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Reproductive conditioning of Chilean scallop (*Argopecten purpuratus*) and the Pacific oyster (*Crassostrea gigas*): effects of enriched diets

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Abstract

The quality of diet fed to bivalve broodstock during reproductive conditioning directly affects growth and survival of their larval and postlarval progeny. The objective of the present study was to improve the quality of larval production in the Chilean scallop (*Argopecten purpuratus*) and the Pacific oyster (*Crassostrea gigas*) by testing of the effects of different experimentally enriched diets on the conditioning and early development of progeny under laboratory conditions. The diets used included protein-rich microalgae and mixtures of microalgae and lipid emulsions. Quality of D-larvae, pediveligers, and 1-mm postlarvae from the variously fed broodstock was measured to evaluate each diet as indicators of growth and survival of the early life stages. Biochemical analyses were carried out on eggs and larvae to determine the effects of the different diets on these parameters. The main results for the scallops and oysters were that a conditioning diet containing algae rich in protein improved larval growth and survival. This effect did not, however, extend past metamorphosis. Also, the best conditioning diet increased the lipid content of the eggs, as well as the protein content of the D-larvae, although the mechanism for this remains unclear.

Keywords: *Argopecten purpuratus*; *Crassostrea gigas*; Reproduction; Broodstock conditioning; Lipid emulsions; Protein-enriched microalgae; Larval quality

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1. Introduction

Composition of molluscan broodstock diets has an important effect on their reproduction and the quality of eggs, as observed in marine species in general (Robinson, 1992a; Samain et al., 1992). Increase in protein in the microalgal diet of the Chilean scallop *Argopecten purpuratus* decreases the time to reach maturity, increases the fecundity in females, and improves the energy balance of pectinid broodstock (Farías et al., 1997). Conversely, with diets low in protein, broodstock of *A. purpuratus* show a state of malnutrition characterized by a high rate of excretion of endogenous protein to make up for the lack of protein in the diet during gonadal maturation, plus a high respiratory rate probably due to an elevated content of carbohydrate in the protein-deficient diet (Farías and Uriarte, 2001). Villalaz (1994) suggested that the adductor muscle stored protein to support the reproductive activity of the gonad in *A. ventricosus*, which supports the concept of importance of protein in the broodstock diet. Martínez et al. (1992) observed a significant reduction in protein in the gonads of *A. purpuratus* conditioned in a hatchery compared with those maintained at sea, which supports the view that scallops conditioned under hatchery conditions are deficient in protein and, for this reason, require protein enrichment. There are no studies on the effects of protein content in the diets used for reproductive conditioning of the Pacific oyster *Crassostrea gigas*.

Another nutritional factor which affects the quality of spawnings in molluscs is the content of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) (Robinson, 1992b). Utting and Millican (1997) observed that the relation EPA/DHA varies between the eggs of species matured in the natural environment and those conditioned in hatcheries. The pattern of fatty acids in eggs reflects that of the lipid in the broodstock diet; for example, in *Chlamys tehuelcha*, a direct correlation was found between the composition of fatty acids in the dominant phytoplankton and their composition in the gonads (Pollero et al., 1979). Lipids in the eggs of marine molluscs are rich in polyunsaturated $n-3$ fatty acids ($n-3$ HUFA), where this component probably arises from dietary diatoms and dinoflagellates (Pollero et al., 1979; Trider and Castell, 1980; Napolitano et al., 1992; Robinson, 1992b). This observation implies that the developing embryos and emerging larvae have high $n-3$ HUFA requirements which are derived from the broodstock diet.

The objective of this study was to compare the efficiencies of protein enrichment and lipid substitution in microalgae used for broodstock conditioning in *A. purpuratus* and *C. gigas*.

2. Materials and methods

A. purpuratus and *C. gigas* broodstock were conditioned in 20-l tanks supplied with UV-treated seawater filtered to 1 μm using CUNO® cartridge filters in a recirculating system (Hernández, 2001). The temperature was maintained with thermoregulatory equipment at 18 ± 1 °C for the scallops and 24 ± 1.5 °C for the oysters in accord with Lara (1990) and Schäfer (1999), respectively. In both cases, food during conditioning was

administered in two daily rations of 200 cells μl^{-1} day^{-1}. The standard diet utilized was a 1:1 (cells) mixture of *Isochrysis aff. galbana* (clone *T-Iso*) and *Chaetoceros neogracile* cultivated in Walne (1960) medium. Diets with high and low protein levels were prepared using the methods of Uriarte and Farías (1999). Lipid emulsions contained 60% lipids, liposoluble vitamins, emulsifiers, preservatives, antioxidants, and water, where the lipids were concentrates of marine oil with 30% $n-3$ HUFA. The DHA-emulsion had a ratio of DHA/EPA = 4 and EPA emulsion a DHA/EPA = 0.6. Both emulsions were mixed at a 1:1 (w/w) ratio to replace the microalgae. For replacement, it was considered that 1 million cells of the microalgal mixture weighed an average of 49 μg. Table 1 shows the protein content of the different diets.

Once conditioning of the broodstock was achieved, they were induced to spawn, and fertilization and incubation were carried out using methods of Uriarte et al. (2001), conserving the origins of the broodstock and the tanks. After hatching, six replicate tanks of 15 l were stocked with D-larvae per each broodstock treatment for Chilean scallop and four replicate tanks for Pacific oyster. During larval development, the larvae were maintained on the standard diet at 19 °C for *A. purpuratus* and 25 °C for *C. gigas*, monitoring the growth rate and survival to the pediveliger phase. At the end of larval growth, larvae from each tank were induced to metamorphosis and distributed into settlement tanks containing netlon® collectors for *A. purpuratus* and shell and fiber collectors for *C. gigas* with care to conserve and separate the origins of broodstock and tanks. After metamorphosis, the postlarvae were maintained on the standard diet at the same temperature as the larvae cultures. After 70 days of culture of the postlarvae, the percentage of juveniles set in collectors was estimated and considered as a measure of postlarval survival. The growth of these individuals was also recorded.

Conditioning experiments using individuals from the same broodstock group were then carried out as follows. For *A. purpuratus*, an experiment with two diets was carried out. One diet (a) included 100% high-protein *T-Iso* + high-protein *C. neogracile* (100% IH/GH) and another (b) included 70% normal *T-Iso* + normal *C. neogracile* plus 30% lipid emulsions enriched in DHA and EPA (70% I/G + 30% DHA/EPA) obtained according to Martínez et al. (2000). The experimental unit was a 20-l tank with three breeder scallops measuring 83.3 ± 2.4 mm in shell height and 111.8 ± 9.5 g in weight. Each treatment

Table 1
Protein content of the microalgal diets obtained by the Lowry et al. (1951) method, expressed as $\mu g\ mg^{-1}$ dry weight

Diet	*n*	Protein content ($\mu g\ mg^{-1}$)
100% IH/GH	7	92.2 ± 5.8
100% I/G	7	32.8 ± 0.7
100% IL/GL	6	11.3 ± 0.8
70% I/G + 30% EPA/DHA	7	24.5 ± 0.3

100% IH/GH: 100% high-protein *T-Iso* + high-protein *C. neogracile*; 100% I/G = 100% normal *T-Iso* + normal *C. neogracile*; 100% IL/GL = 100% low-protein *T-Iso* + low-protein *C. neogracile*; 70% I/G + 30% DHA/EPA = 70% normal *T-Iso* + normal *C. neogracile* plus 30% lipid emulsions enriched in DHA and EPA.
Mean ± one standard error.

included six replicates in a completely randomized design. At the end of the conditioning period, gonadal indexes (GSI = Gonad dry weight:Body dry weight) were calculated after Uriarte et al. (1966).

Two experiments were carried out with *C. gigas*. In the first experiment, three diets with different protein levels were employed as follows: (a) 100% high-protein *T-Iso* + high-protein *C. neogracile* (100% IH/GH), (b) 100% normal *T-Iso* + normal *C. neogracile* (100% I/G), and (c) 100% low-protein *T-Iso* + low-protein *C. neogracile* (100% IL/GL). In the second experiment, two diets were tested, including (a) 100% IH/GH and (b) 70% I/G + 30% DHA/EPA. The experimental unit was a 20-l tank as above containing four breeding oysters measuring 100.8 ± 15.2 mm in shell height and 79.8 ± 17.4 g in weight. Each treatment had six replicates and completely randomized design. At the end of conditioning period, the condition indexes were calculated on a volumetric basis (ICV = Meat dry weight: [Volume live animal − shell volume]) according to Brown and Hartwick (1998).

Condition indexes, larval growth rates, survival and settlement, and biochemical composition were compared between diets using analysis of variance. Percentage data were normalised by arcsine transformation (Sokal and Rohlf, 1981).

3. Results

3.1. A. purpuratus

Similar results were obtained between tests using 100% IH/GH and 70% I/G + 30% DHA/EPA, with 75% of the scallops releasing eggs and 82% releasing sperm. Broodstock fed on each diet matured within 4 weeks (Table 2). The final gonadosomatic index was similar between broodstock which received the high-protein diet and those which received the normal protein plus lipids (Table 2, $P = 0.07$).

Growth rates of larval *A. purpuratus* were significantly affected by the conditioning process applied to the broodstock ($F = 31.76$, $df = 1,10$; $P = 0.0002$). The best growth was

Table 2
Effect of microalgal diets on gonadosomatic index, larval, and postlarval performance of *A. purpuratus*

Diet during reproductive conditioning	GSI	Larval growth (μm day^{-1})	Larval survival (%)	Postlarval growth (μm day^{-1})	Postlarval survival (%)
Onset of experiment	0.04 ± 0.01^{a}, $n = 4$				
100% IH/GH	0.07 ± 0.01^{b}, $n = 6$	5.7 ± 0.2^{a}, $n = 6$	25.6 ± 2.1, $n = 6$	24.9 ± 0.9	2.7 ± 1.0
70% I/G + 30% EPA/DHA	0.05 ± 0.01^{b}, $n = 6$	4.7 ± 0.1^{b}, $n = 6$	23.9 ± 2.2, $n = 6$	22.6 ± 1.3	3.0 ± 0.8

Values in the same column with different superscript letters are significantly different ($P < 0.05$). 100% IH/GH: 100% high-protein *T-Iso* + high-protein *C. neogracile*; 70% I/G + 30% DHA/EPA = 70% normal *T-Iso* + normal *C. neogracile* plus 30% lipid emulsions enriched in DHA and EPA.
Mean ± one standard error of *n* replicates.

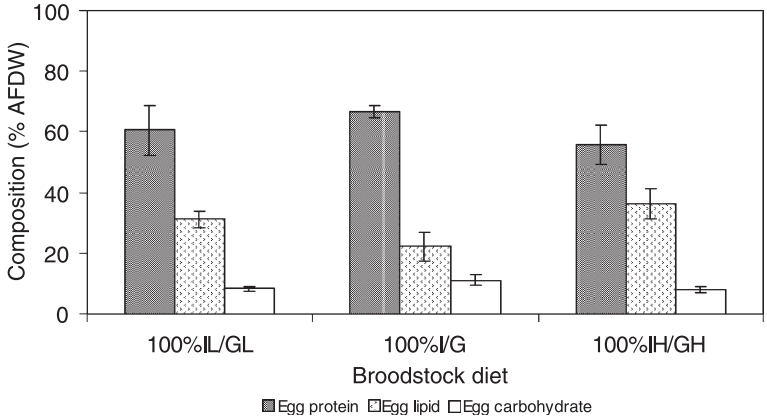

Fig. 1. Biochemical composition of eggs from broodstock of *C. gigas* conditioned with different diets. Mean ± one standard error. AFDW is ash-free dry weight. 100% IH/GH: 100% high-protein *T-Iso* + high-protein *C. neogracile*; 100% I/G = 100% normal *T-Iso* + normal *C. neogracile*; 100% IL/GL = 100% low-protein *T-Iso* + low-protein *C. neogracile*.

recorded for larvae originating from broodstock which received the 100% IH/GH diet (Table 2). Larval survival, postlarval survival, and postlarval growth to 1 mm were not affected by the broodstock dietary conditioning (Table 2).

3.2. C. gigas

In the first experiment with *C. gigas*, egg size was significantly affected by the protein level in the conditioning diets ($F = 9.07$; $df = 2,39$; $P = 0.0006$). Females fed with 100%

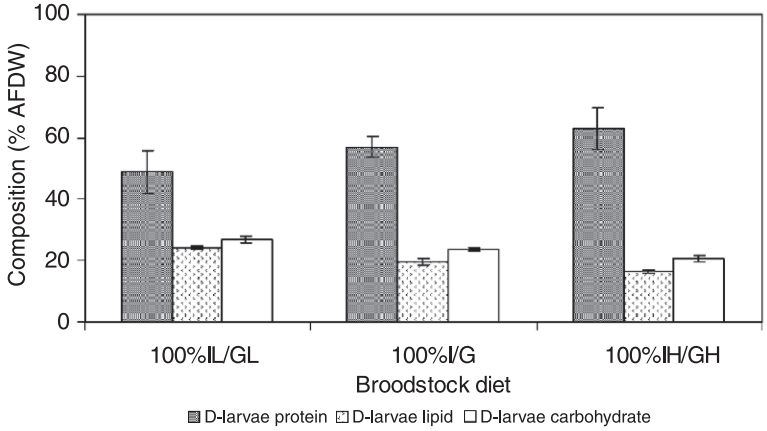

Fig. 2. Biochemical composition of D-larvae of *C. gigas* obtained from broodstock conditioned with different diets. Mean ± one standard error. AFDW is ash-free dry weight. 100% IH/GH: 100% high-protein *T-Iso* + high-protein *C. neogracile*; 100% I/G = 100% normal *T-Iso* + normal *C. neogracile*; 100% IL/GL = 100% low-protein *T-Iso* + low-protein *C. neogracile*.

Table 3
Effect of microalgal diets on conditioning index, larval, and postlarval performance of *C. gigas*

Diet on the reproductive conditioning	ICV	Larval growth (μm day^{-1})	Larval survival (%)	Postlarval growth (μm day^{-1})	Postlarval survival (%)
Onset of experiment	78.6 ± 11.4^{a}, $n=4$				
100% IH/GH	108.0 ± 12.7^{b}, $n=6$	9.6 ± 0.1, $n=4$	55.8 ± 2.9^{a}, $n=4$	54.0 ± 3.3	1.4 ± 0.2
70% I/G + 30% EPA/DHA	110.6 ± 6.2^{b}, $n=6$	8.6 ± 0.3, $n=4$	33.7 ± 6.1^{b}, $n=4$	52.5 ± 0.9	0.9 ± 0.3

Values in the same column with different superscript letters are significantly different ($P<0.05$). 100% IH/GH: 100% high-protein *T-Iso* + high-protein *C. neogracile*; 70% I/G + 30% DHA/EPA = 70% normal *T-Iso* + normal *C. neogracile* plus 30% lipid emulsions enriched in DHA and EPA.
Mean ± one standard error of *n* replicates.

IH/GH and 100% I/G diets produced larger eggs than the females fed with the 100% IL/GL diet (SNK test, $P<0.05$). There were no differences in the fecundity among the females receiving different protein level dietary treatments. The females each produced an average of $34.6 \pm 4.3 \times 10^{6}$ eggs. The eggs of the females fed with the IH/GH diet had greater lipid content ($F=11.00$; $df=2,8$; $P=0.0099$) than those originating from females fed with the 100% I/G and 100% IL/GL diets (Fig. 1). Hatching rates were also significantly affected by the protein content in the diets fed to the broodstock ($F=46.30$; $df=2,6$; $P=0.0002$), with the best values in the larvae originating from the broodstock conditioned with 100% IH/GH diet ($P<0.05$). These larvae also had higher protein ($F=25.00$; $df=2,8$; $P=0.0012$) and lower carbohydrate content ($F=7.49$; $df=2,6$; $P=0.02$) than those coming from broodstock fed with the 100% IL/GL diets (Fig. 2).

In the second experiment with *C. gigas*, broodstock fed on each diet matured within 4 weeks; no differences were recorded in the final condition index between broodstock fed with the two different diets (Table 3). Survival of the oyster larvae originating from the 100% IH/GH diet was significantly higher ($F=7.56$; $df=1,5$; $P=0.04$). The dietary broodstock conditioning did not, however, affect the larval growth rates, although highest total growth was recorded using the 100% IH/GH diet ($P=0.07$). Similarly, the postlarval survival and postlarval growth to 1 mm were not affected by the broodstock diet (Table 3).

4. Discussion

High-protein level microalgae (100% IH/GH) fed during conditioning was a better diet than 70% I/G + 30% DHA/EPA-lipid emulsions for the *A. purpuratus* based on the improvement in the GSI and larval growth. In *C. gigas*, 100% IH/GH was a better diet than 100% I/G and 100% IL/GL or 70% I/G + 30% DHA/EPA-lipid emulsions based on the significant increases in egg diameter, egg lipid content, hatching rate, and larval survival.

Variability in protein content in the *T-Iso* + *C. neogracile* microalgal mix affected the performance of *A. purpuratus* and *C. gigas* broodstock in different ways and also affected

the larval cultures. Working with the same mixture of microalgae (both enriched, normal, and deficient in proteins), Farías and Uriarte (2001) found that female fecundity of *A. purpuratus* increased when using the high-protein diet, while with low-protein diet, the reproductive conditioning was notably delayed by as much as 2 weeks compared to the normal and high-protein diets. Further, when conditioned with low-protein diets, only sperms were released upon induction of spawning, and that high mortality occurred among the broodstock following induction of spawning (Farías and Uriarte, 2001). The present study showed that for *C. gigas*, the variation from IH/GH to IL/GL, with normal protein levels, failed to affect the conditioning time, GSI, or fecundity, demonstrating the capacity for this species to adapt to the nutritional quality of the phytoplankton. Bayne (2002) had previously observed the high plasticity of *C. gigas* in response to the variation in quantity of plankton food when compared with the Sydney oyster *Saccostrea glomerata*. This plasticity is based on better filtration rates at high concentrations of food and better metabolic efficiency for growth. Our study suggests that the high plasticity of *C. gigas* may also explain why the variations in protein content of the diets did not affect gonadal development or larval quality of this species. The Pacific oyster may be considered a ''buffer species'' with regard to environmental variability in diet, which helps explain its great success in culture in various regions of the world.

Eggs of oysters conditioned with the 100% IH/GH diet contained significantly more lipids than eggs of oysters fed with the 100% I/G (normal protein) and 100% IL/GL (low protein) diets. This fact, plus that of the higher rate of larval hatching on this diet, may indicate that the higher lipid reserve in the eggs of *C. gigas* fed with the IH/HG diet promoted better development of the early larval stages. Although the mechanism for this result is not clear, it remains the principal benefit of raising the protein content of the diet.

There are no effects on maturity indexes such as GSI, fecundity, conditioning time, and percentage spawning in *A. purpuratus* when comparing the effects generated by the 100% IH/GH and 70% I/G + 30% EPA/DHA diets. Martínez et al. (2000) have shown that substitution of 30% HUFA-enriched lipid emulsions in the microalgal diet during the reproductive conditioning of *A. purpuratus* produced greater fecundity and better responses to spawning induction than in broodstock receiving the standard 100% I/G diet. Also, $n-3$ HUFA supplements in diets of this species changed the fatty acid profiles in the fatty acids kryacylglycerols in the eggs without producing variations in their phospholipid content (Caers et al., 1999). Our results also show that when *A. purpuratus* broodstock were fed with 100% IH/GH, there was better larval growth than when they were fed with 70% I/G + 30% EPA/DHA, reflecting the improvement of larval development with an increase in protein to the broodstock.

When comparing the same diets in broodstock of *C. gigas*, no effect was observed either in the maturity indexes or in the larval growth rates. There was, however, improved larval survival in the progeny of broodstock fed with 100% IH/GH than in that of broodstock fed with 70% I/G + 30% EPA/DHA. This also demonstrates better larval development as a result of increasing the protein content of the microalgal diet.

The results with both species indicate that administration of $n-3$ HUFA is not required for reproductive conditioning if high-protein microalgal mixtures are used in the process.

Our results also suggest that the diet given to the broodstock might only affect the progeny during the larval phase without influencing postlarval survival and postlarval

growth. Pernet et al. (2001) suggested that the egg triacylglycerol/cholesterol ratio might be a useful predictor of larval survival. Future studies need to determine if the dietary effects on the broodstock have effects on the entire life of the larvae or solely on the early phase of larval life and if these effects are related to the vitelline reserves.

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