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Effect of florfenicol and oxytetracycline treatments on the intensive larval culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819)

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Abstract

The administration of antimicrobials to control bacterial pathologies in Chilean scallop hatcheries is a frequent practice, but their effects on these cultures remained unknown. This study was undertaken to obtain information on the effect of the administration of florfenicol and oxytetracycline on the growth, survival and bacterial content of scallop larvae under farming conditions. Florfenicol-treated cultures exhibited high survival rates (44% after 17 days of culture), whereas cultures not treated or treated with oxytetracycline collapsed after 11 days of culture. Surprisingly, no significant differences in the heterotrophic (Tukey test; $P = 0.226$) and *Vibrio* (Tukey test; $P = 0.666$) concentrations between the oxytetracycline-treated and untreated larval cultures were observed. Otherwise, florfenicol administered directly into rearing tanks produced significantly higher larval growth (Tukey test; $P = 0.0001$) and survival (Tukey test; $P = 0.011$) than bath treatment. When 2 and 4 mg L^{-1} of florfenicol were compared, no significant differences in growth (*t*-test; $P = 0.4596$) and survival (Tukey test; $P = 0.057$) were observed, suggesting that a concentration of 2 mg L^{-1} is sufficient to ensure larval production. The present results demonstrate the efficacy of florfenicol-based therapy to increase larval survival and growth at commercial scale and prompt the necessity to standardize its use in Chilean scallop hatcheries.

Keywords: bacteria, florfenicol, Chilean scallop, shellfish bacteriology, *Argopecten purpuratus*, Chile

Introduction

The culture of Chilean scallop *Argopecten purpuratus* (Lamarck, 1819) is one of the most commercially important industries of Chilean mariculture, and is mainly concentrated in the North region of the country (von Brand, Merino, Abarca & Stotz 2006). Although the major supply of scallop's seeds is the natural environment, 30% comes from controlled condition cultures due to poor catches of natural seeds, which occur cyclically in Chile (Farías, Uriarte & Castilla 1998; Uriarte, Rupp & Abarca 2001). So, it has been necessary to develop a hatchery system to provide a consistent supply of larvae under controlled conditions.

Usually, the most frequent cause of larval mortality in scallop rearing systems is indirect, where the culture conditions or the rearing water quality are responsible to produce the necessary stress for the host susceptibility to opportunistic bacteria (Samain, Cochard, Chevelot, Daniel, Jeanthon, Le Coz, Marty, Moal, Prieur & Salaün 1987; Prieur, Mével, Nicolas, Plusquellec & Vigneulle 1990; Nicolas, Corre, Gauthier, Robert & Ansquer 1996). So, an adequate control and surveillance of the larval quality and its sanitary conditions is of great importance to the scallop hatcheries to obtain a long-term efficient production (Elston 1984; Lodeiros, Bolinches, Dopazo & Toranzo 1987; Sainz-Hernández & Maeda-Martínez 2005). Hatchery-produced mollusc larvae usually suffer massive mortality events, mainly produced by some bacterial pathogens that in some conditions are present in high numbers, causing detrimental effects on the larval culture (DiSalvo, Blecka & Zebal 1978; Riquelme, Toranzo,

Barja, Vergara & Araya 1996; Robert, Miner & Nicolas 1996; Schulze, Alabib, Tattersall-Sheldrakeb & Miller 2006; Rojas, Miranda & Amaro 2009).

Several studies have been performed to assess the effect of antibacterial agents on reared Chilean scallop, but only evaluated the effect of chloramphenicol on larvae (Uriarte, Farías & Castilla 2001) and juvenile (Fierro & Oliva 2009) under laboratory conditions, and no definitive results were obtained. Because of its deletorious effects, such as bone marrow toxicity, the use of chloramphenicol has been banned from aquaculture worldwide.

Usually, in various Chilean scallop hatcheries, larvae mortality events occur after the ninth day of culture, prompting the necessity to administer antibacterial agents in rearing tanks. Most of the scallop hatcheries located in the north region of Chile are currently using florfenicol in their larval cultures, mainly after the fifth day of the larvae rearing period and the drug is directly added into the larvae rearing tanks during the water exchange process that is usually performed every 48 h. Florfenicol was licensed in Chile for the treatment of diverse bacterial infections occurring in Chilean salmon farming and is currently one of the most frequently used antibacterials in Chilean freshwater salmon farming (Bravo, Dölz, Silva, Lagos, Millanao & Urbina 2005; Miranda & Rojas 2007). Before the use of florfenicol, Chilean hatcheries mainly used potentiated sulfonamides, flumequine and oxytetracycline to prevent bacterial pathologies, but the lack of studies evaluating at commercial scale the effect of antimicrobial therapies on reared scallop larvae has obliged scallop hatcheries to operate under a trial/error focus, unknowing completely if its intensive use is probably causing variations in the microbiota associated with scallop larvae, which could increment the possibility of being colonized by bacteria with pathogenic capabilities (Rojas *et al.* 2009).

The main objective of this study was to evaluate the effect of the administration of the antimicrobials oxytetracycline and florfenicol on the growth, survival and bacterial load of scallop larvae under farming conditions.

Materials and methods

Sampling and culture conditions

A commercial scallop hatchery, located in Tongoy Bay at the north of Chile was considered in the study. Larval cultures of the Chilean scallop *A. purpuratus* were done using 5000 L larval rearing tanks and two tanks for each treatment or control were used. According to the hatchery protocols, scallop larvae cultures started at a concentration of approximately 10 larvae per mL. Used water in rearing tanks was treated by filtration using consecutively bag filters of 25, 10 and 5 μm, and finally by UV irradiation using a group of six UV lamps (40 watts each). When necessary, water was heated to 16°C prior to filling the rearing tanks. The scallop larvae were fed daily with unicellular algae *Isochrysis galbana* and *Chaetoceros calcitrans* at 30 000 cells mL^{-1}. Rearing tank water and larval samples were collected each 48 h during the water exchange of rearing tanks, from larval cultures not treated and treated with each antibacterial agent. Larvae were mesh-sized, concentrated and collected using sterile water sampling bottles (APHA 1992). Samples were placed on ice, transported to the laboratory and processed within 2 h after collection.

Effect of the antimicrobial treatments on the larval culture of *A. purpuratus*

The effect of the antibacterials florfenicol and oxytetracycline, on the growth, survival and bacterial load of scallop larvae reared under hatchery intensive conditions, for a period of at least 17 days was determined (Treatment 1, Table 1). Duplicate rearing tanks were treated with 4 mg L^{-1} of florfenicol (Centrovet^{TM}, Santiago, Chile) and 4 mg L^{-1} of oxytetracycline (Centrovet^{TM}) administered each 48 h from day 5 to 17 of larval culture, directly to water tanks after the water exchange process, and larvae samples were collected along the larval growth period prior to the administration of the antimicrobial agent and larval bacterial content was determined at days 5, 9, 11, 13 and 15. In addition, the effect of 2 and 4 mg L^{-1} of florfenicol was determined (Treatment 2, Table 1). Florfenicol was administered directly in the water of rearing tanks from day 5 to 16 of culture and larval samples from treated and control rearing tanks were taken during each water exchange of rearing tanks from day 5 to 16 of larval culture, as previously described.

Having demonstrated that florfenicol was more efficient to improve growth and survival of reared scallop larvae, the efficacy of two ways of drug administration was evaluated (Treatment 3,

Table 1). Scallop larvae cultures were treated from day 5 to 16 of larval culture process, administering directly 4 mg L^{-1} of florfenicol to rearing water tanks during each water exchange and administering by bath 8 mg L^{-1} of florfenicol for 15 min after larval culture was meshed and concentrated.

Bacteriological analysis of scallop larvae culture

Culturable counts of heterotrophic and *Vibrio* spp. were determined by a spread plate method using Plate count agar (PCA; Difco labs, Sparks, MD, USA) added with 2% NaCl, and TCBS agar (Difco labs) prepared using 50% microfiltered (0.22 μm) aged seawater respectively. Scallop larvae samples were aseptically weighed, ground by hand using a sterile glass digester added with 2 mL of sterile physiological saline (0.85%) (PS) to obtain a homogenate according to Nicolas *et al.* (1996). Appropriate tenfold dilutions of the homogenates or water samples in PS were prepared and 0.1 mL aliquots were inoculated in triplicate onto agar plates. All plates were incubated at 20°C for 5 days and the bacterial numbers per g of sample were calculated as described in Miranda and Rojas (2007).

Microscopic analysis of scallop larvae culture

Larval growth and survival were determined in the hatchery using the protocols stated by the scallop farm. A sample of 10 mL was collected from each rearing tank during the tank water exchange when larval cultures were concentrated in a 20 L volume, according the hatchery procedures and a 1/10 dilution was used. Larval growth was determined measuring the larval shell height whereas larval mortality was determined according to Prado, Romalde, Montes and Barja (2005), using a Nikon™ (Kawasaki, Kanagawa, Japan) optical microscope. In addition, in the Aquatic Pathobiology Lab of the Universidad Católica del Norte some larvae samples were analysed for their sizes measuring the average length of 100 larval shells (50 shells per rearing tank), using the *Imaging software* Nis-Elements, version 2.3 (Nikon™) using a Zeiss Axiolab (Carl Zeiss, Jena, Germany) microscope.

Statistical analyses

Culturable counts of total bacteria and *Vibrio* spp. from scallop larvae and rearing tank water samples (Table 1) were transformed to $\log_{10}$ to stabilize the variance in the data. Larval survival percentages were transformed to arc sin of squared roots before their analyses. Bacterial counts, survival percentages and larvae size values were analysed per each specific day of culture under antibacterial treatment using one-way analysis of variance (ANOVA), and then a Tukey's test (Zar 1999) was used to determine the occurrence of differences between treatments. In culture days, when only two treatments were available, a Student's '*t*-test' was used. All statistical analyses were performed using the SPSS version 12.0 computer program (Norusis 2004).

Results

According to the hatchery protocols, all antimicrobial treatments used for intensive scallop larvae rearing started at fifth day of larval culture after the rearing tank water exchange. Growth of

Table 1 Characterization of experimental designs used for the scallop larvae treatment trials

	Number of tanks	Antimicrobial	Concentration (mg L^{-1})	Samples	Sampling* (Rearing day)
Treatment 1	2	None	None	Larvae, Water	5, 9, 11, 13, 15
	2	Florfenicol	4	Larvae, Water	5, 9, 11, 13, 15
	2	Oxytetracycline	4	Larvae, Water	5, 9, 11, 13, 15
Treatment 2	2	None	None	Larvae, Water	5, 7, 9, 12, 14, 16
	2	Florfenicol	2	Larvae, Water	5, 7, 9, 12, 14, 16
	2	Florfenicol	4	Larvae, Water	5, 7, 9, 12, 14, 16
Treatment 3	2	None	None	Larvae	7, 10, 12
	2	Florfenicol	4	Larvae	7, 10, 12
	2	Florfenicol	8	Larvae	7, 10, 12

*Samples for determination of bacteriological levels.

not-treated, florfenicol-treated and oxytetracycline-treated larval cultures before the eleventh day of culture was very similar (Fig. 1). Otherwise, at ninth day of culture, larval survival of florfenicol-treated cultures was significantly (Tukey test; $P = 0.037$) higher than the cultures treated with oxytetracycline, whereas larval survival of control and oxytetracycline-treated cultures was not significantly different (Tukey test; $P = 0.234$), exhibiting 1.11% and 0.47% of larval survival, respectively, and collapsed after 11 days of culture (Fig. 1).

No significant differences in larval sizes at ninth day of culture were observed between the control and treated cultures (ANOVA, $F_{(2,297)} = 1.0958$; $P > 0.05$), but at eleventh day of culture larval sizes of florfenicol-treated culture were significantly higher than those of the control and oxytetracycline-treated groups (ANOVA, $F_{(2,297)} = 63.523$; $P < 0.01$). As shown in Fig. 2, a low dispersion in the frequency of larval size percentages in the florfenicol-treated culture was observed, where 72% of the measured larvae exhibited length values belonging to 145 and 155 μm class marks, differing from the control and the oxytetracycline-treated cultures that predominantly belonged to the 125 and 135 μm class marks (58% and 71% respectively). It must be noted that no larvae samples of untreated (control) and treated-with-oxytetracycline larval cultures could be collected at days 13, 15 and 17 of culture because these cultures exhibited massive mortalities at day 11 of culture and were discarded after sampling.

At ninth and eleventh days of culture, levels of culturable counts of total bacteria in scallop larvae treated with florfenicol were significantly lower than those observed in untreated (Tukey test; $P = 0.009$ and $P = 0.003$ respectively) and oxytetracycline-treated (Tukey test; $P = 0.0001$ and $P = 0.0001$ respectively) larvae (Fig. 3). Surprisingly, no significantly differences between culturable bacterial counts of reared scallop larvae not treated and treated with oxytetracycline were observed at days 9 and 11 of culture (Tukey test; $P = 0.226$ and $P = 0.058$ respectively) (Fig. 3), confirming that this antibacterial is not efficient to reduce bacterial load of scallop larvae reared in hatchery conditions. In addition, florfenicol was significantly (Tukey test; $P = 0.0001$) more efficient than oxytetracycline to reduce Vibrio levels in

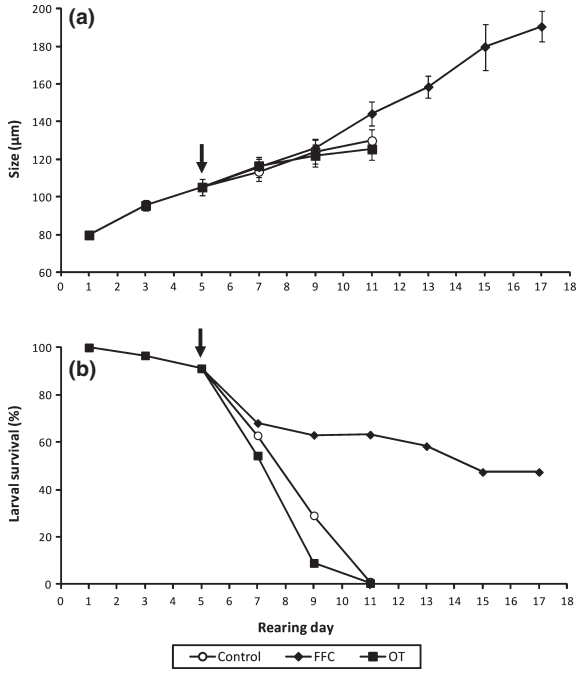

Figure 1 Growth (a) and survival (b) of scallop larval cultures not treated (CO), treated with 4 mg L^{-1} of florfenicol (FFC) and treated with 4 mg L^{-1} of oxytetracycline (OT). Arrows indicate when antibacterial therapy started. Vertical bars in (a) = SD.

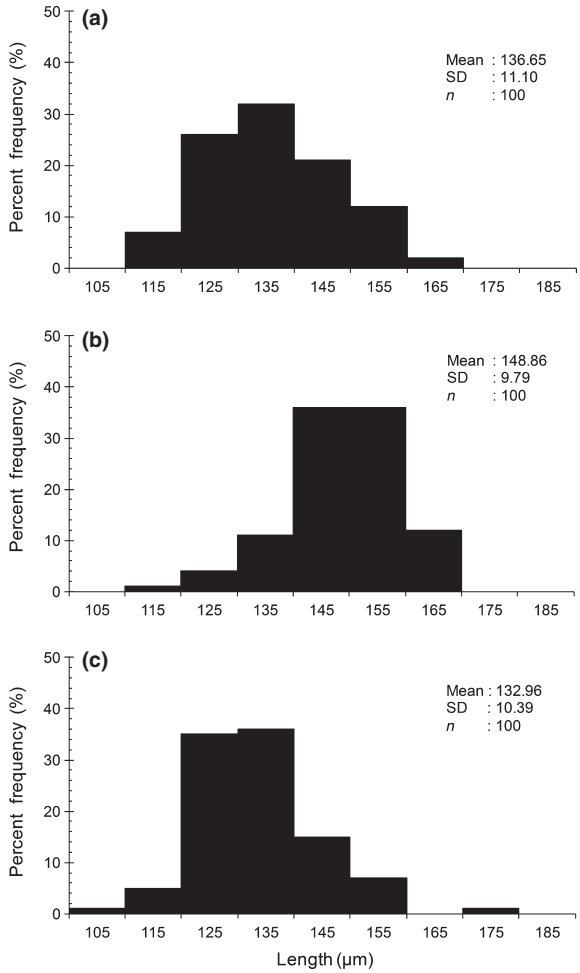

Figure 2 Size distribution of 11-day-old scallop larvae cultures not treated (a), treated with 4 mg L^{-1} of florfenicol (b) and treated with 4 mg L^{-1} of oxytetracycline (c). Larval sizes were determined by measuring the average length of 100 larval shells using the *Imaging software* Nis-Elements, version 2.3 (Nikon™).

larval treated cultures after 11 days of culture (Fig. 3). On the other hand, bacterial culturable counts of rearing tank water samples at ninth and eleventh day of larval culture evidenced variable results, exhibiting no significant differences (Tukey test; $P = 0.633$ and $P = 0.182$ respectively) in total bacterial counts between not-treated and florfenicol-treated rearing tanks, whereas levels of *Vibrio* spp. at days 9 and 11 of culture of water from florfenicol-treated rearing tanks were significantly lower than those determined from not-treated (Tukey test; $P = 0.001$ and $P = 0.0001$ respectively) and oxytetracycline-treated (Tukey test; $P = 0.001$ and $P = 0.0001$ respectively) rearing tanks (Fig. 4).

Growth rates of control culture were slightly lower than larval cultures treated with 2 and 4 mg L^{-1} of florfenicol (Fig. 5). At twelfth day of larval culture, survival rates of scallop larvae cultures treated with 2 and 4 mg L^{-1} of florfenicol were not significantly different (Tukey test; $P = 0.947$), but were significantly (Tukey test; $P = 0.001$) higher than those of the untreated control cultures, which collapsed at day 14 of culture (Fig. 5). In addition, at days 14 and 16 of culture, no significant differences in larval survival of cultures treated with 2 and 4 mg L^{-1} of florfenicol (*t*-test; $P = 0.295$ and $P = 0.057$ respectively) were observed.

At ninth day of culture, sizes of scallop cultures treated with 2 and 4 mg L^{-1} were significantly (one way ANOVA; $F_{(2,297)} = 5.5896$; $P < 0.05$) higher than the size of untreated culture (Fig. 6).

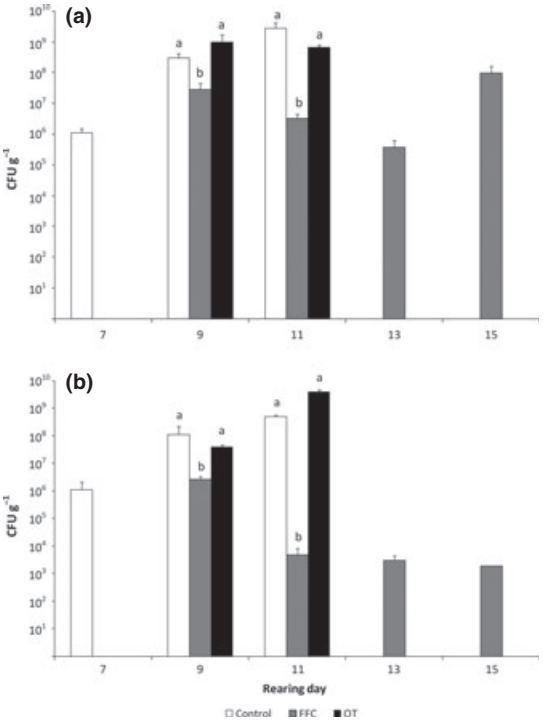

Figure 3 Culturable counts of total bacteria (a) and *Vibrio* spp. (b) from scallop larvae samples of cultures not treated (Control), treated with 4 mg L^{-1} of florfenicol (FFC) and treated with 4 mg L^{-1} of oxytetracycline (OT). Data with different letters are significantly different ($P < 0.05$). Vertical bars = SD.

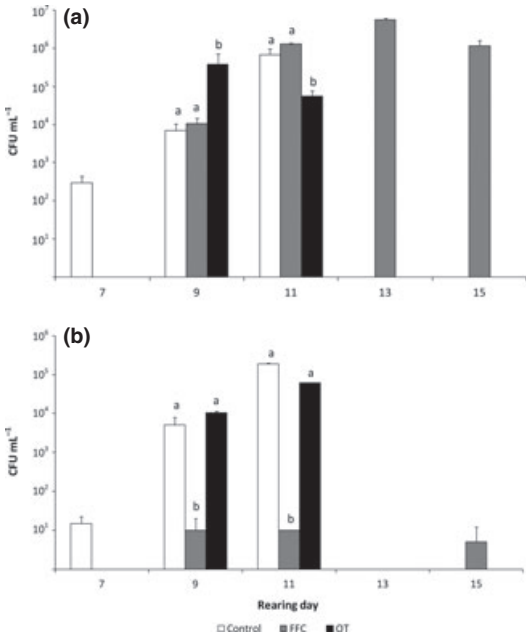

Figure 4 Culturable counts of total bacteria (a) and *Vibrio* spp. (b) from scallop larvae rearing tank water samples of cultures not treated (Control), treated with 4 mg L^{-1} of florfenicol (FFC) and treated with 4 mg L^{-1} of oxytetracycline (OT). Data with different letters are significantly different ($P < 0.05$). Vertical bars = SD.

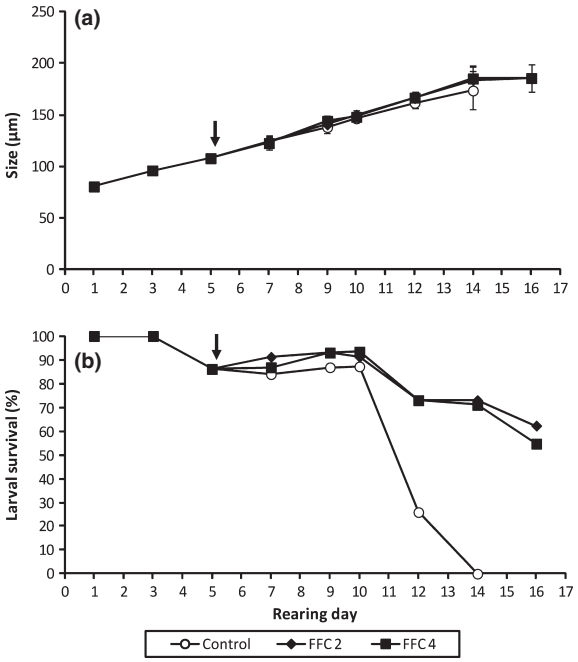

Figure 5 Growth (a) and survival (b) of scallop larvae cultures not treated (CO), and treated with 2 mg L^{-1} (FFC 2) and 4 mg L^{-1} (FFC4) of florfenicol. Arrows indicate when antibacterial therapy started. Vertical bars in (a) = SD.

In addition, at day 16 of culture, no significant differences (*t*-test; $P = 0.4596$) between sizes of reared scallop larvae treated with 2 and 4 mg L^{-1} of florfenicol were observed (Fig. 7).

Culturable counts of total bacteria in scallop larval cultures treated with 2 and 4 mg L^{-1} of florfenicol were significantly lower than those observed in the untreated control cultures at days 12 (Tukey test; $P = 0.0002$ and $P = 0.0003$ respectively) and 14 (Tukey test; $P = 0.015$ and $P = 0.002$ respectively) of culture. In addition, at twelfth day of larval culture, levels of *Vibrio* spp. in scallop larvae of florfenicol-treated cultures were significantly (Tukey test; $P = 0.0002$) lower than those observed in larvae from the untreated cultures (Fig. 8). At day 16 of culture, no culturable counts in untreated cultures were observed, because after fourteenth day of culture the untreated cultures exhibited a massive mortality event, the remaining larvae being discarded by the hatchery personnel as is usually done when high levels of larval mortality occur. Otherwise, at twelfth and fourteenth day of larval culture, total bacterial culturable counts of water samples from rearing tanks treated with 4 mg L^{-1} of florfenicol were not significantly different than those from tanks treated with 2 mg L^{-1} of florfenicol (Tukey test; $P = 0.066$ and $P = 0.130$ respectively), whereas levels of *Vibrio* spp. from water samples from rearing tanks treated with 4 mg L^{-1} were significantly (Tukey test; $P = 0.033$ and $P = 0.018$ respectively) lower than those from rearing tanks treated with 2 mg L^{-1} (Fig. 9).

Having demonstrated that florfenicol is the most effective antibacterial to increase scallop larval survival in commercial hatchery conditions, we decided to determine the effect of the mode of its administration. After starting florfenicol treatment, larval cultures treated by direct administration of the drug always exhibited higher larval sizes than untreated and treated by bath cultures, as evidenced in Fig. 10. At day 12 of culture, scallop larvae cultures treated with florfenicol administered directly in the rearing tanks exhibited levels of survival of 20.00%), whereas untreated and treated larval cultures by bath with 8 mg L^{-1} of florfenicol showed only 0.83% and 3.00% of larval survival, respectively, showing that larval survival of cultures treated by direct administration were significantly higher than those of the untreated (Tukey test; $P = 0.005$) and treated by bath (Tukey test; $P = 0.011$) cultures (Fig. 10). At

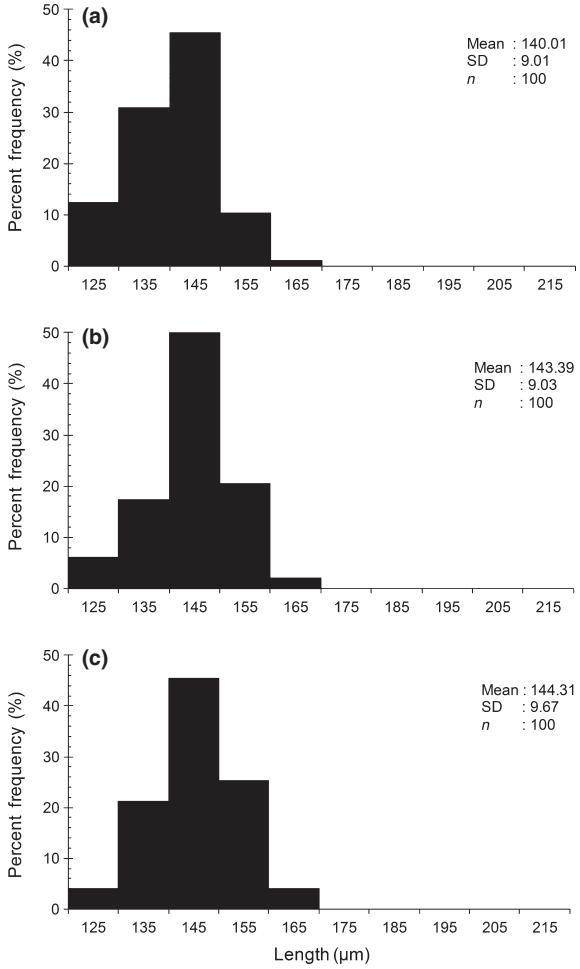

Figure 6 Size distribution of 9-day-old scallop larvae cultures not treated (a), treated with 2 mg L^{-1} of florfenicol (b) and treated with 4 mg L^{-1} of florfenicol (c). Larval sizes were determined by measuring the average length of 100 larval shells using the *Imaging software* Nis-Elements, version 2.3 (Nikon^{TM}).

tenth day of culture, no significant (Tukey test; $P = 0.529$) differences in larval sizes of the control and treated by bath cultures were observed, but larval sizes of larval culture treated by direct administration of florfenicol into the rearing tank showed a highly significant difference with the control and treated by bath cultures (one way ANOVA, $F_{(2,297)} = 16.568$; $P < 0.01$), as evidenced in Fig. 11.

At day 12, culturable counts of total bacteria and *Vibrio* spp. in larval cultures treated with florfenicol by direct administration of the drug into the rearing tank were significantly (Tukey test; $P = 0.0002$ and $P = 0.0002$ respectively) lower than those exhibited by the larvae from the untreated and treated with florfenicol by bath. Surprisingly, at day 12, larval cultures treated by bath evidenced higher culturable counts of total bacteria than the untreated cultures (Fig. 12), demonstrating that administration of florfenicol by bath is not efficient to reduce bacterial loads in reared scallop larvae.

Discussion

Florfenicol is an antibacterial extensively used in aquaculture, mainly in fish culture, but no previous studies have been done to evaluate its activity in shellfish culture. Most of the studies related to use of antimicrobials in scallop larvae culture considered other antimicrobials, such as chloramphenicol and erythromycin. Campa-Córdova, Luna-González, Zarain-Herzberg and Cáceres-Marinez (2005) reported that 6 mg L^{-1} of erythromycin or

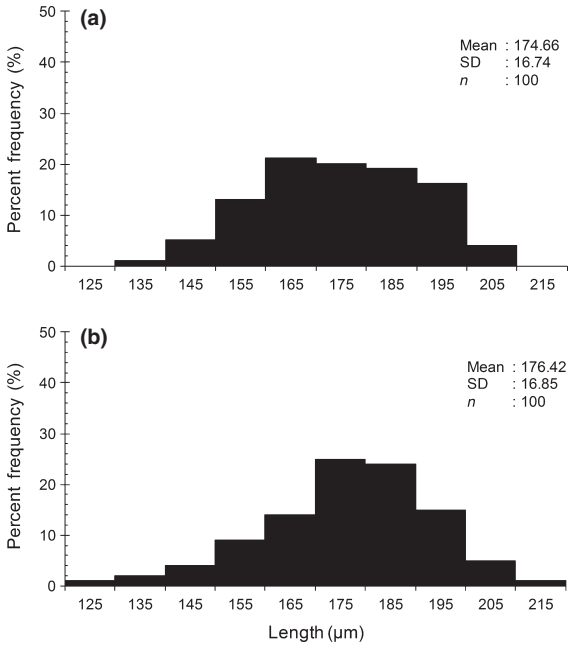

Figure 7 Size distribution of 16-day-old scallop larvae cultures treated with 2 mg L^{-1} of florfenicol (a), and treated with 4 mg L^{-1} of florfenicol (b). Larval sizes were determined by measuring the average length of 100 larval shells using the *Imaging software* Nis-Elements, version 2.3 (Nikon^{TM}).

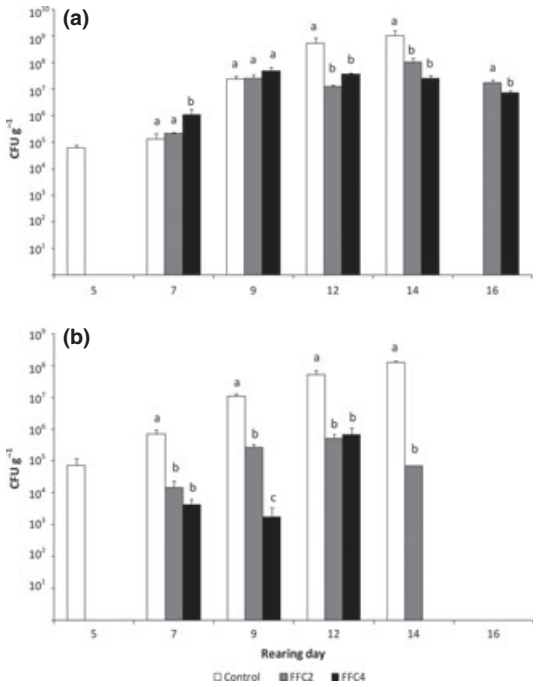

Figure 8 Culturable counts of total bacteria (a) and *Vibrio* spp. (b) from larvae samples of cultures not treated (Control), treated with 2 mg L^{-1} of florfenicol (FFC2) and treated with 4 mg L^{-1} of florfenicol (FFC4). Data with different letters are significantly different ($P < 0.05$). Vertical bars = SD.

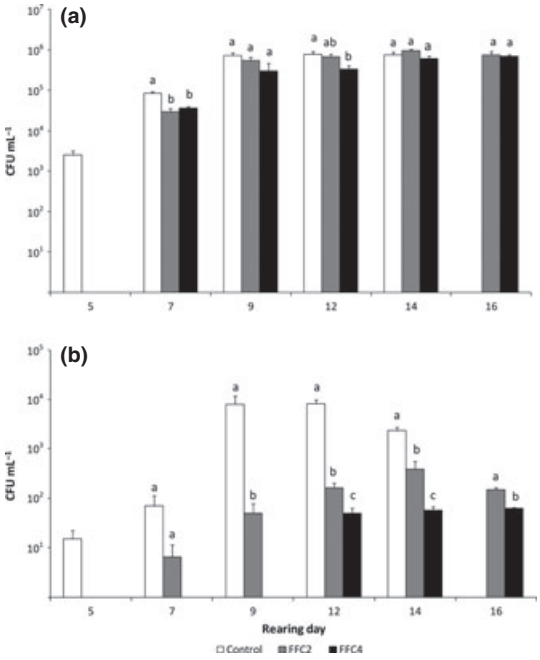

Figure 9 Culturable counts of total bacteria (a) and *Vibrio* spp. (b) from larval rearing tank water samples of cultures not treated (Control), treated with 2 mg L^{-1} of florfenicol (FFC2) and treated with 4 mg L^{-1} of florfenicol (FFC4). Data with different letters are significantly different ($P < 0.05$). Vertical bars = SD.

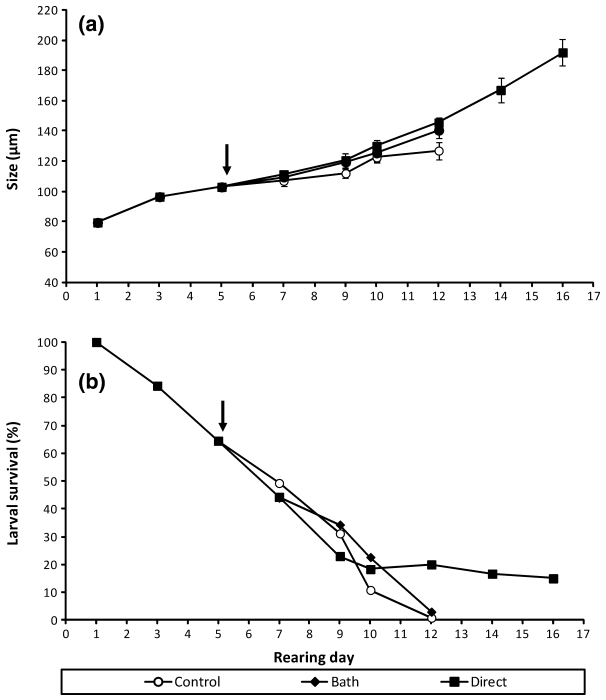

Figure 10 Growth (a) and survival (b) of scallop larvae cultures not treated (Control), treated by bath with 8 mg L^{-1} of florfenicol (Bath) and directly in the rearing tanks with 4 mg L^{-1} of florfenicol (Direct). Arrows indicate when antibacterial therapy started. Vertical bars in (a) = SD.

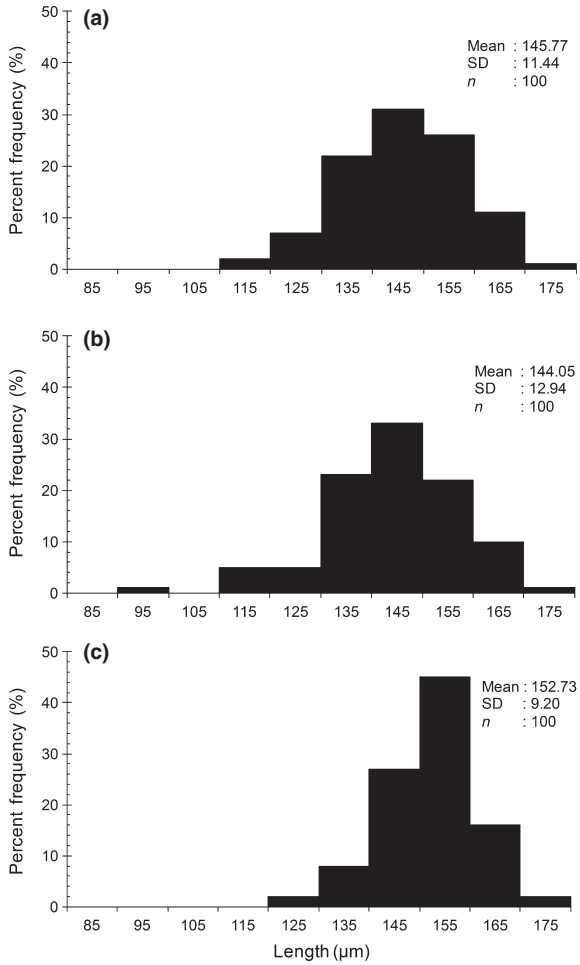

Figure 11 Size distribution of 11-day-old scallop larvae cultures not treated (a), treated by bath with 8 mg L^{-1} of florfenicol (b) and treated directly in the rearing tanks with 4 mg L^{-1} of florfenicol (c). Larval sizes were determined by measuring the average length of 100 larval shells using the *Imaging software* Nis-Elements, version 2.3 (Nikon^{TM}).

chloramphenicol were able to significantly increase larval survival and to reduce levels of potential pathogens in small-scale (60 L at a density of 15 larvae mL^{-1}) larval cultures of *Argopecten ventricosus*, agreeing with the results reported by Robert *et al.* (1996), who found that concentrations of 8 mg L^{-1} of chloramphenicol significantly increased larval survival of *Pecten maximus*, whereas use of erythromycin led to inconsistent larval survival results. In addition, Fierro and Oliva (2009) found that growth rates of scallop juveniles treated with chloramphenicol were inversely proportional to the drug concentration, suggesting a toxic action on the culture, but also could be explained by an increase in the virulence of bacterial strains associated with shellfish culture as a consequence of the drug therapy, as was suggested by Ervik, Thorsen, Eriksen, Lunestad and Samuelsen (1994).

At present, chloramphenicol is not an alternative for scallop larvae treatment due to its high toxicity to humans, whereas erythromycin is a macrolide mainly used to treat Gram-positive mediated pathologies because of its reduced activity against Gram-negative bacteria, so it is questionable that this antibacterial could be effective to reduce levels of *Vibrio*, the genus recognized as the most important pathogenic agent for larvae and juvenile stages of reared molluscs (Elston, Lelbovitz, Relyea & Zatila 1981; Jeffries 1982; Nottage

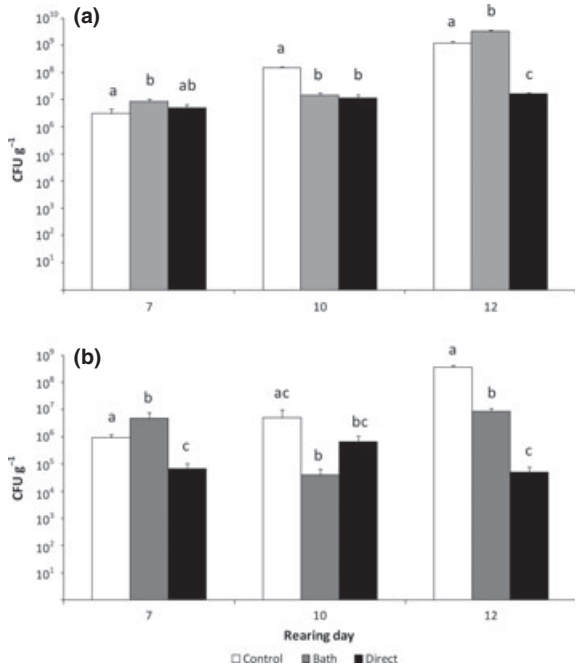

Figure 12 Culturable counts of total bacteria (a) and *Vibrio* spp. (b) from larvae samples of cultures not treated (Control), treated by bath with 8 mg L^{-1} of florfenicol (Bath) and treated directly in the rearing tanks with 4 mg L^{-1} of florfenicol (Direct). Data with different letters are significantly different ($P < 0.05$). Vertical bars = SD.

& Birkbeck 1986, 1987; Riquelme, Hayashida, Toranzo, Vilches & Chavez 1995; Lambert, Nicolas, Cilia & Corre 1998; Sainz, Ascencio & Maeda-Martínez 1998; Estes, Friedmann, Elston & Herwig 2004). In this trend, Uriarte, Farías *et al.* (2001) recommended to use only antibacterial agents that are efficient to reduce levels of *Vibrio* spp. in scallop larval rearing tanks.

Despite that, the exact cause of the mass mortality episodes in Chilean scallop hatcheries remains unknown. It could be explained by a combination of multiple factors including the occurrence of opportunistic pathogenic bacteria mainly characterized by the occurrence of a high diversity of the *Vibrio* species involved. Recently, some strains of *Vibrio splendidus* have been identified as causative of vibriosis outbreaks occurring in reared scallop larvae from Chilean hatcheries (unpublished results), that is in agreement with Torkildsen, Lambert, Nylund, Magnesen and Bergh (2005) who reported that one cluster of strains similar to *V. splendidus* produced high rates of mortality in challenge assays with larvae of *P. maximus*. In addition, different strains phenotypically related to this species have been associated with mortalities of molluscs, such as *P. maximus* (Nicolas *et al.* 1996) and *Crassostrea gigas* (Sugumar, Nakai, Hirata, Matsubara & Muroga 1998; Lacoste, Jalabert, Malham, Cueff, Gélébart, Cordevant, Lange & Poulet 2001; Le Roux, Gay, Lambert, Waechter, Poubalanne, Chollet, Nicolas & Berthe 2002; Gay, Renault, Pons & Le Roux 2004).

Torkildsen *et al.* (2005) observed in a culture of scallop *P. maximus* that only larvae treated prophylactically with chloramphenicol survived to settling, indicating a bacterial causative agent. Torkildsen, Coyne, Samuelsen, Magnesen and Bergh (2002) investigated various antimicrobials, such as florfenicol, oxytetracycline, oxolinic acid and neomycin to reduce high mortality often observed in reared larvae of the great scallop, *P. maximus,* and they found that oxolinic acid was the most effective, although only at high concentrations. In Chilean aquaculture, the use of quinolones is usually restricted because of their high persistence in the environment. In addition, Fitt, Heslinga and Watson (1992) found that development and survival of larvae of *Tridacna derasa* treated with the antibiotics streptomycin, neomycin, penicillin and rifampin during the first 4 days of development was significantly higher than that of the controls, observing that highest survival

occurred when a combination of antibiotics was used, especially with streptomycin and neomycin. Torkildsen, Samuelsen, Lunestad and Bergh (2000) found a significant increase in the MIC values of chloramphenicol, flumequine and trimethoprim/sulfadiazine when 25% seawater was used, concluding that these antimicrobials are antagonized by components in seawater, whereas florfenicol was not inhibited by seawater, confirming its usefulness to be administered in scallop hatcheries.

The reason that oxytetracycline treatment was not effective to significantly reduce bacterial content and enhance survival of reared larvae remains unclear, but our results agree with a recent study of Godoy, Espinoza, Wittwer, Uriarte and Aranda (2011) who found that larval survival of small-scale cultures of *A. purpuratus* in the presence and absence of tetracycline decreased drastically during the 20 days of culture, showing no differences between the treated and untreated cultures.

In most of Chilean scallop hatcheries, the drug is continuously administered each 2 days after the water exchange, in each larval batch until the spat is fixed to Netlon bags. Used water disposal depends on each hatchery, observing that some of them have effluent treatment, whereas other hatcheries discharge their effluents directly into the sea. Considering that use of antibacterial agents in Chilean mollusc culture is not properly regulated and the current lack of knowledge of the real effects of florfenicol treatment on the reared larvae and its potential consequences to the associated microbiota. It is strongly necessary to maintain a strict surveillance of florfenicol use in scallop farming.

Optical microscopy analysis of larval culture evidenced important differences between florfenicol-treated cultures and the other cultures that showed an important incidence of larvae with reduced stomach and empty shells, so this method is highly recommended to hatchery personnel for monitoring reared larvae status to prevent massive mortalities. This is particularly important, considering the usual absence of clinical signs preceding death of scallop larvae in the rearing tanks.

As was stated by Campa-Córdova *et al.* (2005) and Jorquera, Silva and Riquelme (2001), to improve larval survival it is mandatory to decrease bacterial concentrations in larvae rearing tanks, mainly those related to *Vibrio* spp. In this study, it has been demonstrated that the administration of florfenicol in the studied hatchery can avoid a collapse of larval culture. Robert *et al.* (1996) reported that neither a decrease in the larval density nor an increase in the rearing tank water exchange in the larval culture of *P. maximus* led to an increase in the larval survival, stating the necessity to evaluate the feasibility of using florfenicol or oxolinic acid in mollusc larvae culture as alternatives to chloramphenicol therapy.

It is highly important to consider standardizing the methodology used to determine larval size and mortality in scallop hatcheries, to be able to compare larval production from various hatcheries. In each hatchery, larval samples are usually collected and treated using different procedures. In addition, in some hatcheries larval size is determined by measuring the shell height whereas other hatcheries usually use the larval length to determine larval growth.

The feasibility of replacing antibacterial therapy by using probiotic bacteria to control bacterial pathogens in scallop larvae culture has been previously suggested (Riquelme, Araya, Vergara, Rojas, Guaita & Candia 1997; Avendaño & Riquelme 1999), but all reported results were obtained in small-scale experimental cultures and its efficacy in commercial culture still remains to be elucidated.

In conclusion, only a treatment with florfenicol administered directly into the rearing tank has been proved to be efficient to ensure culture survival until larvae were fixed onto Netlon bags prior to their transfer to the sea, and concentrations of 2 mg L^{-1} exhibit a high efficacy to reduce larval mortality in the intensive culture of Chilean scallop demonstrating that it is feasible to reduce the concentration of florfenicol to be administered in scallop hatcheries to 2 mg L^{-1}, considering that amounts of florfenicol currently administered in Chilean scallop hatcheries are very different, varying from 0.3 to 4 mg L^{-1}. Considering that the OIE (Office International des Epizooties) does not consider mollusc larvae bacterial pathogens in its Sanitary Code, no regulations or sanitary guidelines are available for this industry, prompting the necessity to standardize its use in Chilean scallop hatcheries. In addition, it is highly necessary to evaluate the occurrence of antibacterial-resistant bacteria as a consequence of the use of florfenicol to prevent the spread of antibacterial resistance in pathogenic microbiota associated with scallop larvae and rearing hatcheries in Chile.

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References

APHA (1992) Microbiological examination. In: *Standard Methods for the Examination of Water and Wastewater* (ed. by A.E. Greenberg, L.S. Clesceri & A.D. Eaton), pp. 9.1–9.147. American Public Health Association, Washington, DC, USA.

Avendaño R.E. & Riquelme C.E. (1999) Establishment of mixed-culture probiotics and microalgae as food for bivalve larvae. *Aquaculture Research* **30**, 893–900.

von Brand E., Merino G.E., Abarca A. & Stotz W. (2006) Scallop fishery and aquaculture in Chile. In: *Scallops: Biology, Ecology and Aquaculture* (ed. by S.E. Shumway & J. Parsons). *Developments in Aquaculture and Fisheries Science* **35**: 1293–1311. Elsevier, Amsterdam, The Netherlands.

Bravo S., Dölz H., Silva M.T., Lagos C., Millanao A. & Urbina M. (2005) Informe Final. Diagnóstico del uso de fármacos y otros productos químicos en la Acuicultura. Proyecto No. 2003–28. Instituto de Acuicultura, Facultad de Pesquerías y Oceanografía, Univ. Austral de Chile 256pp.

Campa-Córdova A.I., Luna-González A., Zarain-Herzberg M. & Cáceres-Marinez C.J. (2005) Prophylactic use of antibiotics in larval culture of *Argopecten ventricosus* (Sowerby, 1835). *Journal of Shellfish Research* **24**, 923–930.

DiSalvo L.H., Blecka J. & Zebal R. (1978) *Vibrio anguillarum* and larval mortality in a California coastal shellfish hatchery. *Applied and Environmental Microbiology* **35**, 219–221.

Elston R.A. (1984) Prevention and management of infectious diseases in intensive mollusc husbandry. *Journal of World Mariculture Society* **15**, 284–300.

Elston R., Lelbovitz L., Relyea D. & Zatila J. (1981) Diagnosis of vibriosis in a commercial oyster hatchery epizootic: diagnostic tools and management features. *Aquaculture* **24**, 53–62.

Ervik A., Thorsen B., Eriksen V., Lunestad B.T. & Samuelsen O.B. (1994) Impact of administering antibacterial agents on wild fish and blue mussels *Mytilus edulis* in the vicinity of fish farms. *Diseases of Aquatic Organisms* **18**, 45–51.

Estes S., Friedmann C.S., Elston R.A. & Herwig R.P. (2004) Pathogenicity testing of shellfish hatchery bacterial isolates on Pacific oyster *Crassostrea gigas* larvae. *Diseases of Aquatic Organisms* **58**, 223–230.

Farías A., Uriarte I. & Castilla J. (1998) A biochemical study of the larval and postlarval stages of the Chilean scallop *Argopecten purpuratus*. *Aquaculture* **166**, 37–47.

Fierro J. & Oliva D. (2009) Effect of antibiotic treatment on the growth and survival of juvenile northern Chilean scallop, *Argopecten purpuratus* Lamarck (1819), and associated microflora in experimental cultures. *Aquaculture Research* **40**, 1358–1362.

Fitt W.K., Heslinga G.A. & Watson T.C. (1992) Use of antibiotics in the mariculture of giant clams (*F. Tridacnidae*). *Aquaculture* **104**, 1–10.

Gay M., Renault T., Pons A.-M. & Le Roux F. (2004) Two *Vibrio splendidus* related strains collaborate to kill *Crassostrea gigas*: taxonomy and host alterations. *Diseases of Aquatic Organisms* **62**, 65–74.

Godoy F.A., Espinoza M., Wittwer G., Uriarte I. & Aranda C. (2011) Characterization of culturable bacteria in larval cultures of the Chilean scallop *Argopecten purpuratus*. *Ciencias Marinas* **37**, 339–348.

Jeffries V.E. (1982) Three *Vibrio* strains pathogenic to larvae of *Crassostrea virginica* and *Ostrea edulis*. *Aquaculture* **29**, 201–226.

Jorquera M.A., Silva F.R. & Riquelme C.E. (2001) Bacteria in the culture of the scallop, *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture International* **9**, 285–303.

Lacoste A., Jalabert F., Malham S., Cueff A., Gélébart F., Cordevant C., Lange M. & Poulet S.A. (2001) A *Vibrio splendidus* strain is associated with summer mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). *Diseases of Aquatic Organisms* **46**, 139–145.

Lambert C., Nicolas J.L., Cilia V. & Corre S. (1998) *Vibrio pectinicida*, a pathogen of scallops (*Pecten maximus*) larvae. *International Journal of Systematic Bacteriology* **48**, 481–487.

Le Roux F., Gay M., Lambert C., Waechter M., Poubalanne S., Chollet B., Nicolas J.L. & Berthe F. (2002) Comparative analysis of *Vibrio splendidus*-related strains isolated during *Crassostrea gigas* mortality events. *Aquatic Living Resources* **15**, 251–258.

Lodeiros C., Bolinches J., Dopazo C.P. & Toranzo A.E. (1987) Bacillary necrosis in hatcheries of *Ostrea edulis* in Spain. *Aquaculture* **65**, 15–29.

Miranda C.D. & Rojas R. (2007) Occurrence of florfenicol resistance in bacteria associated with two Chilean salmon farms with different history of antibacterial usage. *Aquaculture* **266**, 39–46.

Nicolas J.L., Corre S., Gauthier G., Robert R. & Ansquer D. (1996) Bacterial problems associated with scallop *Pecten maximus* larval culture. *Diseases of Aquatic Organisms* **27**, 67–76.

Norusis M.J. (2004) *Statistical Package for the Social Sciences (SPSS) 12.0 Statistical Procedures Companion*. Prentice Hall, Englewood Cliffs, NJ, USA 601pp.

Nottage A.S. & Birkbeck T.H. (1986) Toxicity to marine bivalves of culture supernatant fluids of the bivalve-pathogenic *Vibrio* strain NCMB 1338 and other marine vibrios. *Journal of Fish Diseases* **9**, 249–250.

Nottage A.S. & Birkbeck T.H. (1987) The role of toxins in *Vibrio* infections of bivalve mollusca. *Aquaculture* **7**, 244–246.

Prado S., Romalde J.L., Montes J. & Barja J.L. (2005) Pathogenic bacteria isolated from disease outbreaks in shellfish hatcheries. First description of *Vibrio neptunius* as an oyster pathogen. *Diseases of Aquatic Organisms* **67**, 209–215.

Prieur D., Mével G., Nicolas J.-L., Plusquellec A. & Vigneulle M. (1990) Interactions between bivalve molluscs and bacteria in the marine environment. *Oceanography and Marine Biology Annual Review* **28**, 277–352.

Riquelme C., Hayashida G., Toranzo A.E., Vilches J. & Chavez P. (1995) Pathogenicity studies on a *Vibrio anguillarum* related (VAR) strain causing an epizootic in *Argopecten purpuratus* larvae cultured in Chile. *Diseases of Aquatic Organisms* **22**, 135–141.

Riquelme C., Toranzo A.E., Barja J.L., Vergara N. & Araya R. (1996) Association of *Aeromonas hydrophila* and *Vibrio alginolyticus* with larval mortalities of scallop (*Argopecten purpuratus*). *Journal of Invertebrate Pathology* **67**, 213–218.

Riquelme C., Araya R., Vergara N., Rojas A., Guaita M. & Candia M. (1997) Potential of probiotic strains in the culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture* **154**, 7–26.

Robert R., Miner P. & Nicolas J.L. (1996) Mortality control of scallop larvae in the hatchery. *Aquaculture International* **4**, 305–313.

Rojas R., Miranda C.D. & Amaro A.M. (2009) Pathogenicity of a highly exopolysaccharide-producing *Halomonas* strain causing epizootics in larval cultures of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). *Microbial Ecology* **57**, 129–139.

Sainz J.C., Ascencio F. & Maeda-Martínez A.N. (1998) Experimental vibriosis induction with *Vibrio alginolyticus* of larvae of the catarina scallop (*Argopecten ventricosus* = *circularis*) (Sowerby II, 1842). *Microbial Ecology* **35**, 188–192.

Sainz-Hernández J.C. & Maeda-Martínez A.N. (2005) Sources of *Vibrio* bacteria in mollusc hatcheries and control methods: a case study. *Aquaculture Research* **36**, 1611–1618.

Samain J.F., Cochard J.C., Chevelot L., Daniel J.Y., Jeanthon C., Le Coz J.R., Marty Y., Moal J., Prieur D. & Salaün M. (1987) Effect de la qualité de l' eau sur la croissance larvaire de *Pecten maximus* en écloserie: observations preliminaries. *Haliotis* **6**, 363–381.

Schulze A.D., Alabib A.O., Tattersall-Sheldrakeb A.R. & Miller K.M. (2006) Bacterial diversity in a marine hatchery: balance between pathogenic and potentially probiotic bacterial strains. *Aquaculture* **256**, 50–73.

Sugumar G., Nakai T., Hirata Y., Matsubara D. & Muroga K. (1998) *Vibrio splendidus* biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* larvae. *Diseases of Aquatic Organisms* **33**, 111–118.

Torkildsen L., Samuelsen O.B., Lunestad B.T. & Bergh Ø. (2000) Minimum inhibitory concentrations of chloramphenicol, florfenicol, trimethoprim/sulfadiazine and flumequine in seawater of bacteria associated with scallops, (*Pecten maximus*) larvae. *Aquaculture* **185**, 1–12.

Torkildsen L., Coyne R., Samuelsen O.B., Magnesen T. & Bergh Ø. (2002) Treatment of the early life stages of scallop (*Pecten maximus*) with antimicrobial agents; searching for an alternative to chloramphenicol. *Aquaculture International* **10**, 399–409.

Torkildsen L., Lambert C., Nylund A., Magnesen T. & Bergh Ø. (2005) Bacteria associated with early life stages of the great scallop, *Pecten maximus*: impact on larval survival. *Aquaculture International* **13**, 575–592.

Uriarte I., Farías A. & Castilla J. (2001) Effect of antibiotic treatment during larval development of the Chilean scallop *Argopecten purpuratus*. *Aquaculture Engineering* **25**, 139–147.

Uriarte I., Rupp G. & Abarca A. (2001) Spat production of Iberoamerican scallops under controlled conditions. In: *Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura* (ed. by A. Maeda-Martinez), pp. 147–171. Edit. Limusa, DF, México.

Zar J.H. (1999) *Biostatistical Analysis*, (4th edn). Prentice-Hall, Upper Saddle River, NJ, USA 931pp.