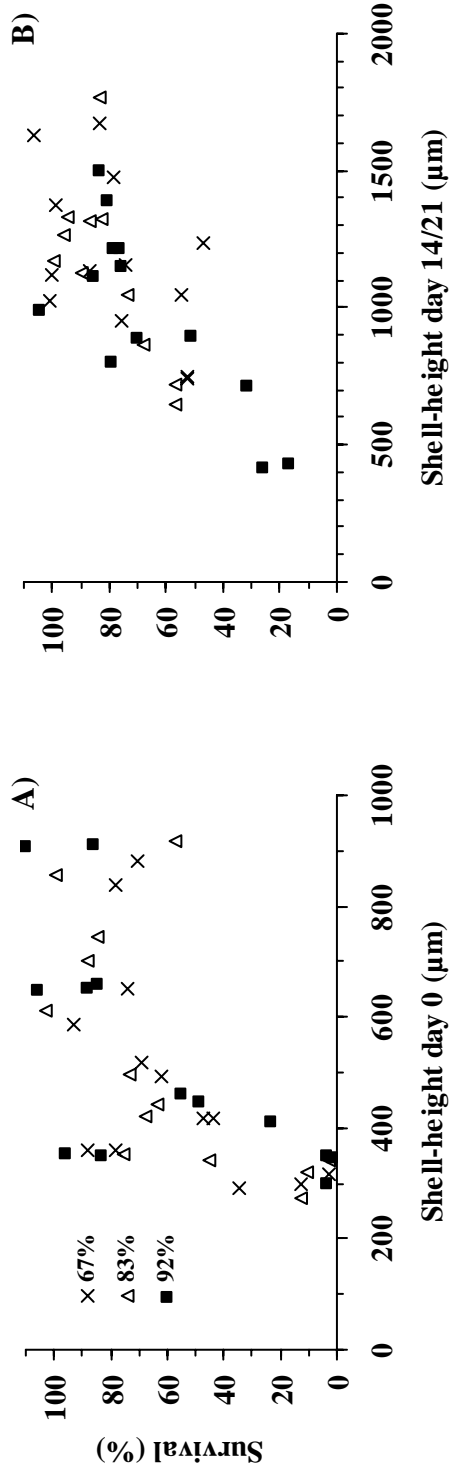


Figure 4. Christophersen, Torkildsen & van der Meer

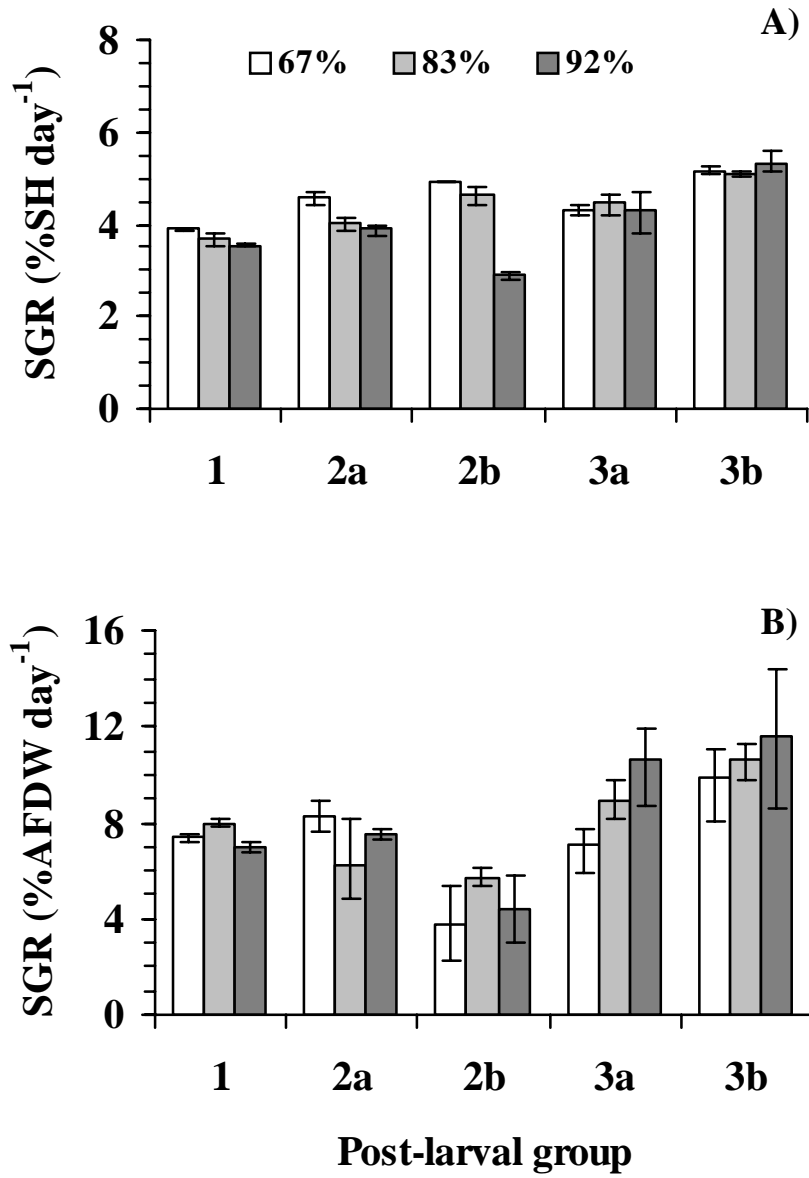


Figure 5. Christophersen, Torkildsen & van der Meeren



Table 1. Experimental design and initial characteristics (SH = shell height, DW = dry weight, AFDW = ash free dry weight) of scallop post-larvae grown at different water recirculation rates (67, 83 and 92 %). Mean values (n=6 or n=9) per sieve  $\pm$  standard deviation (SD) are shown. SH ranges of all measured individuals (n=100) are shown in brackets.

Exp. no.	Post-larval group	Replicate sieves (#)	Settling age (days)	Initial age (days)	Initial SH ( $\mu\text{m}$ )	Initial DW ( $\mu\text{g}$ )	Initial AFDW ( $\mu\text{g}$ )	Initial density ( $\# \text{cm}^{-2}$ )	Exp. time (days)
1	1	2	22	57	886 $\pm$ 34 (440-1480)	75.7 $\pm$ 10.0	9.6 $\pm$ 1.6	10 $\pm$ 4	28
	2a	3	24	50	642 $\pm$ 65 (280-1260)	41.4 $\pm$ 10.8	6.5 $\pm$ 2.3	17 $\pm$ 4	28
	2b	2	27	50	353 $\pm$ 7 (240-620)	29.5 $\pm$ 16.2	6.2 $\pm$ 3.8	16 $\pm$ 6	28
2	3a	3	22	43	446 $\pm$ 32 (152-1041)	12.6 $\pm$ 6.9	2.1 $\pm$ 1.1	29 $\pm$ 4	34
	3b	3	24	43	316 $\pm$ 27 (176-772)	6.1 $\pm$ 4.0	1.1 $\pm$ 0.7	34 $\pm$ 8	34

Table 2. Recirculation rates in post-larvae rearing tanks and corresponding volume of new water with algae feed and recirculated water added and water exchange rates.

<b>Recirculation rate (%)</b>	<b>New water added to sieve (<math>l\ min^{-1}</math>)</b>	<b>New water added to tank (<math>l\ min^{-1}</math>)</b>	<b>Recirculated water added to tank (<math>l\ min^{-1}</math>)</b>	<b>Exchange of tank volume (<math>times\ day^{-1}</math>)</b>
67	1	14	28	8
83	0.5	7	35	4
92	0.25	3.5	38.5	2

Table 3. Algae cells removed (cells introduced to sieves-cells in tank water outlet) from rearing tanks at different recirculation rates (67, 83 and 92 %). Data are shown as mean algae concentration (cells  $\mu l^{-1}$ ) and percentage (%) of algae removed  $\pm$  SD, n=9.

<b>Experiment no.</b>	<b>Cells into tanks (<math>\mu l^{-1}</math>)</b>	<b>Recirculation rate (%)</b>	<b>Cells into sieves (<math>\mu l^{-1}</math>)</b>	<b>Cells removed</b>	
				<b>(<math>\mu l^{-1}</math>)</b>	<b>(%)</b>
1	$10 \pm 2.9$	67	$7.90 \pm 1.88$	$0.17 \pm 2.13$	$1.7 \pm 23.4$
		83	$6.64 \pm 1.70$	$1.08 \pm 1.41$	$15.2 \pm 18.8$
		92	$5.91 \pm 2.03$	$0.51 \pm 0.98$	$8.0 \pm 17.0$
2	$15 \pm 3.5$	67	$13.03 \pm 1.95$	$1.33 \pm 1.63$	$10.0 \pm 12.0$
		83	$9.31 \pm 2.87$	$2.40 \pm 2.32$	$27.2 \pm 22.0$
		92	$7.53 \pm 3.30$	$1.07 \pm 1.51$	$10.2 \pm 16.6$

Table 4. Shell-height (SH), ash free dry weight (AFDW) and density of scallop at sampling date (period 1) and termination (period 2) of the experiment. Mean values per sieve  $\pm$  standard deviation (SD) are shown for pooled data of post-larvae grown at different water recirculation rates (67, 83 and 92 %).

Exp. no.	Group	Period 1 (days)	SH ( $\mu\text{m}$ )	AFDW ( $\mu\text{g}$ )	Density ( $\# \text{cm}^{-2}$ )	Period 2 (days)	SH ( $\mu\text{m}$ )	AFDW ( $\mu\text{g}$ )	Density ( $\# \text{cm}^{-2}$ )
1	1	0-14	1549 $\pm$ 168	36.1 $\pm$ 6.5	8 $\pm$ 3	14-28	2453 $\pm$ 79	70.9 $\pm$ 3.1	7 $\pm$ 3
	2a	0-14	1200 $\pm$ 144	20.6 $\pm$ 4.2	15 $\pm$ 4	14-28	2012 $\pm$ 142	45.4 $\pm$ 7.4	11 $\pm$ 3
	2b	0-14	619 $\pm$ 151	4.5 $\pm$ 0.8	12 $\pm$ 6	14-28	1134 $\pm$ 282	20.0 $\pm$ 5.7	6 $\pm$ 4
2	3a	0-21	1173 $\pm$ 140	12.8 $\pm$ 2.7	15 $\pm$ 4	21-34	1905 $\pm$ 169	33.3 $\pm$ 6.6	14 $\pm$ 4
	3b	0-21	918 $\pm$ 105	8.3 $\pm$ 1.6	3 $\pm$ 2	21-34	1712 $\pm$ 164	27.7 $\pm$ 6.9	2 $\pm$ 2

Table 5. Results from Pearson product-moment correlations for initial density, ash free dry weight (AFDW) and shell-height (SH) and SH at sampling date after growth period 1 vs. survival, growth (daily SGR) and final biomass (AFDW weight).

Pearson correlation coefficient	Survival (%)		SH growth (%)		AFDW growth (%)		Final biomass (AFDW)	
	r	p	r	p	r	p	r	p
$Density_{init}$ ( $\# \text{ cm}^{-2}$ )	-0.575	0.000	0.628	0.000	0.539	0.001	-0.781	0.000
$AFDW_{init}$ ( $\mu\text{g}$ )	-0.613	0.000	-0.442	0.006	-0.685	0.000	0.891	0.000
$SH_{init}$ ( $\mu\text{m}$ )	0.786	0.000	-0.594	0.000	-0.181	0.285	0.918	0.000
$SH_{\text{samp}}$ ( $\mu\text{m}$ )	0.643	0.000	-0.144	0.397	-0.192	0.254	0.755	0.000

Table 6. Condition index (CI) and chemical content (total lipid, protein and carbohydrate) of scallop post-larvae at termination of the experiments. Data are shown as means per sieve  $\pm$  SD.

Post-larval group	n	CI (%)	Lipid ( $\mu\text{g mg}^{-1}$ AFDW)	Protein ( $\mu\text{g mg}^{-1}$ AFDW)	Carbohydrate ( $\mu\text{g mg}^{-1}$ AFDW)
1	6	8.1 $\pm$ 0.3	334 $\pm$ 215	752 $\pm$ 74	86 $\pm$ 7
2a	9	8.5 $\pm$ 0.3	275 $\pm$ 61	925 $\pm$ 119	95 $\pm$ 12
2b	6 (CI), 4	11.4 $\pm$ 1.8	305 $\pm$ 45	692 $\pm$ 169	98 $\pm$ 13
3a	9	7.3 $\pm$ 0.4	330 $\pm$ 140	877 $\pm$ 122	94 $\pm$ 15
3b	7 (CI), 3	7.9 $\pm$ 0.2	294 $\pm$ 19	783 $\pm$ 20	92 $\pm$ 26