ELSEVIER

Aquaculture 166 (1998) 37–47

Aquaculture

A biochemical study of the larval and postlarval stages of the Chilean scallop *Argopecten purpuratus*

A. Farías, I. Uriarte [1], J.C. Castilla

Estación Costera de Investigaciones Marinas, P. Universidad Católica de Chile, Las Cruces, Chile

Accepted 16 December 1997

Abstract

Eggs, larvae and postlarvae of northern scallop, *Argopecten purpuratus*, were collected from the experimental hatchery of the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile at Las Cruces to compare their protein, lipid and carbohydrate composition. In the egg protein, lipid and carbohydrate accounted for 38.2, 34.1 and 1.5% ash-free dry weight (AFDW), respectively. Protein in the larvae increased from 43.9 to 55.9% AFDW throughout larval development, and after metamorphosis values varied between 39.8 and 62.1% AFDW, with highest values in 22 day-old postlarvae. Lipids were constant at 20% AFDW during larval development, decreasing to 7.4% AFDW in the premetamorphosis period and increasing again to a constant value of 15% AFDW during the 22 days after metamorphosis, then decreasing to 2.5% AFDW. Carbohydrate contents were lower than lipid and protein in all larval stages, varying between 5.1 and 16.5% AFDW; during the postmetamorphosis period, carbohydrate content varied between 6.6 and 14.4%, but they were higher than lipid by day 60 after metamorphosis. The premetamorphosis period was characterized by a considerable decrease in lipid accompanied by a small decrease in protein and carbohydrate. The planting-out stage (commercial seed) was characterized by a high content of protein and a reduced content of lipid in relation to carbohydrate. This work provides basic information on the role played by the balance of energy reserves in the metabolism of scallop larvae.

Keywords: Argopecten purpuratus; Scallop hatchery; Energy reserves; Biochemical composition

[1] Facultad de Oceanografía y Pesquerías, Universidad Austral de Chile. P.O. Box 1327, Puerto Montt, Chile. Fax: +56-65-255-583; e-mail: iuriarte@uach.cl.

0044-8486/98/$19.00
PII S0044-8486(98)00204-X

1. Introduction

Since 1982, the suspended culture of the Chilean scallop in Chile has greatly increased, with an annual production of 8264 tonnes in 1995 and 9788 tonnes in 1996 accounting for almost 100% of the total Chilean scallop production in Chile (Servicio Nacional de Pesca, SERNAP, 1996). Currently more than 70% of the scallop production comes from natural seed settlement at the beginning of summer. Occasionally, scallop recruitment is boosted 60 or more times that of 'normal' years by the El Niño phenomenon (Wolff, 1987; Castilla and Camus, 1992). A constant output of spat through managed hatcheries is necessary to ensure further expansion of the scallop culture in the country.

Hatchery research on the Chilean scallop has been greatly stimulated by the growing economic importance of the species. At present most of the technical problems of hatchery production of Chilean scallop have been solved, although, few studies have been published (Uriarte and Farías, 1995; Uriarte et al., 1996).

Martinez (1991) reported changes in biochemical composition of muscle, gonad, and digestive of the Chilean scallop at different ages and seasons. As pointed out by many authors, the shell growth and biochemical composition of larvae give clear indications about environmental conditions, both in the laboratory and in the field; changes in biochemical composition reflect changes in the quality of the environment (Gabbott and Holland, 1973; Mann and Gallager, 1985; Gallager et al., 1986; His and Maurer, 1988; Napolitano et al., 1988; Ferreiro et al., 1990). Furthermore, study of the biochemical composition of larvae and spat is an important tool to evaluate the energy metabolism and the nutritional condition of hatchery-reared bivalves.

2. Materials and methods

During the summer of 1991–1992, broodstock of *Argopecten purpuratus* were transferred from the long-lines of the Fishery Pesquera San José in Tongoy (IV Region, 30°15′S, 71°35′W) to the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile at Las Cruces (33°15′S, 71°38′W) to perform an experimental hatchery study. The larvae and spat were fed and reared following Castilla et al. (personal communication) and were collected on nylon mesh. Since no published data on the biochemical composition of *A. purpuratus* larvae and spat exist, our aim was to study its changes through larval and postlarval development to obtain a baseline to work on future nutrition experiments. The broodstock consisted of 50 adults between 8 and 10 cm height acclimatized to hatchery conditions. They were held for two days in filtered, UV-sterilized seawater without food, then stimulated to spawn using temperature shock and serotonin injections in the adductor muscle Castilla et al. (personal communication).

2.1. Larval culture

Batches of scallop spat were obtained between November 1991 and March 1992. Larvae and postlarvae were reared in 500 l propylene tanks at densities of 3.5 larvae

ml^{-1} and 1 postlarvae ml^{-1}. Seawater, filtered to 1 μm, at 20 ± 1°C and salinity 35 g l^{-1}, was supplied to the culture systems and changed every other day. Food was added to each tank at concentrations varying between 30 cells μl^{-1} and 120 cells μl^{-1} (depending on the size of the larvae or postlarvae) delivered in two daily doses. Four species of microalgae were used: *Isochrysis aff. galbana*, *Pavlova lutheri*, *Chaetoceros calcitrans* and *Thalassiosira minima*, in a mixture 1:1:1:1 in cell concentration, considered optimal for *A. purpuratus* (Castilla et al., personal communication). Algal cells were counted with a hemocytometer prior to feeding.

2.2. Biochemical study

Samples of eggs, larvae and microalgae to be used for biochemical analysis were obtained by filtration onto ashed and weighed Whatman GF/C filters. Postlarvae and spat were collected directly from the settlement substrate, when the water was changed. All samples were washed with 5% ammonium formate, transferred to labelled flasks, frozen at −20°C, freeze-dried and stored at −20°C.

Samples of 30 ml of the concentrated microalgae mixture were filtered through 2.5 cm Whatman GF/C filters. The process was the same for the larvae sampled for chemical composition. Dry weight was determined by differences between dried filters before and after samplings, with the exception of the postlarvae where dry weight was determined for a known number of individuals of the same shell height. Ash content was determined after combustion at 500°C for 12 h after drying samples at 100°C for 24 h.

Lipids were extracted following Bligh and Dyer (1959), with the modifications of Utting (1985). Total lipids were determined with phosphovanillin using the method of Barnes and Blackstock (1973). Protein was assayed as described by Lowry et al. (1951) after hydrolysis with 0.5 NaOH for 24 h at room temperature. Total carbohydrates were quantified as glucose by the phenol sulfuric acid method as modified by Hellebust and Craigie (1978).

2.3. Statistical analysis

Statistical analysis of the data was carried out through Analysis of Variance and the Newman–Keuls test. The values for percentages of AFDW were converted by the arcsine transformation (Sokal and Rohlf, 1981). Since the main objective was to test for differences through scallop development, age as a variable was entered as total age in days after spawning, both for larvae and postlarvae. Total dry weights and the size of eggs and larvae analysed during the summer of 1991–1992 enabled the calculation of the regression equations to obtain dry weight from the shell height. These regressions permitted an estimation of the quantity of individuals per sample to calculate the biochemical contents per individual. Values of ash-free dry weight (AFDW) enabled calculation of the linear regression equations to obtain AFDW from the dry weights of larvae and spat. From these regressions, it was possible to estimate the biochemical contents as percentage of AFDW. All data are presented as means $\pm$ s.e.

3. Results

Larvae of *A. purpuratus* increased in dry weight from 0.015 to 0.980 μg during the development period (Fig. 1A), which normally lasts 18 days from fertilization to metamorphosis at 20°C. The mean diameter of the eggs was 66.8 μm $\pm$ 0.3 ($n = 115$). Veligers of height of 81.0 μm $\pm$ 0.5 ($n = 91$) were observed 48 h after hatching. The first appearance of pediveliger larvae, at a mean height of 211.2 μm $\pm$ 0.6 ($n = 105$), occurred 12 days after hatching.

At the postmetamorphosis stage, the newly metamorphosed postlarvae had a mean height of 388.4 μm $\pm$ 15.0 ($n = 131$). During the 15–60 days after metamorphosis, juveniles held under laboratory condition increased in dry weight from 0.015 to 105.53 mg with increments in mean height from 0.56 to 10.04 mm, respectively (Fig. 1B).

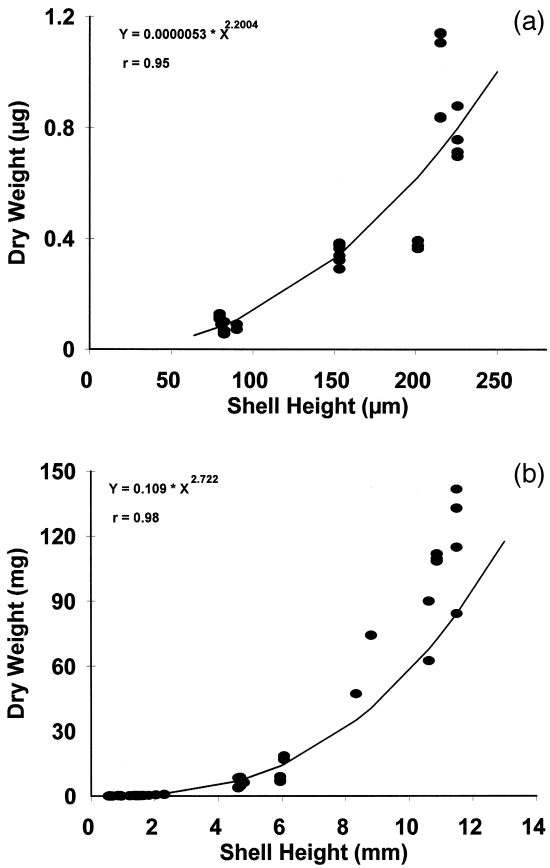

Fig. 1. *A. purpuratus*. Allometric relationship between shell height and dry weight: (a) from eggs to 16-day-old pediveliger larvae, and (b) from 3-day-old metamorphosed larvae (plantigrade) to 57-day-old spat (planting-out juvenile). The curves were fit by multiplicative regression, the real points are showed, r indicates the correlation coefficient with the model.

During the larval period, AFDW increased from 0.04 μg egg^{-1} to 0.45 μg larva^{-1}. Ash was not detected in the eggs and accounted for less than 46% of total dry weight in the early trochophore stages, increasing to about 69% at the pediveliger stage (Fig. 2A).

After metamorphosis mean AFDW increased considerably, from 4.04 μg spat^{-1} to 10.42 mg spat^{-1}. Ash increased from 75% of total dry weight at the first postlarva (Fig. 2B) to 92% at the commercial seed of 10 mm height, mainly due to the effect of increase in the shell thickness of the postlarva.

The protein content increased during larval development from 5.9 ± 0.2 ng protein egg^{-1} in eggs to 11.3 ± 0.8 ng protein larva^{-1} in newly hatched larvae, and to 99.1 ± 8.5 ng larva^{-1} in pediveliger larvae (Table 1). Postlarvae attained a protein content of 0.79 ± 0.01 μg protein spat^{-1} by day 3, increasing to a significant maximum value of 3.06 ± 0.43 mg spat^{-1} by day 60.

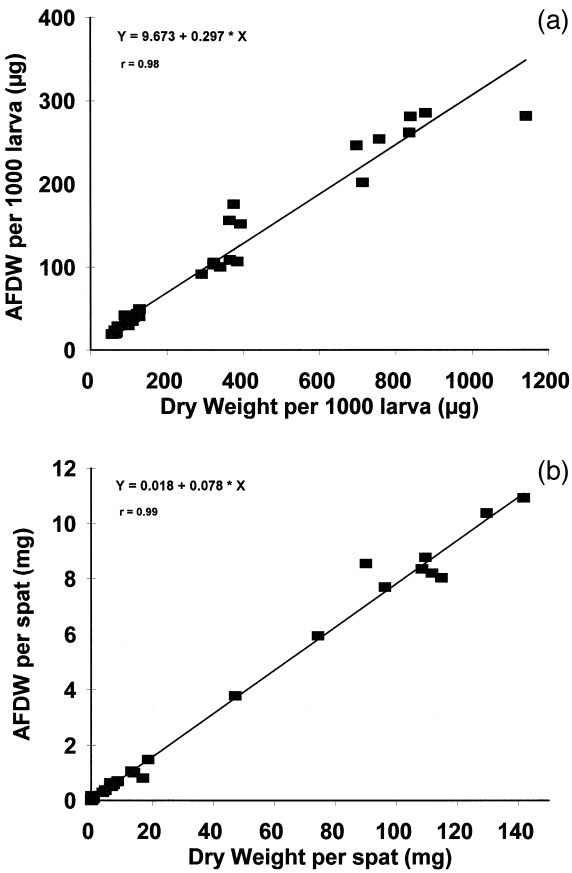

Fig. 2. *A. purpuratus*. Relationship between organic dry weight (AFDW) and total dry weight: (a) throughout larval development. Values were expressed per 1000 individuals, and (b) after metamorphosis from plantigrade to planting-out juvenile. The curves were fit by linear regression, the real points are showed, *r* indicates the correlation coefficient with the model.

Table 1
Total protein in *A. purpuratus* eggs, larvae and postlarvae from different spawnings in summer 1991/1992

Stage	Age (days)	N	mg individual^{-1}	Percentage of AFDW (%)
After spawning				
	0	5	0.0059 (0.0002)	38.21 (1.11)
	1	7	0.0113 (0.0008)	43.89 (2.56)[a]
	3	7	0.0203 (0.0029)	64.99 (8.92)[a]
	12	8	0.1200 (0.0081)	56.22 (4.00)
	16	3	0.0991 (0.0085)	55.94 (4.81)
After metamorphosis				
	3	3	0.787 (0.006)	45.00 (1.70)
	22	3	14.772 (1.694)	39.85 (1.64)
	57	3	3057.981 (431.928)[a]	62.12 (5.89)[a]
			$F_{age} = 102.36$	$F_{age} = 3.30$
			d.f. = 7, 38	d.f. = 7, 38
			$P < 0.0001$	$P = 0.01$

The value in parenthesis is the standard error. AFDW is ash-free dry weight. *N* is the number of samples from 3 batches. [a]Significant group by the Newman–Keuls test.

Protein in the eggs accounted for $38.2 \pm 1.1\%$ of the AFDW, and the percentage of protein increased from 43.9 ± 2.5 to $55.9 \pm 4.8\%$ AFDW throughout larval development (Table 1). After metamorphosis, protein decreased from 45.0 ± 1.7 to $39.8 \pm 1.6\%$ AFDW in the first 22 days, but then there was a significant increase by day 60 on the commercial spat, with $62.1 \pm 5.9\%$ AFDW ($F_{age} = 3.30$; d.f. = 8, 31; $P = 0.01$).

Lipid content increased during larval development from 5.1 ± 0.1 ng lipid egg^{-1} in eggs to 59.3 ± 1.9 ng lipid larva^{-1} in early pediveliger larvae, then decreased to 16.5 ± 1.9 ng larva^{-1} at the end of the pediveliger stage. Postlarvae attained a lipid content of 0.26 ± 0.01 μg lipid spat^{-1} by day 3, increasing significantly to 68.4 ± 0.61 μg spat^{-1} by day 60 (Table 2).

Total lipid in the eggs accounted for $34.1 \pm 0.6\%$ AFDW, falling significantly by the trochophore stage to $19.8 \pm 0.9\%$ AFDW (Table 2). During the first 12 days of larval development, the lipids were constant at 20% AFDW, but the percentage decreased to $7.4 \pm 0.8\%$ AFDW in the premetamorphosis period by day 16. After metamorphosis, lipids were constant in the first 22 days at about 15% AFDW, after which there was a significant decrease to 2.5% AFDW ($F_{age} = 8.90$; d.f. = 8, 33; $P < .0001$)

The carbohydrate content increased during larval development from very low values in the eggs (0.22 ng egg^{-1}) to 27.4 ± 2.31 ng larva^{-1} in pediveliger larvae. Postlarvae attained a carbohydrate content of 0.14 ± 0.002 μg carbohydrate spat^{-1} by day 3, increasing significantly to 322.6 ± 21.8 μg carbohydrate spat^{-1} by day 60 (Table 3).

The total carbohydrate fraction in the eggs was significantly small, accounting for $1.5 \pm 0.04\%$ AFDW. One sampling of eggs, not included in the averages of Tables 1–3, resulted in an unsuccessful larval culture, and the eggs had a high content of carbohydrate and a low content of lipid, 5.12 ± 0.30 and $16.58 \pm 0.59\%$ AFDW, respectively. During the veliger stage, the carbohydrates were constant at about 5% AFDW, increasing significantly to $12.6 \pm 0.87\%$ AFDW in the early pediveliger and decreasing to

Table 2
Total lipid in *A. purpuratus* eggs, larvae and postlarvae from spawnings in summer 1991/1992

Stage	Age (days)	N	mg individual^{-1}	Percentage of AFDW (%)
After spawning				
	0	5	0.0051 (0.0001)	34.07 (0.61)[a]
	1	7	0.0063 (0.0003)	19.83 (0.95)
	3	7	0.0074 (0.0015)	20.22 (3.93)
	12	8	0.0593 (0.0019)[a]	20.69 (0.69)
	16	3	0.0165 (0.0019)	7.44 (0.85)[a]
After metamorphosis				
	3	3	0.259 (0.012)	15.51 (0.73)
	22	3	5.600 (0.208)[a]	15.92 (2.05)
	57	3	68.425 (0.616)[a]	2.50 (0.45)[a]
			$F_{age} = 38{,}320.25$	$F_{age} = 9.91$
			d.f. = 7, 38	d.f. = 7, 38
			$P < 0.0001$	$P < 0.0001$

The value in parenthesis is the standard error. AFDW is ash-free dry weight. *N* is the number of samples from 3 batches. [a]Significant group by the Newman–Keuls test.

$8.5 \pm 0.93\%$ AFDW by the late pediveliger stage (Table 3). During 22 days of postmetamorphosis, carbohydrates increased from 10.30 ± 0.01 to $14.35 \pm 0.23\%$ AFDW, then decreased significantly to $6.56 \pm 0.39\%$ AFDW, but were more abundant than lipids by day 60.

Food was added to the larval culture when newly shelled veligers appeared. The biochemical composition of the microalgal mixture used as food throughout scallop

Table 3
Total carbohydrate in *A. purpuratus* eggs, larvae and postlarvae from different spawnings in the summer 1991/1992

Stage	Age (days)	N	mg individual^{-1}	Percentage of AFDW (%)
After spawning				
	0	5	0.00022 (0.00001)	1.48 (0.04)[a]
	1	7	0.00139 (0.00013)	5.48 (0.59)
	3	7	0.00249 (0.00053)	5.76 (0.84)
	12	8	0.02737 (0.00231)	12.59 (0.87)[a]
	16	3	0.01500 (0.00164)	8.46 (0.93)
After metamorphosis				
	3	3	0.138 (0.002)	10.30 (0.07)
	22	3	5.010 (0.093)	14.36 (0.23)
	57	3	322.602 (21.81)[a]	6.57 (0.39)[a]
			$F_{age} = 854.84$	$F_{age} = 25.93$
			d.f. = 7,36	d.f. = 7, 36
			$P < 0.0001$	$P < 0.0001$

The value in parenthesis is the standard error. AFDW is ash-free dry weight. *N* is the number of samples from 3 batches. [a]Significant group by the Newman–Keuls test.

Table 4
Gross biochemical composition (% of dry weight) of the microalgal mixture composed by *I. aff. galbana*, *P. lutheri*, *C. calcitrans* and *T. minima*, harvested during the exponential growth phase

Biochemical composition	Sample dates			
	24.01.1992	10.02.1992	29.02.1992	11.03.1992
Protein	35.1 (0.5)	25.7 (1.1)	24.3 (0.6)	27.7 (1.3)
Lipid	29.1 (3.1)	28.1 (0.2)	23.7 (1.8)	27.5 (0.9)
Carbohydrate	10.8 (0.4)	4.8 (1.1)	7.0 (0.7)	6.3 (0.1)
Ash	29.3 (4.5)	20.9 (1.4)	28.5 (4.0)	28.9 (0.9)
Dry weight (mg per million of cells)	22.2 (1.5)	20.7 (0.1)	32.2 (2.3)	29.8 (0.9)

The values are the means of 2–4 samples.

development (Table 4) showed a mean dry weight of $26.86 \pm 0.70 \times 10^6$ μg cells^{-1}, with a mean values of 6.57 ± 0.12 μg of protein, 6.38 ± 0.22 μg of lipid and 1.71 ± 0.07 μg of carbohydrate per million cells, while 26.9 ± 1.8% of total dry weight was ash. The biochemical composition of the microalga mixture during the rearing period showed differences for protein and carbohydrate contents, while lipid remained constant (Table 4).

The percentage of dry weight explained by the biochemical content and ash was about 70–79%, both in samples of animals and microalgae.

4. Discussion

Eggs of *A. purpuratus* showed high values for lipid and protein, while carbohydrate was very low. Newly hatched *A. purpuratus* larvae showed an increase in protein and carbohydrate content and a decrease in lipid during the first 48 h of development, after which gradual increases in all components were observed, with both lipid and protein content exceeding carbohydrate content. Total carbohydrate was much lower than protein and lipid levels during the larval phase and at the beginning of postlarval development, but at the stage of planting-out juveniles (commercial seed), carbohydrate exceeded total lipid content.

The high lipid content of eggs of *A. purpuratus* probably represents reserves available for supporting swimming and embryonic development during the first trochophore stage (Utting and Doyou, 1992). The increase in protein and carbohydrate content during the first 48 hours suggests DOM uptake in the scallop embryos (Manahan, 1990). The third hatching of eggs from the ECIM hatchery on January 1991 resulted in a unsuccessful culture, and the samples of eggs did not show the normal pattern with a high lipid content; in this case, carbohydrate was higher than lipid, although the protein content was the same as that of normal eggs. Ripe females of *A. purpuratus* showed a significantly higher content of lipid and protein than ripe males (Martinez, 1991), which suggests that these components are important for the larval stages, probably the high energy reserves in eggs assessed by utilization through embryogenesis in a high morphogenetic activity (Whyte et al., 1987).

Tables 1–3 show that all larval stages have low carbohydrate reserves, and differ mainly in their lipid and protein content. The biochemical composition of scallops in different developmental stages is consistent with that of other bivalve larvae such as mussels and oysters (Ferreiro et al., 1990), characterized by high lipid reserves and low carbohydrate in early larvae and high levels of protein and very low lipid in the pediveliger.

The low levels of protein, lipid and carbohydrate in the pediveliger may be a good indicator that metamorphosis in *A. purpuratus* requires all the energy reserves of the organism, while in *Ostrea edulis* metamorphosis causes only lipid (Holland and Spencer, 1973) or protein depletion (His and Maurer, 1988; Rodriguez et al., 1990). In various invertebrate larvae, lipids have been shown to be the main biochemical component mobilized when an energy imbalance exists. It is possible that lipid may be an important source of energy during periods of stress, and a lipid index may be useful to document the physiological condition of bivalve larvae (Gallager et al., 1986).

Scallops are subtidal organisms showing low tolerance to anoxia (Bricelj and Shumway, 1991). This is related to low levels of glycogen in the adductor muscle, not more than 18 or 25% dry weight for *Chlamys islandica* and *A. irradians*, respectively. In the whole organism, we observed the highest value for carbohydrate after metamorphosis, reaching 14% AFDW. This value is lower than those reported for mussels and oysters, but is consistent with the observations of Bricelj and Shumway (1991) on pectinids.

The high protein content found in the *A. purpuratus* postlarva by day 60 and its significant difference relative to postlarvae by day 22 may be related to a change in the use of energy reserves between early postmetamorphosis stages and juveniles. Comparative analysis between postlarvae of *A. purpuratus* (this work) and juveniles of the same species (Martinez, 1991) has shown the same trend, i.e., a relatively high protein content in storage tissues.

For postlarvae the increase in relative importance of carbohydrate as an energy reserve was marked, exceeding lipid at day 60. This is consistent with the pattern for metamorphosed bivalves described by Holland and Hannant (1974). The increase in carbohydrate after metamorphosis is probably to provide a substrate for energy production during exposure in intertidal environments. It has been shown that the main energy role of lipid (especially triacylglycerols) is to supply energy for bivalve larval growth and metamorphosis (Holland and Spencer, 1973; Napolitano et al., 1992), while after metamorphosis energy for normal growth in juvenile and adult bivalves is supplied by glycogen (Holland and Hannant, 1974).

The low value of dry weight explained by the biochemical content in larva and postlarva of *A. purpuratus* could be produced by an overestimation of biomass caused for losses of inorganic material with the method used (Palmerini and Bianchi, 1994).

On the other hand, the quality of food greatly influences the storing of reserves and the survival of the resultant spat (Whyte et al., 1987, 1989, Ferreiro et al., 1990). Food is an important environmental factor influencing the biochemical composition of bivalve larvae and accounting for their survival and development. It has been shown (Gallager and Mann, 1981, Gallager et al., 1986; Whyte et al., 1987; Ferreiro et al., 1990) that during 20 days of larval development, if the food source is insufficient to support body

and shell growth, scallops show a decrease in their metabolic and digestive processes and resort to their energy reserves. From this point of view, the microalgal mixture used as a diet in our cultures was the best (experimentally tested) for the growth and survival of *A. purpuratus* larvae (Castilla et al., personal communication). The biochemical analysis showed a high caloric content as a result of high lipid levels (46% of organic weight), moderate levels of protein (38% of organic weight) and low levels of carbohydrate (8% of organic weight). Furthermore, a high percentage of ash was observed, probably due to the presence of two diatoms in the diet.

In bivalves, it is possible that storage and utilization of biochemical components in relation to energy metabolism is affected by adverse rearing conditions (Gallager and Mann, 1981). In our work, optimal conditions were used to rear *A. purpuratus* (Castilla et al., personal communication). The objective was to produce healthy larvae with a good balance between storage and utilization of energy, to give base information about the biochemical composition and energetic metabolism of the larvae and postlarvae of Chilean scallop through development of the species under standard rearing conditions. Future work should be focused on the role of nutrition before and after metamorphosis in *A. purpuratus*, looking for a significant role played by carbohydrate, lipid and protein in the balance of nutrients in the production of energy.

Acknowledgements

This work was supported by a grant from the Fishery Pesquera San José. We are greatly indebted to A. Abarca and Q. Medina, who produced the massive cultures of scallop at ECIM, and to M. Bronfman, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, who allowed us to use his biochemistry laboratory facilities. We also acknowledge useful corrections and comments by G. Román (Spain) and R. Thompson (Canadá).

References

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin methods for total lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bricelj, V.M., Shumway, S., 1991. Physiology: energy acquisition and utilization. In: Shumway, S. (Ed.), Scallop Biology and Culture. International Pectinid Workshop 1989, pp. 305–346.

Castilla, J.C., Camus, P.A., 1992. The Humboldt-El Niño scenario: coastal benthic resources and anthropogenic influences, with particular reference to the 1982/1983 enso. S. Afr. J. Mar. Sci. 12, 703–712.

Ferreiro, M.J., Perez-Camacho, A., Labarta, U., Beiras, R., Planas, M., Fernández-Reiriz, M.J., 1990. Changes in the biochemical composition of *Ostrea edulis* larvae fed on different food regimes. Mar. Biol. 106, 395–401.

Gabbott, P.A., Holland, D.L., 1973. Growth and metabolism of *Ostrea edulis* larvae. Nature 241, 475.

Gallager, S.M., Mann, R., 1981. Use of Lipid-Specific Staining technique for assaying condition in Cultured bivalve Larvae. J. Shellf. Res. 1, 69–73.

Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as index of growth and viability in three species of bivalve larvae. Aquaculture 56, 81–103.

Hellebust, J.A., Craigie, J.S., 1978. Physiological and biochemical methods. Handbook of Phycological Methods. Cambridge U.P.

His, E., Maurer, D., 1988. Shell growth and gross biochemical composition of oyster larvae (*Crassosstrea gigas*) in the field. Aquaculture 69, 185–194.

Holland, D.L., Hannant, P.J., 1974. Biochemical changes during growth of the spat of the oyster, *Ostrea edulis* L. J. Mar. Biol. Assoc. U.K. 54, 1007–1016.

Holland, D.L., Spencer, B.E., 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L., during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assoc. U.K. 53, 287–298.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Manahan, D.T., 1990. Adaptations by invertebrate larvae for nutrients acquisition from seawater. Am. Zool. 30, 147–160.

Mann, R., Gallager, S.M., 1985. Physiological and biochemical energetics of larvae of *Teredo navalis* L. and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). J. Exp. Mar. Biol. Ecol. 85, 211–228.

Martinez, G., 1991. Seasonal variation in biochemical composition of three size classes of the Chilean scallop *Argopecten purpuratus* Lamarck, 1819. Veliger 34, 335–343.

Napolitano, G.E., Ratnayake, W.M.N., Ackman, R.G., 1988. Fatty acid components of larval *Ostrea edulis* (L.): importance of triacylglycerols as a fatty acid reserve. Comp. Biochem. Physiol. 90B, 875–883.

Napolitano, G.E., MacDonald, B.A., Thompson, R.J., Ackman, R.G., 1992. Lipid composition of eggs and adductor muscle in giant scallops (*Placopecten magellanicus*) from different habitats. Mar. Biol. 113, 71–76.

Palmerini, P., Bianchi, C.N., 1994. Biomass measurements and weight-to-weight conversion factors: a comparison of methods applied to the mussel *Mytilus galloprovincialis*. Mar. Biol. 120, 273–277.

Rodriguez, J.L., Sedano, F.J., García-Martín, L.O., Perez-Camacho, A., Sánchez, J.L., 1990. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. Mar. Biol. 106, 109–111.

Servicio Nacional de Pesca, 1996. Anuario estadístico de pesca (Fishery Statistical Annuary), SERNAP, Ministerio de Economía, Fomento y Reconstrucción, Santiago-Chile.

Sokal, R.R., Rohlf, F.J., 1981. Biometry. The Principles and Practice of Statistics in Biological Research, 2nd edn. Freeman, New York.

Uriarte, I., Farías, A., 1995. Effect of broodstock origin and postlarval diet on postlarval growth and physiological performance of the Chilean scallop *Argopecten purpuratus*. In: Lavens, P., Jaspers, E., Roelants, I. (Eds.), Larvi'95—Fish and Shellfish Larviculture Symposium, EAS, Special Publication 24, pp. 69–72.

Uriarte, I., Farías, A., Muñoz, C., 1996. Estado actual del cultivo en hatchery y preengorde del ostión del norte, *Argopecten purpuratus* (Lamarck, 1919), en la X región (Current status of hatchery and grow-out culture of the Chilean scallop, *Argopecten purpuratus*, in the South of Chile (Xth Region). Rev. Biol. Mar. (Valparaíso) 31, 81–90.

Utting, S.D., 1985. Influence of nitrogen availability on the biochemical composition of three unicellular marine algae of commercial importance. Aquacult. Eng. 4, 175–190.

Utting, S.D., Doyou, J., 1992. The increased utilization of egg lipid reserves following induction of triploidy in the Manila clam (*Tapes philippinarum*). Aquaculture 103, 17–28.

Whyte, J.N.C., Bourne, N., Hodgson, C.A., 1987. Assessment of biochemical composition and energy reserves in larvae of the scallop *Patinopecten yessoensis*. J. Exp. Mar. Biol. Ecol. 113, 113–124.

Whyte, J.N.C., Bourne, N., Hodgson, C.A., 1989. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture 78, 333–347.

Wolff, M., 1987. Population dynamics of the Peruvian scallop *Argopecten purpuratus* during the El Niño phenomenon of 1983. Can. J. Fish. Aquatic Sci. 44, 1684–1691.