Effect of antibiotic treatment during larval development of the Chilean scallop Argopecten purpuratus

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Abstract

The requirement for antibiotic use in a culture depends principally on the quality of water available and on the use of strict husbandry of the materials closely related with the culture. The purpose of the present study was to determine the dose of chloramphenicol resulting in better survival and growth rates of Chilean scallops between the early larvae and pediveliger stages cultured in closed systems with manual dosing of food two times per day. Two experiments with antibiotic application during larval development of the Chilean scallop (Argopecten purpuratus) were conducted. The experiments were carried out at the early larval stage (86 μm) and at the eyed stage (213 μm). The antibiotic concentration ranged between 0 and 8 mg l⁻¹ chloramphenicol (CHL) per day. The survival and growth rates of the larvae were monitored for 10 days at each stage. In the experiment with eyed larvae, larval settlement and percent metamorphosis were measured. Use of an antibiotic on the early larvae resulted in significantly better growth and survival. Growth rates were 2.3 ± 0.3 and 2.6 ± 0.2% per day when using 2 and 8 mg l⁻¹ CHL per day, respectively, compared with 1.3% ± 0.2 per day for the larvae without antibiotic. Survival was also better with antibiotic treatment reaching 50% compared with 35% without antibiotic. The metamorphosis was highest using 8 mg l⁻¹ CHL day⁻¹, compared with treatment without antibiotic. Between 75 and 79% of the metamorphosed larvae were found settled on the nets in the treatments using 2 and 8 mg l⁻¹, while only 55.5% were settled in the nets in the treatment without antibiotic. The results of the experiments indicate that concentrations of 2 and 8 mg l⁻¹...
CHL demonstrated effective control of larval contamination. Moreover, the condition of the postlarvae was improved by the addition of 8 mg l\(^{-1}\) CHL from eyed larvae to postlarvae. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords**: Chloramphenicol; Larviculture; *Argopecten purpuratus*; Hatchery

1. Introduction

The quality of seawater must be tested before the installation of a hatchery or pilot plant, through an inspection of the chemical and organic composition, to know the presence of bacteria, fungus and protozoa, which are the principal causes of bivalve larval mortality during culture (Prieur et al., 1990). When the sources of contamination in the seawater inflow to larval culture have been discarded, only rest to focus attention on good management of cleaning and sterilization of all the materials that come in contact with the cultures. In this manner, the costs of preventing bacterial contamination of the cultures can be reduced.

There is abundant evidence that bacterial contamination can cause mortality of shellfish larvae (Brown, 1973; Austin et al., 1988; Jeathon et al., 1988; Riquelme et al., 1995). The factors that may increase the risk of contamination that can destroy the cultured larvae include parental bacterial transfer (Riquelme et al., 1994) and culture conditions such as elevated temperature, quality and sterile condition of the diet, concentration of inorganic nutrients (introduced with the diet), and larval density (Jeathon et al., 1988).

Normally, periodical changes of filtered sterilized seawater, using filters from 10 to 1 μm and ultraviolet light irradiation, prevent bacterial infections in the larval cultures. Nonetheless, the use of antibiotics also routinely accompanies these preventive measures. The requirement for antibiotic use in a culture depends principally on the quality of water available. Strict cleaning, sterilization and handling of the culture is also necessary (Elston, 1984; Toranzo, 1990).

The larval culture of pectinids is initiated 48 h after spawning with veliger larvae with a characteristic D shape. As of this phase, the larva is able to feed finishing the larval period as pediveliger larva or eyed larva. These larvae that already have reached 220–250 μm seek an adequate substrate which they can recognize and on which they set until metamorphosis. At this stage, the larvae begin to secrete the final seashell adopting the characteristic form of the adult individual as they reach the post-larval stage (Uriarte et al., 2001). To obtain high survival, growth and setting rates during these phases, rigorous cleaning and aseptic conditions are required. Careful control of the water temperature, salinity and pH, quantity and quality of the food, as well as the larval density during cultivation, are also very important. From the point of view of sterility, the quality of the water is controlled by means of processing with filters, autoclave and germicidal systems to eliminate particulate material and micro-organisms alien to the culture. Aeration is another factor to consider in the cultivation of larvae, since it increases water oxygenation and assists in maintaining food particles in suspension in the water column. The
temperature of the water should be regulated within narrow margins. Prior to the larval settling phase, the substrates where the larvae are going to settle down must be prepared (Uriarte et al., 2001).

Despite the risk of selecting bacterial resistant strains, chloramphenicol (CHL) is the antibiotic most frequently used in culture laboratories (Le Pennec and Prieur, 1977) and in hatcheries (Buestel et al., 1982). In Chile, Chilean scallop larvae has been cultured in hatcheries for more than 15 years. Disalvo et al. (1984) were the first to report the use of antibiotics with cultures of *Argopecten purpuratus* using 25 mg l$^{-1}$ CHL when bacterial infection was suspected. The purpose of this study was to determine the dose of chloramphenicol required to improve survival and growth rates of Chilean scallops between the early larvae and pediveliger stages cultured in a closed system.

2. Materials and methods

The seawater was earlier filtered through a CUNO system with a battery of cartridges of 10, 5, 1 and 0.45 μm and then UV-sterilized. Tanks, sieves, pipes and tank caps were carefully disinfected every 2 days using 10% HCl (technical grade). The water temperature during the larval and postlarval experiments was maintained at 22 ± 1 °C by heating the seawater with titanium heaters and maintaining the room temperature at 22 °C. Microalgae diet was dosed twice a day at concentrations varying between 30 and 85 cells μl$^{-1}$, from D-stage to eyed larvae.

2.1. Qualitative monitoring of microalgae

The bacteria of microalgae cultures was controlled two ways—first, by using only strains of microalgal species that give negative growth in TCBS agar, and secondly, by applying a microscopic qualitative index, with values between 1 and 4. The index was assessed by microscopic observation of two samples, at 100 × magnification, as described by Varas (2000). A quality value 1 indicated absence of bacteria in all fields observed, value 2 showed at least one field of the sample showed bacteria, a value 3 indicated that each observed field of the sample had bacteria, and a value 4 indicated excessive bacteria in every field observed in the sample. We also eliminated the microalgae culture if protozoa were present. We gave this quality a value $X$. Only microalgal cultures of quality 2 were used because we did not obtain cultures of quality 1. Those cultures of accepted quality were quantified for microalgal cell concentration and dosed to the tanks. Daily microscopic cell counts were made with hemocytometer prior to feeding. The dosing was done manually twice daily.

2.2. Early larvae

The experiment started with 145 000, 4-day-old veligers of 85.7 ± 1.1 μm average size. Four treatments were conducted using 0, 0.2, 2 and 8 mg CHL per liter of
water containing the larval culture. These antibiotic doses were administered daily. The experiment lasted 10 days, with sampling every 2 days. The larvae were cultured at an initial density of 11 larvae per ml in 1-l tanks using three randomly distributed replicates. The larvae were fed with a microalgal mixture of *Isochrysis aff galbana* (clone T-Iso), *Chaetoceros calcitrans*, *Paclova lutheri* and *Thalassiossira minima*, (1:1:1:1 ratio).

2.3. Eyed pediveliger

The experiment started with 11 700 eyed larvae with an initial mean size of 212.6 ± 2.29 μm divided in 1 l tanks at a concentration of 1.6 larvae ml⁻¹. The temperature was maintained at 22 ± 1 °C. The seawater was sterilized and filtered to 0.45 μm. Tanks, pipes, sieves and airstones were sterilized every 2 days, and the seawater was changed. Sampling for larval settlement and metamorphosis was also conducted at this time.

During the settling phase, the larvae were fed with a mix of standard microalgae at concentration of 30 cells μl⁻¹. Feeding was considered necessary during the settling stage to prevent starvation of larvae late in metamorphosis, or those metamorphosing prematurely. The treatments applied were 0, 2 and 8 mg l⁻¹ CHL with three replicates per treatment. The settling substrate was planktonic nets supported by PVC-frames suspended in seawater by fishing-lines. Each replicate contained 10 settling units.

All dependent variables were analyzed by ANOVA. Percentage data were normalized by arcsine transformation (Sokal and Rohlf, 1981).

3. Results

3.1. Early larvae

Concentration of the chloramphenicol had a significant effect on the larval growth rate of the Chilean scallop (*F* = 14.53; df = 3, 15, *P* < 0.00001). A larger size was achieved by the larvae cultured with 2 and 8 mg l⁻¹ of chloramphenicol (Table 1 Fig. 1).

<table>
<thead>
<tr>
<th>Chloramphenicol dose (mg l⁻¹)</th>
<th>Growth rate (μm day⁻¹)</th>
<th>Specific growth rate (% per day)</th>
<th>Final survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.19 ± 0.20</td>
<td>1.3 ± 0.2</td>
<td>34.9 ± 3.3</td>
</tr>
<tr>
<td>0.2</td>
<td>1.59 ± 0.30</td>
<td>1.7 ± 0.3</td>
<td>29.5 ± 2.0</td>
</tr>
<tr>
<td>2.0</td>
<td>2.29 ± 0.28</td>
<td>2.3 ± 0.3</td>
<td>50.2 ± 3.0</td>
</tr>
<tr>
<td>8.0</td>
<td>2.64 ± 0.23</td>
<td>2.6 ± 0.2</td>
<td>49.8 ± 1.3</td>
</tr>
</tbody>
</table>

Mean value ± S.E. of three replicates.
Additionally, the antibiotic had a highly significant effect on the larval survival ($F = 8.31; \text{df} = 3, 15, P = 0.0003$). Highest survivals of $50.2 \pm 3.0$ and $49.8 \pm 1.3\%$ were observed at the end of 10 days of culture in the treatments of 2 and 8 mg $l^{-1}$ of CHL (Table 1). Significant differences were also observed in the changes in survival and growth relative to antibiotic concentration and duration of the treatment. This interaction was interpreted as a better survival and growth in the larvae treated with 8 mg $l^{-1}$ of antibiotics in the first 2 days of culture, while there were no significant differences observed between the concentrations of 2 and 8 mg $l^{-1}$ of culture during the remainder of the experiment.

The analysis with TCBS agar demonstrated that the treatments of 2 and 8 mg $l^{-1}$ of CHL did not contain colonies of *Vibrio*, while bacterial colonies were detected in the treatments receiving 0 and 0.2 mg $l^{-1}$ of the antibiotic.

### 3.2. Eyed pediveliger

The mean percent of larval settlement increased significantly with the addition of chloramphenicol (Fig. 2, $F = 37.42; \text{df} = 9, 18, P < 0.0000001$). Highest settlement was obtained in the 8 mg $l^{-1}$ dose ($5.73 \pm 0.57\%, n = 3$), which was significantly higher than in the 2 mg $l^{-1}$ dose ($2.73 \pm 0.23\%, n = 3$) (Tukey’s test, $P = 0.0004$). The lowest rate of settlement was observed in the larvae without antibiotic ($1.43 \pm 0.57\%, n = 3$).

Differences in percent metamorphosis were also significant (Fig. 3, $F = 37.42$, $\text{df} = 2, 18, P < 0.0000001$), varying from $6.30\% \pm 0.94\%, n = 3$ without antibiotic to $7.97\% \pm 0.66\%, n = 3$ in the 8 mg $l^{-1}$ concentration.

Of the total observed larvae, only 55.5% were found settled in the nets in the treatment without antibiotics, the other 45% were deposited on the bottom. In contrast, 75 and 79% of larvae were found settled in the nets, at the concentrations of 2 and 8 mg $l^{-1}$, respectively.
Fig. 2. *A. purpuratus*. Total larval settlement at different chloramphenicol concentrations (mg CHL l$^{-1}$). Each point is an average of three replicates. Bar is the standard error.

4. Discussion

It is important to compare the survival and growth rates obtained with the experimental application of antibiotic to those obtained with only routine disinfection of water and materials, that consider disinfecting materials with 10% HCl and the work surfaces and hands with 90° alcohol plus 1% iodine. Our results show enhanced survival and growth, and better condition, when early larvae and eyed pediveliger are treated with chloramphenicol. This improvement depends on antibiotic concentration, and the concentration required was a function of the larval age.

Many research laboratories, pilot plants and commercial hatcheries emphasize the importance of seawater quality in the success of larval cultures. This quality lies in microbiology and ecotoxicology aspects. Low quality of the water in any of these
two aspects (high bacteria concentration or presence of contaminants) can inhibit larval growth, even when nutrient requirements are being satisfied.

In general, the treatment of the seawater by filtration and UV sterilization, significantly reduce the concentration of certain types of bacteria such as *Vibrio*, and others naturally found in seawater, that can be opportunistic pathogens (Munro et al., 1999). Nonetheless, treatment with antibiotics may be necessary when production scale cultures, involving cleaning of large tanks, sieving of millions of larvae and massive culture of microalgae, increase the risk of contamination by manipulation. However, therapeutic use of antibiotics to stop bacterial infection must be carried out with appropriate antibiotic sensitivity screening (Prieur et al., 1990).

The detaching of the larvae before or after metamorphosis, and their sinking and accumulation on the bottom of tanks, favors the fast multiplication of bacteria able to attack and invade moribund larvae. Montes and Lara (1989), described mortalities of 90% of Chilean scallop larvae occurring in the bottom of tanks, Riquelme et al., (1996) proposed that larvae are initially attacked by the exotoxins released by the *Vibrio alginolyticus* strains and then the accumulation of moribund larvae and the high temperature of the cultures hasten the proliferation of pathogenic bacteria such as *Aeromonas hydrophila*. Since only *V. alginolyticus* have been shown to be sensitive to chloramphenicol (Riquelme et al., 1996), and our results showed 75% increased survival when the antibiotic was administered, we can infer that *Vibrio* was likely the prevalent bacteria present in our cultures of *A. purpuratus* larvae.

Although the use of antibiotics, in addition to systems of sterilization, may be beneficial to avoid pathogens in larval cultures, its use must be reviewed with more care according to the literature (Prieur et al., 1990; Araya et al., 1999). There has been evidence of secondary effects of antibiotics, like an agent producer of resistant bacteria and increase of the virulence of some strains, which could dramatically change the pathogenic nature of the strains (Ervik et al., 1994). Another reason for concern is that the product tends to become more expensive when antibiotics are used. For these reasons, chloramphenicol application is advised only when there are *Vibrio* positive tests in the culture tanks, or during periods of the year when *Vibrio* reaches maximum abundance in the seawater source of the hatchery (Videla, 1993).

When the sources of contamination in the seawater inflow to larval culture have been eliminated, it is necessary to focus attention on good management of cleaning and sterilization of all the materials that go in contact with the cultures.

Another source of bacterial contamination to larval culture is the vertical transmission of bacteria from the parents to the eggs (Riquelme et al., 1994). Then it may be expected that D-larvae initiate the culture with some bacteria cover similarly to that described by Munro et al. (1999) for rotifers, where each rotifer could carry a flora of about 10^4 external bacteria (almost 1% of the individual mass).

Furthermore, the microalgae used as nourishment in the bivalve hatcheries commonly are non-axenic. Thus, each cell has some bacteria cover and bacteria are also present in the culture medium. Therefore, it has been shown that microalgae can be a source of introduction of bacterial diseases to shellfish larvae. In our study
we used a qualitative index of bacterial presence in microalgal cultures. This index has been highly efficient to select cultures suitable for larva, postlarva and broodstock (Farías et al., 1994).

The results indicate that various conditions exist at the hatchery that make the application of antibiotic efficient. Concentrations of 2 and 8 mg l$^{-1}$ of chloramphenicol demonstrated effective control of larval contamination because it reduced mortality and increased growth rate. An antibiotic concentration between 2 and 8 mg l$^{-1}$ is recommended for massive cultures, using the highest concentration in the first days of culture. By the time the larvae reach 100 μm, the concentration may be reduced to 2 mg l$^{-1}$, by the time. Moreover, there was advantage to the application of antibiotic during the settlement stage and larval metamorphosis. In particular, the condition of postlarvae could be improved by addition of 8 mg l$^{-1}$ of chloramphenicol from eyed larvae to postlarvae of 300 μm in the massive cultures.

Use of chloramphenicol or other antibiotic in larval cultures may be avoided when three basic points of husbandry and technique are closely controlled: (1) improvement of the zootechnic handling of the larvae and postlarval cultures; (2) availability of equipment and systems for seawater filtration and sterilization; and (3) maintenance of a program of microbiological monitoring of the larval cultures and seawater inflow in the pumping section of the hatchery (Elston, 1984). In some countries the use of chloramphenicol is not permitted in food animals because of the known toxicity of the drug to humans. However, for the treatment of larval and early juvenile stages cultured in controlled systems, the use of chloramphenicol may not be relevant, provided that the bivalve hatchery produce seeds of 2 mm, which will be later grown to commercial size at sea, and that the hatchery has an effluent treatment system to prevent environmental contamination (Uriarte and Machulas, 2000).

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References


