

# Nursery growth, survival and chemical composition of great scallop *Pecten maximus* (L.) spat from different larval settlement groups

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## Abstract

Scallop *Pecten maximus* spat (1.3–2.1 mm shell height) from different settlement groups were transferred from hatchery to land-based nursery at different ages and sizes. Chemical content, growth and survival were compared at transfer time and after 1 and 8 weeks of nursery growth. Growth was lowest and mortality highest in the first week after transfer. Mean shell height growth was 21.5–71.4  $\mu\text{m day}^{-1}$  and ash-free dry weight (AFDW) growth – 2.7 to 10.3  $\mu\text{g day}^{-1}$ . Spat from the first settlement group attained a larger size and weight than spat from larvae settled 3 days later, but had a lower daily growth rate (%). Keeping the late-settled spat a longer time in the hatchery to reach a bigger size before transfer seemed not to improve subsequent nursery growth. Survival showed a large variation with mean survival ranging from 32% to 74%. A substantial reduction in lipid content was found after transfer to the nursery. Sterol content at transfer was the only lipid class correlating with survival in the nursery. Based on the results, it is justified that spat groups of different settlement age are included in production of 15-mm great scallop spat if they are transferred from the hatchery at the same age.

**Keywords:** *Pecten maximus*, scallop spat, nursery growth, chemical content, lipid classes

## Introduction

The success of scallop aquaculture depends on a reliable spat source, making hatchery production an op-

tion for predictable spat supply (Bourne 2000). The substantial increase in food demand, resulting from rapid growth in biomass during the hatchery growth phase, necessitates that intensive larval rearing be followed by growth to commercial sized spat in more cost-effective rearing systems or nurseries. In Norway, the use of a manipulated land-locked heliothermic marine basin, called a 'poll' (Strand 1996), has been considered as a water supply and food production system for nursery growth of scallop *Pecten maximus* spat from 2-mm shell height to 15–20 mm.

During the larval growth phase, culling takes place. At water changes, the larvae are sieved through fine mesh screens, with increasing mesh size as the larvae grow. Poorly developed and the slowest growing larvae are successively eliminated from the batch, while the faster growing larvae retained on the mesh are allowed further growth. Reaching the pediveliger, or the 'ready to settle' stage, the larvae can be transferred to another growth system to settle and metamorphose. Larvae will be ready to settle at different times resulting in different settlement groups from each larval batch. How the larval growth rate of the scallop *P. maximus*, expressed as the length of the larval growth period, affects the growth rate in the subsequent growth phases lacks documentation. A positive relationship between larval and spat growth rates has been shown for the oysters *Crassostrea virginica* (Newkirk, Haley, Waugh & Doyle 1977; Losee 1979) and *Crassostrea gigas* (Collet, Boudry, Thebault, Heurtebise, Morand & Gérard 1999), whereas a negative correlation between length of larval period and subsequent growth

to commercial size is suggested for the European oyster *Ostrea edulis* (Newkirk & Haley 1982). The merits of the larval culling practice in bivalve hatcheries is disputed, as fast-growing adults of *Mercenaria mercenaria* and bay scallop *Argopecten irradians* were demonstrated to produce slower growing larvae than unselected parents (Heffernan, Walker & Crenshaw 1991, 1992). A study on the clam *M. mercenaria* (Deming & Russell 1999) likewise indicated the culling of slow-growing larvae to be counterproductive. Whether *P. maximus* spat originating from slow-growing larvae should be retained is therefore an issue of significance in the production of commercial-sized scallops.

Transition of spat from hatchery to nursery involves alteration in the environmental conditions. The food supply changes from cultivated algae to naturally produced food, and the nutritional value will be influenced by season. The chemical content of scallop juveniles has been shown to be affected by diet (Martínez, Torres, Uribe, Díaz & Pérez 1992; Ryan, Parsons & Dabinett 1998; Uriarte & Fariás 1999) and may reflect the nutritive condition of the spat. Lipids are important as an energy reserve to be consumed during critical stages in early scallop life (Whyte, Bourne & Hodgson 1987; Delauney, Marty, Moal & Samain 1992; Whyte, Bourne, Ginther & Hodgson 1992; Fariás, Uriarte & Castilla 1998; Lu, Blake & Torres 1999; Robert, Nicolas, Moisan & Barbier 1999). Less is known about lipid composition and how lipid content affects growth in the post-metamorphic scallop stages. Growth of *Placopecten magellanicus* juveniles has been associated with polyunsaturated fatty acids (22:6-3, 20:5-3 and 20:4-6) and total lipid in the diet (Parrish, McKenzie, MacDonald & Hatfield 1995; Parrish, Wells, Yang & Dabinett 1999). The level of the acetone-mobile polar lipid class (glycolipids and monoacylglycerols) is suggested as an indicator of stress (Parrish *et al.* 1999). Quantification of lipid classes rather than total lipid in the scallop spat may thus give more detailed information about which components are metabolized during growth.

In the present study, *P. maximus* spat originating from different settlement groups of two larval batches were transferred from intensive hatchery to semi-intensive nursery conditions. The main purpose was to compare the subsequent nursery growth and survival of spat with different length of larval growth period (i.e. fast- and slow-growing larvae). Further, the objective was to quantify chemical composition in terms of protein, carbohydrate, total lipid and lipid classes.

## Materials and methods

The scallop *Pecten maximus* spat were obtained from two spawnings at Scalpro AS scallop hatchery. Broodstock scallops originated from the local population in Hordaland, western Norway, and were induced to spawn in March and May 1996. Eyed pediveliger larvae were transferred to a settlement system when retained on 150- $\mu\text{m}$  mesh. The post-larval growth was in partly submerged PVC cylinders (sieves) fitted with 140- $\mu\text{m}$  mesh (height 15 cm, diameter 39.5 cm). Airlifts provided for a downwelling flow ( $1 \text{ L min}^{-1}$ ) and recirculation (67%) of water with algae in the rearing tanks ( $1 \text{ m} \times 1 \text{ m} \times 6 \text{ m}$ ). The diet consisted of a mix of the monocultured algae *Pavlova lutheri*, *Isochrysis galbana* and *Skeletonema costatum* in a 1:1:2 ratio. The spat were continuously fed a concentration of 10 algae cells  $\mu\text{L}^{-1}$ . Rearing temperature was kept at 15 °C and salinity at 32 until transfer to the nursery.

Transportation of spat to the commercial nursery Sealife AS was in moist atmosphere in a cooler box (Christophersen 2000). The transport lasted 4–5 h, and the temperature was kept at 10–15 °C. Immediately after arrival at the nursery, the spat were relayed onto rectangular trays (length 57 cm, width 37 cm) covered with 500- $\mu\text{m}$  mesh to hinder escape. Stacks of trays were placed on the bottom in a land-based raceway nursery system ( $0.6 \text{ m} \times 0.8 \text{ m} \times 12 \text{ m}$ ) with horizontal water flow ( $\approx 400 \text{ L min}^{-1}$ ). The water was taken from a 67 000- $\text{m}^3$  land-locked heliothermic marine basin (described by Strand 1996) and filtered through 100- $\mu\text{m}$  mesh size before entering the raceways. During the experimental growth period in the nursery, temperature averaged 15.9 °C ( $\pm \text{SD } 1.4$ , range 13–19 °C) and salinity 30.2 ( $\pm \text{SD } 0.3$ ). The food production system provided particle concentration of  $>10 \mu\text{L}^{-1}$  throughout the period from June to October. Algae cell counts from July and August ranged from 3 to 17  $\mu\text{L}^{-1}$ . The diatoms (*Skeletonema costatum*, *Chaetoceros* spp. and *Thalassiosira* sp.) were shown to be in the majority from June to mid-July and at the end of August, whereas dinoflagellates (*Gymnodinium* spp. and *Prorocentrum* spp.) and other small flagellates were dominant for the rest of the time.

From each of the two spawnings (March and May), two settlement groups of larvae were transferred to triplicate sieves in the settlement system. Fast-growing larvae (March 1 and May 1) reached the 'ready to settle' stage 3 days earlier than slow-growing larvae (March 2 and May 2) (Table 1). The settlement group 2

**Table 1** Larval age when transferred to settling system, age, shell height (SH), ash-free dry weight (AFDW) at the time of transfer from hatchery to nursery and specific daily growth rates (% day<sup>-1</sup>) of shell height (SH) and AFDW are shown for the total nursery period (weeks 0–8), the first week (0–1) and the period in the nursery (weeks 1–8)

Parameter	Spat group						
	March 1	March 2a	March 2b	May 1	May 2a	May 2b	
Settling age (days)	21	24	24	22	25	25	
Transfer date	June 11	June 11	June 28	August 1	August 1	August 16	
Age at transfer (days)	74	74	91	77	77	92	
SH at transfer (μm)	1891 (±478)	1349 (±429)	2091 (±642)	1699 (±524)	1391 (±416)	2147 (±819)	
AFDW at transfer (μg)	44 (±2)	24 (±1)	65 (±4)	41 (±8)	28	69	
SH (% day <sup>-1</sup> )	Week 0–8	1.8 (±0.04)	2.0 (±0.01)	1.6	2.0 (±0.06)	2.1	1.9
	Week 0–1	1.4 (±0.21)	1.5 (1.13)	1.2 (±0.40)	1.2 (±0.42)	3.4	1.9
	Week 1–8	1.8 (±0.05)	2.1 (±0.24)	1.7	2.1 (±0.01)	1.9	1.8
AFDW (% day <sup>-1</sup> )	Week 0–8	4.2 (±0.11)	4.3 (±0.05)	3.3	4.6 (±0.18)	4.5	3.8
	Week 0–1	3.7 (±0.81)	3.9 (±0.23)	2.5 (±0.69)	-2.1 (±1.14)	6.4	-4.5
	Week 1–8	4.3 (±0.20)	4.4 (±0.11)	3.5	5.7 (±0.27)	4.2	5.1

Mean values of three sieves or trays (*n* = 3) are shown with standard deviation (±SD) in brackets. Where SD is missing, *n* = 1.

was divided into two subgroups (March 2a and March 2b, May 2a and May 2b). After about 8 weeks growth in the settlement system, spat groups 1 and 2a were transferred to the nursery. These spat were the same age but differed in size at transfer time (Table 1). Spat groups 2b were allowed to grow for two more weeks to reach the size of ≈ 2 mm shell height before transfer. The density in the sieves ranged from 4 to 7 and 1 to 4 spat cm<sup>-2</sup> for settlement groups 1 and 2, respectively, at the time of transfer. The scallops in the experimental sieves were transferred to corresponding triplicate trays in the nursery at densities of 0.5–4 spat cm<sup>-2</sup>. The sieves and trays in our study were considered replicates. Owing to high mortality in the post-larval phase in the settlement system, the spat from the three sieves of the May 2a and May 2b groups were pooled and transferred to one tray in the nursery.

Growth and survival parameters were monitored after 7–10 and 55–59 days, hereafter expressed as 1 and 8 weeks, in the nursery. Survival was calculated as a percentage of initial count on the day of transfer from the hatchery. The numbers of spat were estimated from counting the individuals in 20 square areas (1 cm<sup>2</sup> on sieves and 4 cm<sup>2</sup> on trays) randomly

scattered on the bottom area of the sieve or tray. Total number was calculated as the average number per square multiplied by the total area. Spat to be analysed were detached and removed from an arbitrary area on the mesh bottom using a small piece of transparency film. The shell height (SH) of 100 specimens was measured from each replicate under a dissection microscope with a calibrated eyepiece micrometer. Specific growth rate (% day<sup>-1</sup>) was calculated using the equation  $G = (e^g - 1) \times 100$ , where the instantaneous daily growth coefficient  $g = (\ln SH_{\text{final}} - \ln SH_{\text{initial}}) \text{ days}^{-1}$  (Ricker 1979; Claus 1981). Dry weights were recorded for the whole group of 100 spat using a Sartorius micro M3P balance. Total dry weight (DW) and ash weight (AW) were determined after drying at 60 °C for 72 h and reweighing after combustion at 490 °C for 6 h. Total ash-free dry weight (AFDW) was obtained by subtracting AW from DW. Weight-specific growth rate (% day<sup>-1</sup>) was calculated by replacing SH with AFDW in the equation above.

Spat for chemical analyses were immediately frozen to -80 °C. Lipid, protein and carbohydrate content were analysed at transfer time from the hatchery and after 1 and 8 weeks in the nursery. The material

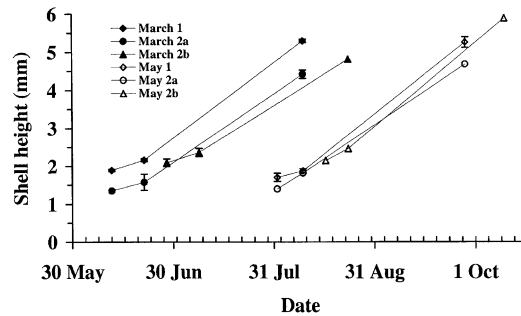
was freeze dried before analysing groups of whole scallops, shell included. Groups of 25–100, 45–300 and 50–100 individuals were required for the lipid, carbohydrate and protein analyses respectively. The freeze-dried material for lipids and protein was dry ground to powder in a glass tube with a glass rod before analyses, whereas the material for carbohydrate analyses was homogenized in distilled water. Lipids were extracted according to the method of Rønnestad, Finn, Lein, & Lie (1995), and total lipids were determined gravimetrically. Lipid classes were detected using an Iatrosan thin-layer chromatograph (Iatrosan MK-5) with a flame ionization detector (FID). An amount of 1  $\mu\text{L}$  of chloroform solution of the total lipid extract was spotted on silica rods, and the neutral lipids were separated before the polar lipids (Rønnestad *et al.* 1995). Chromatograms were registered and interpreted with the help of MAXIMA integrating software. Proteins were calculated after determination of nitrogen using a nitrogen analyser (Perkin-Elmer 2410 Series II). Amount of nitrogen was multiplied by a factor of 6.25 to get the protein content. Total content of carbohydrates was determined colorimetrically by the phenol-sulphuric acid method (Dubois, Gillies, Hamilton, Rebers & Smith 1956) and absorption measurements at 490 nm and 600 nm. The chemical components were calculated to express the amount in organic matter ( $\mu\text{g mg}^{-1}$  AFDW).

Statistical testing was carried out using STATISTICA, version 5. Analyses of variance (one-way ANOVA) were performed on mean values. Final size and AFDW means were based on samples of 100 scallop spat from each replicate, survival data on estimated total count in each tray, and the chemical data from pooled samples of 25–300 animals per tray according to the amount required for each analysis. The lipid class data were again based on the means of three Iatrosan detections per sample. Tukey HSD test was used to compare the spat groups, and effects were considered significant at a probability level of  $\alpha < 0.05$ . Survival and growth were related to the different chemical fractions at transfer time by calculating Pearson product-moment correlation coefficients.

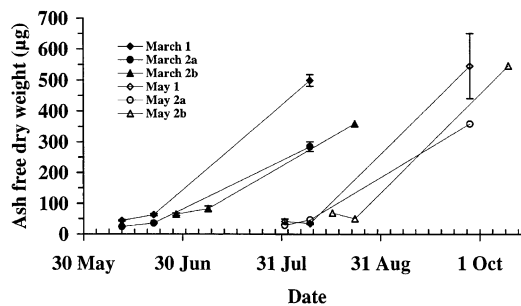
## Results

### Growth

After 8 weeks growth in the nursery the spat had reached mean sizes from 4.4 to 5.9 mm shell height (Fig. 1) and from 285 to 547  $\mu\text{g}$  AFDW (Fig. 2). The



**Figure 1** Shell height increase of scallop spat originating from fast-growing larvae (March 1 and May 1) and slow-growing larvae (March 2a, 2b and May 2a, 2b) during nursery growth. Symbols show mean values of three trays ( $n = 3$ ) and vertical bars standard deviation (SD). Where SD is missing,  $n = 1$ .



**Figure 2** Ash-free dry weight (AFDW) increase of scallop spat originating from fast-growing larvae (March 1 and May 1) and slow-growing larvae (March 2a, 2b and May 2a, 2b) during nursery growth. Symbols show mean values of three trays ( $n = 3$ ) and vertical bars standard deviation (SD). Where SD is missing,  $n = 1$ .

shell height increased at a rate of 21.5–53.2  $\mu\text{m day}^{-1}$  during week 1, and 55.5–71.4  $\mu\text{m day}^{-1}$  during weeks 1–8. The corresponding growth rates for AFDW were  $-2.7$  to  $1.9 \mu\text{g}$  and  $5.1$ – $10.3 \mu\text{g day}^{-1}$ . The spat groups from the March spawning differed significantly ( $P < 0.05$ ) in shell height and AFDW at transfer time and after 8 weeks of nursery growth, whereas no significant differences ( $P \geq 0.05$ ) were found between the spat groups from the May spawning.

The final size and AFDW of the first settlement group (March 1 and May 1) were superior to those of the later settled spat group, except for the May 2b spat, which ended up with a higher shell height than the others (Figs 1 and 2). On the other hand, a higher growth rate was found for the spat of smallest size (March 2a and May 2a) at transfer (Table 1).

The specific growth rate (% day<sup>-1</sup>) was lower during the first week of nursery growth than during the following 7 weeks, with the exception of the May 2a spat, which showed a higher growth rate initially (Table 1). A reduction in AFDW of the spat groups May 1 and May 2b was found in the first week of nursery growth, which resulted in negative growth rates during this period (Table 1).

### Survival

After 1 week of nursery growth, mean survival ranged from 59% to 93% of the number of scallops transferred (Fig. 3). The survival was further reduced for all spat groups after 8 weeks (32–74%), but there was a great variation between trays, expressed by the somewhat high standard deviations (Fig. 3).

Spat from the March spawning showed a trend of reduced survival related to size at transfer (March 2a) and time held in the hatchery (March 2b) before transfer to nursery (Fig. 3). However, survival was, not significantly different after 1 week but, after 8 weeks of nursery growth, the March 1 and March 2b groups showed significant differences. The same trend was not shown for the spat that originated from the May spawning.

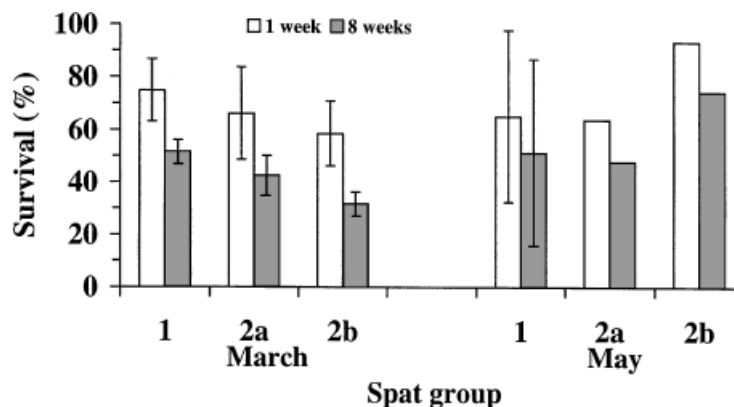
### Chemical composition

When added up, the spat content ( $\mu\text{g mg}^{-1}$  AFDW) of lipid, protein and carbohydrate accounted for  $104.3 \pm 10.0\%$  (SD) of total AFDW. Generally, the AW increased with time and the organic matter (i.e. AFDW) decreased. AFDW averaged  $10.3 \pm 1.1\%$  (SD)

of total dry weight (DW) at transfer time from the hatchery and  $8.7 \pm 1.1\%$  and  $7.7 \pm 0.8\%$  after 1 and 8 weeks of nursery growth respectively. The correlation between shell height growth during the 8 weeks and AW at transfer time was significant but negative ( $r = -0.91$ ,  $P < 0.001$ ).

Protein was the major chemical component varying between 650 and 1050  $\mu\text{g mg}^{-1}$  AFDW (Table 2). The March 2b spat kept a stable protein level around 855  $\mu\text{g mg}^{-1}$  AFDW throughout the experiment, whereas the other groups built up an additional 100–200  $\mu\text{g mg}^{-1}$  AFDW during the nursery growth period. The increase in protein with time was significant for the March 1 and March 2a spat. Considering all spat groups, the protein level was insignificantly different after 8 weeks in the nursery. Within the March spawning, the March 1 and March 2b spat groups contained significantly more protein  $\text{mg}^{-1}$  AFDW at transfer time than the March 2a spat but, after 8 weeks in the nursery, both spat groups from settlement group 2 held less protein than the March 1 spat (Table 2). Such differences were not shown within the May spawning. The shell height growth in the nursery was negatively correlated with the spat protein content at transfer time ( $r = -0.79$ ,  $P = 0.007$ ).

A significant reduction in total lipid content was found for all spat groups after 8 weeks of growth in the nursery (Table 2). The drop in lipid from  $\approx 200$ –300 to 110–150  $\mu\text{g mg}^{-1}$  AFDW was already significant after 1 week in the nursery for the March spat groups. Initially, the March spat held more lipids than the May spat but, after 8 weeks of nursery growth, the amount related to organic matter was evened out (Table 2). There was no significant difference in



**Figure 3** Survival of scallop spat in land-based nursery. Mean survival of three trays ( $n = 3$ ) after 1 and 8 weeks is shown as a percentage of the number of scallops transferred from the hatchery. Vertical bars show standard deviation (SD). Where SD is missing,  $n = 1$ .

**Table 2** Chemical content of scallop spat at transfer time from hatchery (0) and after 8 weeks growth in the nursery (8)

Parameter		Spat group					
		March 1	March 2a	March 2b	May 1	May 2a	May 2b
Protein	0	813 (±52)	672 (±42)	858 (±23)	745 (±32)	656	831
	8	1013 (±45)	902 (±15)	851	865 (±144)	778	1053
Lipid	0	244 (±43)	254 (±49)	182 (±15)	179	191	162
	8	112 (±4)	122 (±39)	122	121 (±12)	114	135
Carbohydrate	0	31 (±8)	28 (±4)	22 (±4)	28 (±3)	22	24
	8	28 (±1)	26 (±4)	31	21 (±8)	NA	31

Mean values of three sieves or trays ( $n = 3$ ) ± standard deviation (SD) of total protein, lipid and carbohydrate ( $\mu\text{g mg}^{-1}$  AFDW) are shown. Where SD is missing,  $n = 1$ . NA, data not available.

total lipid content between or within the March and May spat groups at transfer time or after 8 weeks.

Quantitatively, the carbohydrates contributed the least to the chemical composition ranging from 16 to 40  $\mu\text{g mg}^{-1}$  AFDW with an average of 26  $\mu\text{g mg}^{-1}$  AFDW (Table 2). The content did not change significantly with time for any of the spat groups, and there were no significant differences between the spat groups at either date.

### Lipid classes

The neutral fraction of the lipids in AFDW was high in the hatchery and decreased substantially after growth in the nursery (Table 3). The neutral part counted for  $\approx 40\%$  of the total lipids at transfer time and 20% or less after 8 weeks of nursery growth. Correspondingly, the polar fraction increased to about 80% of the total lipids (Table 3). The percentage neutral and polar lipids of the total showed no significant differences between spat groups at transfer time or after 8 weeks.

Before transfer from the hatchery, the major neutral lipid class in the scallop spat was triacylglycerol (TAG), which accounted for 50–69% of the neutral lipids. After 8 weeks in the nursery, the TAG fraction accounted for 13–20%, which corresponded to a reduction to about one-tenth of the amount ( $\mu\text{g mg}^{-1}$  AFDW) found before leaving the hatchery conditions

(Table 3). Sterol ester (SE) also decreased considerably during the nursery growth period. After 8 weeks, the SE content ( $\mu\text{g mg}^{-1}$  AFDW) was approximately equal to the TAG content. SE was the only neutral lipid class analysed that showed significant differences within the spawning groups. The March 2b and May 2b spat groups held a lower SE content at transfer time than the 1 and 2a spat groups and, after 8 weeks in the nursery, a higher level was found in the March 2a spat compared with the other March groups. Sterol (ST) was the largest neutral fraction at the end of the nursery growth period, and the only part not changing significantly after transfer to the new environment (Table 3). The relative ST content increased from 14–21% to 55–68%. Survival after 8 weeks correlated significantly with the amount of sterol (ST) in the scallop spat at transfer from the hatchery ( $r = 0.73$ ,  $P = 0.017$ ).

Considering all spat groups, the total quantity of polar lipids ( $\mu\text{g mg}^{-1}$  AFDW) did not change significantly with time. After 8 weeks of nursery growth, the phosphatidylethanolamine (PE) component had increased, whereas the glycolipid and unknown polar lipids (other PL) decreased (Table 3). The different polar lipid classes changed significantly in amount from transfer time to after 8 weeks in the nursery, except for the PC (phosphatidylcholine) and other PL within the May group. Within the March group, a significantly higher PC level was found at transfer time in the March 1 spat than in the March 2b spat.

**Table 3** Lipid classes ( $\mu\text{g mg}^{-1}$  AFDW) of scallop spat at transfer time from hatchery (0) and after 8 weeks of growth in the nursery (8)

Lipid	March spat group		May spat group	
	0 (n = 9)	8 (n = 7)	0 (n = 5)	8 (n = 5)
SE	10.9±3.8	3.0±0.5	6.6±1.4	2.4±0.6
TAG	25.3±4.3	2.6±0.3	20.0±11.1	1.9±0.3
ST	6.9±0.6	7.8±0.8	6.1±2.3	8.8±1.6
% NL	39.9±2.7	19.6±1.3	38.5±4.8	16.9±1.2
PE	18.6±1.9	25.9±2.8	15.7±4.8	32.7±5.3
PC	21.3±2.6	17.7±2.1	19.2±4.7	20.1±3.0
Other PL	24.3±3.0	11.5±0.9	15.1±3.4	11.2±1.0
% PL	60.1±2.7	80.4±1.3	61.5±4.8	83.1±1.2

SE, sterol ester; TAG, triacylglycerol; ST, sterol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; other PL, glycolipid and unknown polar lipids. Total neutral (NL) and polar lipids (PL) are shown as percentage of total lipid content. Data shown are means  $\pm$  SD.

Relatively, the PE lipid class increased from 26–36% to 45–52% of total polar lipids, whereas the other PL fell from 26–39% to 16–22%. The PC content contributed to 30–40% of total polar lipids throughout the experimental period. AFDW growth was positively and significant correlated with initial content of other PL ( $r = 0.71$ ,  $P = 0.020$ ) during the first week of nursery growth.

## Discussion

Growth was shown to be lowest and mortality highest during the first week after transfer of spat from the hatchery to the nursery. During 8 weeks of nursery growth, the spat originating from larvae of the first settlement group (March 1 and May 1) attained a larger shell height and AFDW than spat from larvae that settled 3 days later (March 2a and May 2a). Nursery growth was not improved by prolonging the growth time in the hatchery, as spat from settlement group 2b (March 2b and May 2b) showed inferior growth to the spat of the earliest settlement group. The survival of spat originating from the March spawning was found to be lower for the later settlement group, whereas survival of the spat groups obtained from the May spawning was comparable. The final survival of 32–74% was lower than 93–99%, which could be expected according to emersion trials with *P. maximum* of the same size (Christophersen 2000). The spat in these experiments were replaced into the hatchery water conditions after simulated transport with emersion period as the only deviant. Therefore, the change in environment may be a major cause of mortality in our study.

The lipid class composition stayed equivalent between spat groups from the same spawning, probably because of cultivation in the same environment. Juvenile mussels, originating from different habitats and inhabiting different lipid class compositions, have also been shown to reach comparable levels after being raised in the same environment for 1–2 months (Freites, Fernández-Reiriz & Labarta 2002). No clear evidence was found showing that the nutritional status of the spat when transferred from the hatchery could be associated with subsequent growth and survival in the nursery. The total lipid content was greatly reduced during the nursery growth period, but the final level agreed with the lipid percentage found in other scallop spat *Argopecten purpuratus* and *Crassadoma gigantea* grown in the sea (Martínez *et al.* 1992; Whyte *et al.* 1992). Our results showed that the protein contributed most to the energy of the spat, total lipid the second most and carbohydrate the least, as shown for rock scallop *C. gigantea* spat (Whyte *et al.* 1992). In Chilean scallop *A. purpuratus* spat, the carbohydrate content is, on the contrary, reported to be higher than the lipid (Martínez *et al.* 1992; Fariás *et al.* 1998). The difference might be species specific, diet or locality dependent. In the study by Martínez *et al.* (1992), *A. purpuratus* spat transferred to the sea reduced their content of lipids and carbohydrates compared with spat kept in the hatchery, but had better growth. Except for the sterol content that showed a significant positive correlation with survival in the nursery, the initial chemical content seemed, in the present study, to be of minor importance to subsequent growth and survival. The correlation of protein and ash content with

shell growth is related to the general increase in size and is therefore of no importance to survival. On the other hand, the great variability in survival implies a great production potential by optimizing the growth conditions.

The shell growth rates during the nursery growth period in the present work are within the same range as those found in studies of *P. maximus* spat (Andersen & Naas 1993; Laing & Psimopoulos 1998; Robert & Gérard 1999) and other scallop juveniles (Heasman, O'Connor & Frazer 1996; Lu & Blake 1996) of the same size. The shell growth obtained was, however, lower ( $55\text{--}71\ \mu\text{m day}^{-1}$ ) than growth rates found for *P. maximus* spat of equal size transferred to a sea-based nursery ( $70\text{--}125\ \mu\text{m day}^{-1}$ ) at the same time of the year (Christophersen & Magnesen 2001). Similarly, growth of 23–30 mm juvenile *P. maximus* was shown to be better at sea locations compared with suspended cultivation in a fertilized, shallow sea-water pond (Andersen & Naas 1993). As in our study, it was shown that pearl oyster *Pinctada margaritifera* spat transferred to an ocean-based nursery early after settlement performed better in shell height growth than spat transferred after being kept for a longer time in the hatchery (Pit & Southgate 2000). The environmental conditions in the nursery will be critical, and Pit & Southgate (2000) suggested that their results reflect superior nutritional conditions in the sea compared with the hatchery. Martínez *et al.* (1992) also concluded that the natural environment held a better balanced diet than could be offered in the hatchery.

Other factors than food availability, biotic and abiotic, may also have affected nursery growth and survival (Chauvaud, Thouzeau & Paulet 1998; Grecian, Parsons, Dabinett & Couturier 2000). Temperature and salinity are the two main environmental factors affecting the performance of early juvenile *P. maximus*, but the salinity of 30 and temperature of  $16\ ^\circ\text{C}$  in the nursery most probably provided good growth conditions (Strand, Solberg, Andersen & Magnesen 1993; Laing 2000, 2002; Christophersen & Strand 2003). The shell height growth for the two spat groups in the present study is relatively similar, but the AFDW growth tends to be higher in the May spawning group than in the March group. Similar shell growth rate for the two spat groups was expected as the temperature and salinity were kept stable during the entire experimental period. Changes in the food quality with time could explain the growth differences in AFDW, as shell growth is suggested to reflect metabolism and not somatic

tissue production (Lewis & Cerrato 1997). The nursery growth system and equipment used during our study differed from suspended culture in the water column in terms of filtering the incoming water and flow characteristics. This may have affected growth indirectly by reducing food availability compared with sea conditions (Cahalan, Siddall & Luckenbach 1989; Brake & Parsons 1998; Grecian *et al.* 2000).

The higher production of organic matter (AFDW) in the spat transferred to the nursery in August instead of in June could be season related. The food supplied to the nursery was dependent on natural primary production, and the composition and amount was likely to change during the experimental period (Strand, Solberg & Magnesen 1996). Compared with results from feeding trials, our growth rates were comparable with those obtained for small scallop spat fed single-species algae diets (Lu & Blake 1996; Laing & Psimopoulos 1998). The best growth was found when the scallops were fed a mixed algae diet. The growth rates we obtained also coincide with the results of *P. maximus* spat grown at  $16\ ^\circ\text{C}$  and fed a very low ration compared with optimum conditions (Laing 2000). These results indicate suboptimal food conditions in the poll water during our study. Parrish *et al.* (1995) suggested a relation between lipid content in seston of natural waters and growth of scallop *Placopecten magellanicus*. They found growth in the sea to be seasonal, and that it could be linked to the presence of 22:6(n-3) fatty acid supplied by cryptophytes (flagellates) rather than total lipids. An experimental study with spat of the same species showed a relation between diet and scallop lipid classes and growth (Parrish *et al.* 1999). By adding the diatom *Chaetoceros muelleri* to the flagellate *Isochrysis galbana* food, spat growth was initiated and the proportion of phospholipids increased, as they are essential components of membranes.

Substantial reductions in neutral lipid, more specifically the TAG content, further imply suboptimal feeding conditions in the nursery compared with the hatchery. The relative amount of TAG to total lipids was within the range found in *P. magellanicus* spat fed different diets (Parrish *et al.* 1999), whereas starvation is shown to result in total depletion of TAG in juvenile oysters and clams (Caers, Coutteau & Sorgeloos 2000). TAG represents energy storage reserves and was likely to be expended during the handling and transport stress and adaptation to the new environment. The TAG content of  $2\text{--}3\ \mu\text{g mg}^{-1}$  AFDW found after 8 weeks of nursery growth seems, however, not to have been critically low, as growth



increase occurred for all spat groups during the experimental period.

Sterol is another important neutral lipid class expected to increase with membrane anabolism along with polar lipids. The levels of these lipid classes increased with spat growth in our study, as the quantity per  $\mu\text{g}$  AFDW showed no significant differences with time, while their relative contribution did. The correlation of sterol content with weight supports data reported for fish, bivalve and crustacean larvae (Fraser 1989). Based on lipid class composition, Fraser (1989) suggested using the TAG:sterol ratio as a condition index as an alternative to expressing the TAG content relative to size or dry weight. Likewise, the sterol conservation of polar compared with neutral lipids has been shown for scallop *P. magellanicus* (Parrish *et al.* 1999) and oyster and clam (Caers *et al.* 2000) spat. Sterols are found to account for 10–19% of total lipids in *P. magellanicus* spat (Parrish *et al.* 1999). Our data on *P. maximus* spat were similar and in the range 6–12%. Napolitano, Ackman & Silva-Serra (1993) found that a major change in diet is necessary to alter the anatomical distribution of sterols in adult *P. magellanicus*. A constant level of cholesterol was maintained in the female *P. maximus* gonad regardless of the diet and was suggested to be important for later use of larvae (Soudant, Marty, Moal, Robert, Quéré, Le Coz & Samain 1996). Cholesterol has also shown indications of playing an important role in Pacific oyster spat metabolism (Knauer, Barrett, Volkman & Southgate 1999). The significant positive correlation with initial sterol content and survival found for spat in our work also indicate a great importance of the sterol lipid fraction.

A clear conclusion, that spat originating from slow-growing larvae should be omitted from production, may not be drawn from the findings in our experiment. Assuming continuation of the growth rates found in the present work, 15-mm spat could be attained after 2 months of further growth for spat originating from both fast- and slow-growing larvae. Thus, the indications are that growth rate in the larval phase is unrelated to the spat stage growth, as shown for clams, oysters and mussels (Newkirk & Haley 1982; Strömberg & Nielsen 1989; Hilbish, Winn & Rawson 1993; Deming & Russell 1999). For the same reason, spat from the second settlement group might, during nursery growth, catch up with spat from the first settlement group when transferred from the hatchery at the same age. Hence, both spat groups should be included in production. For rock oysters *Saccostrea commercialis*, it was found that growth

rate was not affected by initial size class, implying the initial differences in size resulted from temporary environmental stunting (Mason, Reid & Nell 1998). Deming & Russell (1999) even suggested elimination of slow-growing larvae from production to be counterproductive, because they found an inverse growth relationship between larvae and post-settlement spat of hard clam. It seems, however, of little advantage to keep the latest settled scallop spat for a prolonged period in the hatchery to reach a bigger size before transfer.

Based on the large variation in survival shown in the present study, it is also questionable whether spat originating from the slower growing larvae should be eliminated from production. Spat from the March spawning tended towards reduced survival for the late settlement group, whereas spat from the May spawning showed relatively high survival of spat from both larval groups. Consequently, the results justify spat groups of different settlement age being included in production, although it remains to be seen how the scallops will perform at later stages in the sea until market size is attained.

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