Effect of Increasing Salinity on Weight-Specific Filtration Rate of Juvenile Scallop *Argopecten purpuratus* Reared at Two Temperatures: Is Any Effect Related to Ammonia Buildup?

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EFFECT OF INCREASING SALINITY ON WEIGHT-SPECIFIC FILTRATION RATE OF JUVENILE SCALLOP *ARGOPECTEN PURPURATUS* REARED AT TWO TEMPERATURES: IS ANY EFFECT RELATED TO AMMONIA BUILDUP?

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ABSTRACT Larvae of the northern Chilean scallop *Argopecten purpuratus* (Lamarck, 1819) can be produced in hatcheries in closed aquaculture systems (CAS), and then early juveniles transferred to the sea for further grow-out. A new scallop mass production method from larvae to early juveniles that applies recirculation aquaculture system (RAS) technology has been developed at Universidad Catolica del Norte. RASs might lose water by evaporation, which increases salinity. Water temperature will increase as well if it is not controlled. An experiment was performed to determine the effect of varying salinity and temperature over water quality parameters and scallop physiological processes. Feeding behavior of *A. purpuratus* was determined under CAS with daily water exchange for hatchery-produced juvenile scallops (mean shell height, 26.5 mm; SD, 1.9) reared at salinities of 34, 38, and 42 g/L, and temperatures at 16°C and 22°C. Weight-specific filtration rates (WFRs) were analyzed at day 12 and again at day 55. Scallops were fed *Isochrysis galbana* and *Chaetoceros calcitrans* (ratio, 1:1). Results shows that WFRs analyzed for a 24 h period had a tendency to be lower at 0 h than at 24 h, for both temperatures; WFRs were higher at 22°C than at 16°C. A significant positive regression was also found between final WFR and total ammonia nitrogen (both NH_3-N and NH_4^+-N) levels. Results presented here should be interpreted with caution outside the shell height range of 26.5 ± 1.9 mm. These results are applicable to the management of *A. purpuratus* under controlled conditions, such as a RAS.

KEY WORDS: *Argopecten purpuratus*, juvenile scallops, filtration rates, ammonia levels, hypersalinity

INTRODUCTION

The northern Chilean scallop *Argopecten purpuratus* (Lamarck, 1819) is mainly produced through aquaculture practices in Chile (Merino et al. 2001, von Brand et al. 2006). Rearing of this species has increased significantly during the past decades in part as a result of broodstock conditioning, as well as hatchery production of larvae and juvenile scallops (DiSalvo et al. 1984, von Brand et al. 2006). Larvae of *A. purpuratus* produced in hatcheries are generally reared using closed aquaculture system (CAS) technology—meaning, daily 100% water exchange (Avendaño et al. 2001, Riquelme et al. 2001, Uriarte et al. 2001) and, after a few weeks, early juveniles (less than 1 mm in shell height (SH)) are transferred to the sea for further grow-out. The scallop industry relies on an efficient year-round supply of larvae and juveniles as well as on appropriate reproductive conditioning of the parental stock, and requires natural seawater on a daily basis (Avendaño et al. 2001, Merino et al. 2001, Riquelme et al. 2001). On the other hand, recirculating aquaculture systems (RASs) have been tried on an experimental basis for parasite transmission studies in shellfish (MacMillan et al. 1994), for maintenance of nursery-sized scallops *Argopecten irradians* (Lamarck, 1819) (Widman 1998) and *Pecten maximus* Linné (1758) (Christophersen et al. 2006), and for studying effects of environmental conditions on scallop physiology with *A. purpuratus* (Soria et al. 2007). Recently, the aquacultural engineering team at Universidad Católica del Norte developed RAS technology for *A. purpuratus* mass rearing from egg to the early-juvenile stage with very promising results (Merino et al. 2009).

In RAS processes there is an evaporative water loss that increases salinity when seawater is used (Merino 2005). Variations in salinity and temperature may change water-quality parameters, such as dissolved oxygen (DO), CO_2 levels, the NH_3-to-NH_4^+ ratio, and pH (Lawson 1995). Salinity and temperature can also affect survival, growth, and physiological processes related to the energy acquisition (ingestion and absorption) and utilization (respiration and excretion) (Navarro & González 1998, González et al. 2002, MacDonald et al. 2006, Soria et al. 2007).

Pectinids are osmoconformers, in which hemolymph is close to the osmotic pressure and ionic composition of seawater. In pectinids, it has been reported that ammonia excretion increases with decreasing salinity (Bricelj & Shumway 1991) and decreases as salinity increases (Shumway 1977, Singnoret-Brailowski et al. 1996, Soria et al. 2007). The effect of decreasing salinity on several scallop species is well documented (Paul 1980, Tettelbach & Rhodes 1981, Strand et al. 1993, Laing 2002, Christophersen & Strand 2003, Rupp & Parson 2004), but information related to scallop responses to hypersaline situations is scarce.

Knowledge about how the filtration rate of juvenile scallops is affected by increased salinity at different temperature conditions is an important factor for optimum cultivation of this species. *A. purpuratus* is able to maintain similar filtration rates until salinities fall below 27 g/L, but significant reductions on filtration rate is observed below this limit (Navarro & González 1998, Fernández-Reiriz et al. 2005). This phenomenon is found in other scallop species, such as *P. maximus* (Strand et al. 1993,

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Laing 2002). On the other hand, Martínez et al. (1995) have reported that juveniles of *A. purpuratus* fed 1 or 2 portions per day will experience a higher cell density of algae (depressed clearing rate) for an initial period of time than when the same total amount of algae is provided continuously. After cell density exceeds the upper critical level, the feed provided to *A. purpuratus* was not filtered and ingested efficiently. In general, filtration rates have been reported to be affected by algae concentration and ration strategies, which in turn have a direct impact on scallop growth rate (Martínez et al. 1995, Rheault & Rice 1996, Riisgård 2001, Laing, 2004).

This article examines the feeding behavior of *A. purpuratus* juveniles cultured under increasing saline conditions at two temperatures—culture conditions that likely occur in RAS hatchery settings (Merino 2005, Merino et al. 2009). Our focus was on salinity and temperature, so we chose a closed system because it allowed us to control the effect of the other potential water-quality explanatory variables. For instance, ammonia, nitrate, nitrite, alkalinity, and other water-quality parameters are usually controlled within specific ranges in standard RAS used for fish rearing according to a given optimal rearing environment. However, the physiological effect of these water-quality parameters on *A. purpuratus,* as well as on other pectinid species, are poorly understood. The information presented in this article is an extension and complements the results previously reported by Soria et al. (2007).

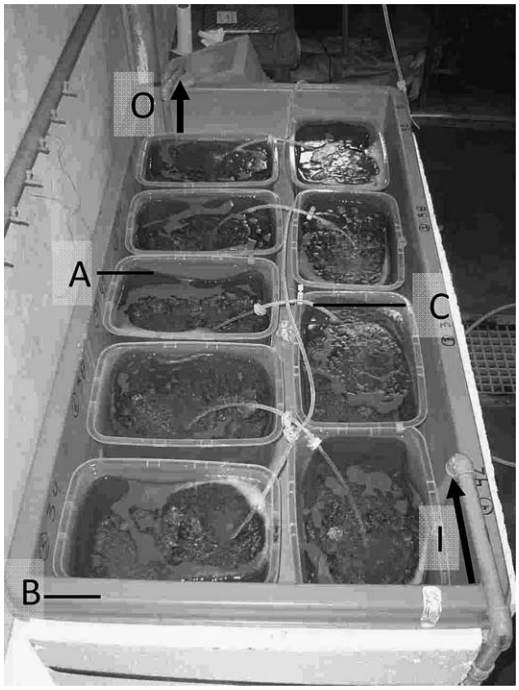

Figure 1. Experimental set up at 16°C. Triplicate batch of juvenile scallops were reared at 3 salinities (34, 38, and 42 g/L) in 15-L net cages within pails (a) placed in a water bath tank (b). Each rearing unit was air supplied (c). Arrows show the inlet (i) and the outlet (o) of water from the chiller unit (not shown).

MATERIALS AND METHODS

Hatchery-produced juvenile scallops were provided by Universidad Católica del Norte (UCN), Coquimbo, Chile (30° LS), where the research was conducted. Juvenile scallops (mean SH, 26.5 ± 1.9 mm (SD)) were close cultured at 3 salinities (34, 38, and 42 g/L) and two temperatures 16°C (chiller unit Azoo CH 200 W, Aquatic Ecosystems, Apopka, FL) and 22°C (submersible heater Tetratec HT 300 W, Aquatic Ecosystems, Apopka, FL) with 3 replications per treatment. Seawater used was 1-μm filtered and UV treated (80 W; Aquatic Ecosystems). Scallops were stocked in plastic net cages into 15-L plastic pails (25 × 30 × 24 cm). Pails were introduced into 2 water bath containers (250 L, 160 × 64 × 26 cm), one for each temperature (Fig. 1). Twenty juvenile scallops were assigned to each net cage, corresponding to a mean total wet scallop biomass, including shell, of 5.83 g/L. Cultures were changed every 24 h by transferring the net cages directly into a clean pail containing new seawater at the appropriate salinity and temperature.

Hypersaline water was prepared by evaporating seawater. Air stones were used to provide aeration to maintain DO levels above 80% saturation. Scallops were fed with *Isochrysis galbana* and *Chaetoceros calcitrans* (ratio, 1:1; cell to cell) in a quantity equivalent to 10% of the scallop's dry weight per day, corresponding to 360×10^6 cell/L/day (11.11 mg dry matter/L, 92% of organic amount). Daily feed ratio allotment was divided into thirds, provided morning (0800 HR), noon (1200 HR), and evening (1800 HR). Before running the experiment, scallops were acclimated to different experimental conditions by increasing salinity and temperature by 1 g/L and 1°C per day. After 55 days, when differences in survival rates were significant, the experiment was terminated (Soria et al. 2007).

Samples of 50 juveniles were used for initial dry tissue weight (W_{dry}, in grams). Scallops were rinsed with isotonic ammonium formate (3.4%) to remove salt, and all tissue components were removed and dried at 70°C to a constant weight. At the end of the experiment, scallops were sacrificed to obtain final dry tissue weights following the same procedure just described.

Salinity, temperature, and DO were measured daily using a multiparameter hand sensor (YSI 85; Yellow Spring Inc.). Solórzano's (1969) phenol–hypochlorite method was used to determine total ammonia (sum of NH_3 and NH_4^+) at time 0 h on fresh seawater and again after 24 h prior to a full water exchange. Water samples were collected in acid-washed glassware, and total ammonia was measured immediately after collection. A pH meter (calibrated with buffer solutions of pH 7 and pH 10 before use; HI 9023C, Hanna Instruments) was used to measure water pH at the time of collection. Unionized ammonia (NH_3) was indirectly estimated from appropriate temperature, pH, and salinity according to Fivelstad (1988). Ionized ammonia (NH_4^+) was estimated as a difference between total ammonia and NH_3. Values for total ammonia, un-ionized ammonia, and ionized ammonia were expressed on a nitrogen basis and recorded as total ammonia nitrogen (TAN), NH_3-N, and NH_4^+-N (Colt & Armstrong 1981).

Measurements for filtration rate were acquired after an acclimation period of 12 days and again at day 55. Filtration rate was determined from an analysis of mass balance for a closed system using a static system as (Coughlan 1964, Merino 2005)

$$\mathrm{FR} = \frac{\mathrm{V}}{n}\left[\frac{(\ln C_0 - \ln C_t) - S}{t}\right] \quad (1)$$

where FR is the filtration rate equivalent to a volume of seawater cleared of particles per unit of time (measured in

TABLE 1.

Mean values of water quality in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C.

	16°C			22°C		
Treatment	**34 g/L**	**38 g/L**	**42 g/L**	**34 g/L**	**38 g/L**	**42 g/L**
Temperature (°C)	15.9 (0.1)	16.0 (0.1)	15.9 (0.1)	22.1 (0.2)	22.0 (0.1)	22.0 (0.2)
Salinity (g/L)	33.8 (0.1)	37.9 (0.1)	41.8 (0.2)	34.0 (0.2)	38.1 (0.1)	42.0 (0.2)
DO (%)	92.0 (6.5)	91.0 (6.40)	90.3 (5.9)	93.5 (3.9)	92.6 (4.3)	92.0 (4.4)
DO (ml/L)	5.18 (0.38)	4.99 (0.36)	4.83 (0.33)	4.8 (0.33)	4.57 (0.23)	4.4 (0.21)
pH	7.95 (0.08)	7.95 (0.07)	7.90 (0.10)	8.04 (0.06)	7.99 (0.06)	7.99 (0.06)
NH_4^+-N (mg/L)	0.97 (0.1)	0.91 (0.02)	0.84 (0.03)	1.47 (0.36)	1.26 (0.09)	1.43 (0.08)
NH_3-N (mg/L)*	0.022 (2.1)	0.02 (0.5)	0.017 (0.8)	0.068 (1.7)	0.05 (3.7)	0.056 (3.1)

* Values of SD $\times 10^{-3}$.
Values in parentheses are SD.

milliliters per minute); V is the volume (measured in milliliters); n is the number of scallops; t is time (measured in minutes); C_0 and C_t are the initial and final algal concentration (measured in cells per milliliter), respectively; and S is the rate of sedimentation in controls. A particle counter (Coulter Counter Z2) fitted with a 100-µm orifice tube was used to count particles ranging from 2–10 µm. Filtration rate determinations for scallops fed *I. galbana*, in a quantity equivalent to one-third of the daily ration ($\sim 120 \times 10^3$ cell/mL), were taken at time 0.5, 1, 3, 6, and 24 h after the addition of algae. After 24 h, scallops were fed a second ration consisting of the same diet, without a water exchange, and filtration rate was determined once more at the same times described earlier. Control close-culture beakers without scallops (one for each combination of salinity and temperature) were used to calculate algae settlement rates. Finally, the weight-specific filtration rate (WFR; measured in milliliters per hour per gram) was determined as

$$\mathrm{WFR} = \frac{\mathrm{FR}}{W_{dry}} \tag{2}$$

I. galbana was harvested from a 35-L semicontinuous photobioreactor made with a 12.4-m transparent hose (diameter, 1.9 cm), with an algae yield capacity that ranged from 25×10^6–45×10^6 cell/mL. Twenty-four-hour illumination was provided by 8 cool-white fluorescent lights. The circulation of the algae culture was ensured by an airlift system. Algae cells were counted daily using a particle counter (Coulter Counter Z2) fitted with a 100-µm orifice tube to count particles ranging from 2–10 µm, prior to harvesting at either logarithmic or stationary phases of growth and before feeding larvae. *C. calcitrans* was batch cultured in 6-L glass flasks with 1-µm filtered, chlorine-sterilized seawater (salinity, 34–35 g/L) and f/2 media.

To test for significant effects of salinity, temperature, and time on WFR, we conducted repeated-measures analysis of variance (ANOVA) followed by an HSD Tukey test. We conducted all statistical tests with $\alpha = 0.05$, using STATISTICA version 6.

RESULTS

After the acclimation period, experimentally set temperature and salinity values remained stable (Table 1). DO was higher than 90% in all treatments, and both NH_3-N and NH_4^+-N were higher at 22°C than 16°C, exhibiting a slight inverse relationship with salinity (Table 1).

Filtration Rate

Filtration rate per scallop showed a tendency to increase between days 12 and 55 day when reared at 16°C and at 34 g/L, whereas at 38 g/L and 42 g/L, the filtration rate decreased either at 0 h or at 24 h (Table 2). Filtration rate values registered at 22°C between days 12 and 55 showed a tendency to increase at all salinities at 0 h; however, filtration rate values increased at

TABLE 2.

Mean values of filtration rates per scallop (milliliters per hour per scallop) in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C at 12 and 55 days after the first (0 h) and second (24 h) algae delivery.

		16°C			22°C		
Day	**Treatment**	**34 g/L**	**38 g/L**	**42 g/L**	**34 g/L**	**38 g/L**	**42 g/L**
12	0 h	2,507.1 (276.1)	2,577.6 (148.7)	2,289.0 (254.6)	2,785.7 (206.1)	2,499.3 (400.0)	2,167.6 (407.7)
	24 h	2,367.2 (558.8)	2,732.0 (31.3)	2,761.6 (741.1)	3,574.8 (598.8)	4,102.3 (813.2)	2,621.5 (541.5)
55	0 h	2,858.8 (455.0)	2,053.7 (337.3)	2,148.5 (602.5)	3,550.1 (747.4)	3,304.2 (798.6)	2,697.6 (189.2)
	24 h	3,030.7 (879.1)	2,398.2 (375.9)	2,329.8 (277.0)	3,734.4 (336.4)	3,417.7 (712.6)	2,596.6 (683.3)

Values in parentheses are SD.

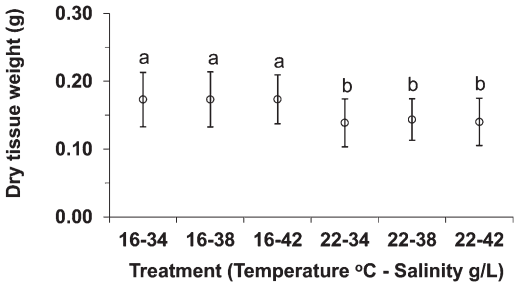

Figure 2. Final mean dry tissue weight in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C. Treatments with different letters indicate significantly different values (Tukey, $P < 0.05$).

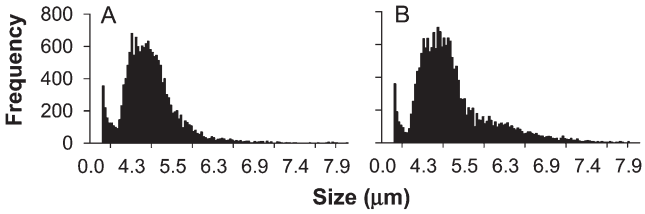

Figure 3. Frequency size distribution of *I. galbana* delivered to *A. purpuratus* juveniles on day 12. (A, B) First delivery (0 h; A) and second delivery (24 h; B).

24 h only at 34 g/L, decreased at 38 g/L, and were basically unchanged at 42 g/L (Table 2). In general, filtration rate 0-h values on day 12 show a tendency to be similar for scallops reared at both temperatures; filtration rate 24-h values on day 12 show a tendency to increase only for scallops reared at 22°C at a salinity of 34 g/L and 38 g/L (Table 2). For similar salinities, filtration rate 0-h and 24-h values on day 55 were higher for scallops reared at 22°C (Table 2).

Dry Tissue Weight

Initial mean dry tissue weight was 0.102 ± 0.02 g, corresponding to an initial mean SH of 26.5 ± 1.9 mm, and was used to calculate the initial WFR. Final mean dry tissue weight was significantly higher when juveniles were reared at 16°C ($P < 0.001$). Neither salinity ($P = 0.72$) nor the interaction between the main factors ($P = 0.98$) affected final mean dry tissue weight. There were no significant differences ($P = 0.725$) between final mean dry tissue weights when juvenile scallops were reared at 16°C at different salinities. Similar patterns were found for scallops reared at 22°C (Fig. 2). Final mean dry tissue weights were used to divide the final filtration rate values determined on day 55 to calculate final WFR.

Weight-Specific Filtration Rate

At the beginning of the experiment (day 12), scallops received a diet around 360×10^6 cell/L/day, equivalent to a total dry weight of 11.11 mg/L with an organic proportion of 10.22 mg/L (92%). The first delivery of algae showed a mean size of 4.79 ± 0.6 μm; the second algal delivery showed a mean size value of 5.03 ($P = 0.98$) 0.8 μm (Fig. 3A and B, respectively). An important reduction in algal concentration within all treatments (Fig. 4A–F) with scallops occurred on day 12, 0.5 h from the first delivery of *I. galbana*. Similarly, a considerable reduction in algae numbers was quantified when analyzing plotted algae size frequency distributions after the second delivery (Fig. 4G–L).

WFR calculated at 0.5 h after the first feeding ranged from 607.76–835.50 mL/h/g. Evidence was found that salinity affected WFR (2-way ANOVA, 1-sided $P = 0.047$, $F_{2,12} = 3.97$). Convincing evidence was found that neither temperature (2-way ANOVA, 1-sided $P = 0.92$, $F_{1,12} = 0.01$) nor the interaction of main factors (2-way ANOVA, 1-sided $P = 0.14$, $F_{2,12} = 2.3$) affected WFR (Fig. 5).

WFR calculated at 0.5 h after the second feeding with algae ranged from 650.32–1,200.20 mL/h/g. Higher values of WFR were found after 0.5 h for scallops fed at 24 h when compared with those that were fed at 0 h (repeated-measures ANOVA, $P = 0.012$); however, no effect was found to be related to the interaction between factors ($P > 0.06$ for temperature × time; $P > 0.3$ for salinity × time and temperature × salinity × time). Higher WFRs were found for the groups of scallops reared at 22°C (2-way ANOVA, 1-sided $P = 0.02$, $F_{1,12} = 6.88$), particularly for scallops reared at 34 g/L and 38 g/L salinity (Fig. 5).

At the end of the experiment (day 55), WFR was determined for scallops fed with an equivalent amount of *I. galbana* with similar size distribution frequency (Fig. 6). A high depletion of algal cells was quantified after 0.5 h for both: the first (at 0 h)

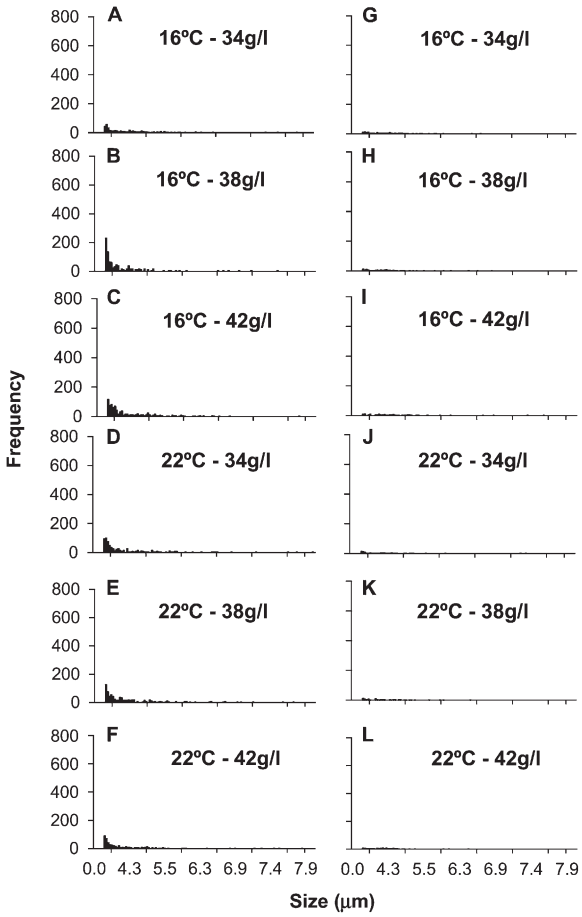

Figure 4. (A–L) Size frequency distribution of *I. galbana* determined 0.5 h after the first (A–F) and second (G–L) algae deliveries on day 12 in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and, 42 g/L) at 16°C and 22°C.

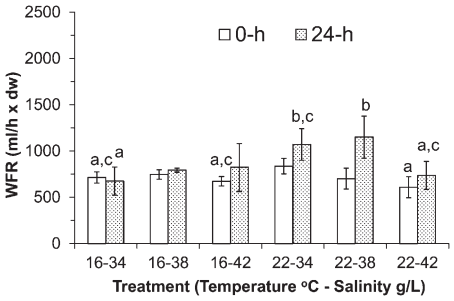

Figure 5. Weight-specific filtration rate (WFR) in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C on day 12 determined 0.5 h after the initial feeding (0 h) and determined again 0.5 h after 24 h (24 h). Treatments with different letters indicate significantly different values (Tukey, $P < 0.05$), and treatments without letters indicate no difference between treatments.

and second (at 24 h) algae deliveries (Fig. 7). A significantly lower WFR value (627.57 mL/h/g) was found at 0.5 h after the first algae delivery in the group of scallops reared at 16°C compared with those reared at 22°C (1,622.98 mL/h/g; $P < 0.001$). In addition, WFR was significantly affected by salinity ($P = 0.031$), but no significant interaction between factors was found ($P = 0.28$; Fig. 8). WFRs determined 0.5 h after the second algae delivery was higher (1,714.72 mL/h/g) in scallops reared at 22°C ($P < 0.0001$). Final WFR was significantly affected by salinity ($P = 0.004$) as well as by the interaction between factors ($P = 0.046$; Fig. 8). When comparing mean values of WFR, it was found that WFR values were higher after the second algal delivery compared with the initial WFR values (repeated-measures ANOVA, $P = 0.047$; Fig. 8). As with the WFR values determined on day 12, WFR on day 55 was not affected by the interaction between any combination of factors ($P > 0.78$ in all cases; temperature × time, salinity × time, and temperature × salinity × time).

A significant effect of time ($P = 0.002$, repeated-measures ANOVA) was found on days 12 and 55 for WFR after the first (at 0 h) algal delivery, as well as a significant interaction between temperature and time ($P = 0.001$, repeated-measures ANOVA; Fig. 9). No significant effect related to the interaction between salinity × time ($P = 0.20$) or temperature × salinity × time ($P = 0.73$) were detected. Mean WFR values were higher on day 55, particularly for scallops reared at 22°C at salinities of 34 g/L and 38 g/L of salinity (1,622.98 mL/h/g and 1,124.23 mL/h/g, respectively; Fig. 9).

Relationships between mean WFR values, at the end of the experiment, with NH_3-N and NH_4^+-N mean values (Table 1),

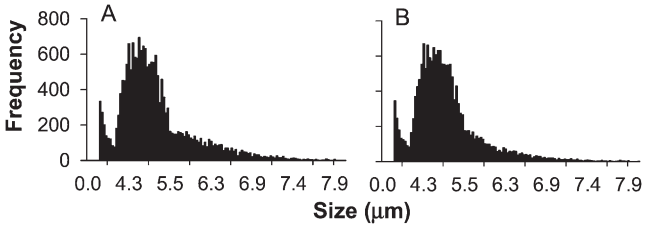

Figure 6. Frequency size distribution of *I. galbana* delivered to *A. purpuratus* juveniles on day 55. (A, B) First delivery (0 h; A) and second algae delivery (24 h; B).

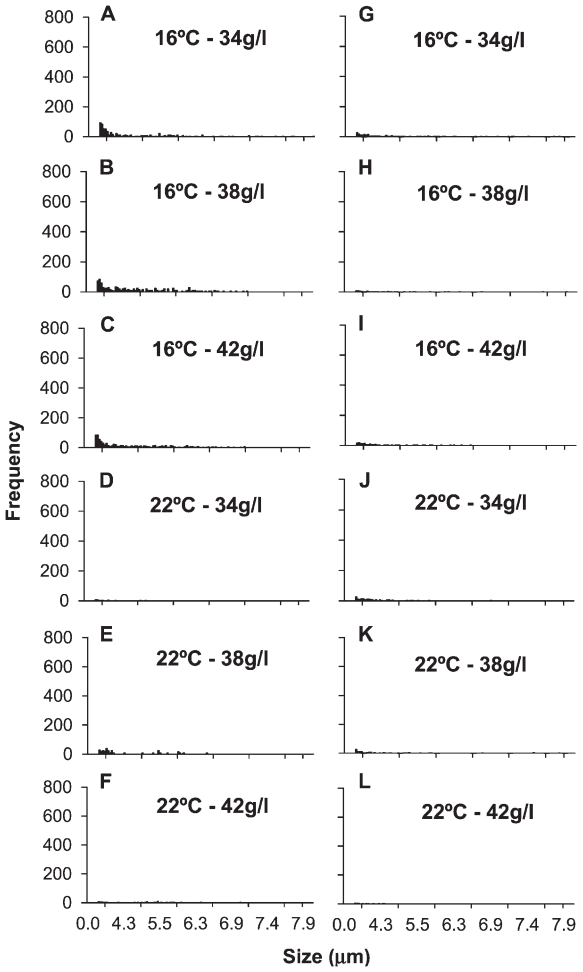

Figure 7. (A–L) Size frequency distribution of *I. galbana* determined 0.5 h after the first (A–F) and the second (G–L) algae delivery on day 55 in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C.

throughout the experiment, were estimated by using a linear regression model. When WFR data were pooled together (16°C and 22°C) against NH_3-N as well as against NH_4^+-N a positive slope was shown. A significant increasing trend in WFR was

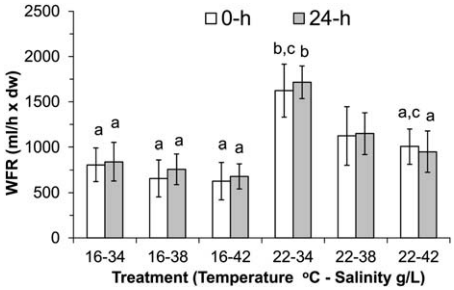

Figure 8. Weight-specific filtration rate (WFR) in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C on day 55 measured 0.5 h after the first (0 h) and second (24 h) algae delivery. Treatments with different letters indicate significantly different values (Tukey, $P < 0.05$); treatments without letters indicate no differences between treatments.

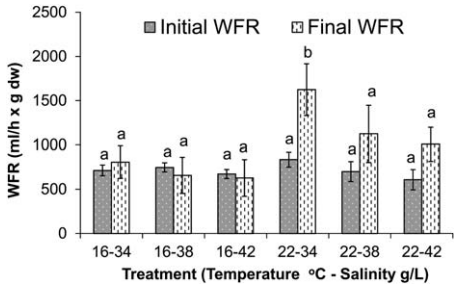

Figure 9. Weight-specific filtration rate (WFR) in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C determined 0.5 h after the first feeding on day 12 (initial) and on day 55 (final). Treatments with different letters indicate significantly different values (Tukey, $P < 0.05$); treatments without letters indicate no differences between treatments.

observed with a temperature increase in relation to both NH_3-N ($P = 0.02$; WFR = 15,645·NH_3-N + 358.62; $R^2 = 0.85$) and NH_4^+-N levels ($P = 0.03$; WFR = 1,186·NH_4^+-N + 390.9; $R^2 = 0.76$; Fig. 10).

DISCUSSION

In scallops, filtration rate can vary in relation to salinity, temperature, algal quantity, supply, and flow rate (MacDonald et al. 2006), but will not be affected within certain ranges (Bricelj & Shumway 1991). Although this work did not evaluate the effects of TAN (NH_4^+-N + NH_3-N) levels in the WFR response of *A. purpuratus* juveniles, we measured the TAN values throughout the experiment. Both ionized (NH_4^+-N) and un-ionized (NH_3-N) ammonia can be toxic, affecting survival, growth, and physiological parameters such as filtration rate, oxygen consumption, or ammonia excretion (Colt & Armstrong 1981). The fraction of TAN in the un-ionized or ionized form is dependent on pH, temperature, and salinity. The un-ionized form is proportionally more toxic and is facilitated by an increase in pH and temperature. An increase in salinity, on the other hand, produces the opposite effect (Colt & Armstrong 1981, Lawson 1995). Concentrations higher than 0.05 NH_3-N mg/L and 1 NH_4^+-N mg/L have been shown to be toxic to most fish, crustaceans, and molluscs (Colt & Armstrong 1981, Lawson 1995, Huchette et al. 2003). In the abalone *Haliotis midae* (Linnaeus, 1758), it has been reported that 0.0074 NH_3-N mg/L reduced the specific growth rate in about 50% (Reddy-Lopata et al. 2006). In addition, juvenile greenlip abalone, *Haliotis laevigata* (Donovan, 1808) showed 5% and 50% growth reductions when reared at 0.041 NH_3-N mg/L and 0.158 NH_3-N mg/L, respectively (Harris et al. 1998). Juvenile *Argopecten irradians irradians* (Lamarck, 1819) (7.2–26.4 mm) exposed to concentrations greater than 1.0 mg NH_3-N mg/L resulted in 100% mortality within 72 h (Widman et al. 2008). Our experimental closed-culture design could be viewed as a chronic and cyclical exposure to NH_3-N levels (Table 1) resulting from the 24-h accumulation of this compound between water exchanges at 22°C, which had higher NH_4^+-N and NH_3-N concentrations than at 16°C. Throughout the experiment, NH_3-N and NH_4^+-N reached values (Table 1) reported as affecting growth rates in other mollusc species (Harris et al. 1998, Reddy-Lopata et al. 2006). However, there are no reports on specific safety ranges of nitrogen compound concentrations for rearing *A. purpuratus*. This allows us to draw some conclusions regarding the relative importance of pH, salinity, temperature, and ammonia as factors influencing scallop filtration rates. Like TAN levels, DO has been reported as another key factor limiting production in aquaculture (Colt & Armstrong 1981, Lawson 1995). Soria et al. (2007) reported that metabolic rates of *A. purpuratus* juvenile scallops did not change when it decreased to 85% DO. DO levels in our experiment were higher than 90% (Table 1) in all treatments, and we assume there was no effect on filtration rates resulting from DO levels.

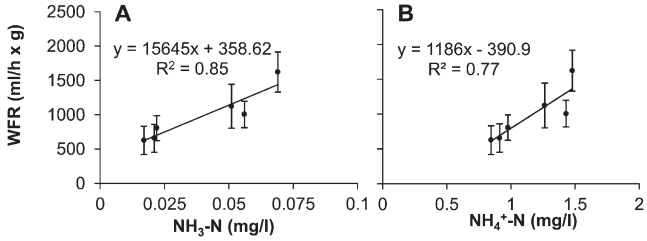

Figure 10. (A, B) Relationship between mean final WFR and NH_3-N (A) and NH_4^+-N (B) concentration values measured throughout the experiment in *A. purpuratus* juveniles reared at 3 salinities (34, 38, and 42 g/L) at 16°C and 22°C. All WFR data were pooled against NH_3-N as well as against NH_4^+-N. Each NH_3-N or NH_4^+-N value is based on 18 measurements on 6 occasions. Vertical lines represent SDs.

Epifanio and Srna (1975) reported that filtration rate for both *Mercenaria mercenaria* (Linnaeus, 1758) and *Crassostrea virginica* (Gmelin 1791) juveniles were more affected than adults when exposed to TAN levels from 110–880 mg/L. Although these values are higher than TAN values (NH_3-N plus NH_4^+-N) determined in our study (Table 1), a positive significant regression was found between WFR with both NH_3-N and NH_4^+-N increments throughout the experiment (Fig. 10). It is possible that TAN levels observed in this study for *A. purpuratus* were not high enough to reduce WFR, considering that pH values (near 8.0) were within a range where most TAN is present as NH_3-N (Merino 2005).

Navarro and González (1998) reported that *A. purpuratus* filtration rate ranging in SH from 25–100 mm (0.1–5.7 g dry tissue weight) was not affected by decreasing salinity from 30 g/L to 27 g/L, but was significant reduced to less than 27 g/L to 18 g/L of salinity when reared in CAS at 12°C and fed *I. galbana*. A similar pattern was confirmed by Laing (2002) for *P. maximus* juveniles (SH, 6–10 mm). Laing (2002) suggests that maintaining filtration rate requires a higher energy demand on scallops and can have a detrimental effect on survival and growth. In *P. maximus* juveniles (SH, 30 mm), filtration rate varied little when scallops were reared in CAS at salinities between 33 g/L and 23 g/L at either 9°C and 5°C, but was 4–5-fold lower at 5°C. At 9°C, filtration rate response capacity is decreased at 20 g/L salinity. Filtration rate (standardized to 3 g in dry tissue weight) did not differ between 16°C and 22°C for *A. purpuratus* parental stock reared in CAS and fed a continuous regimen for reproductive conditioning (Navarro et al. 2000).

On days 12 and 55, scallops showed an increasing trend in WFR between the first algae delivery to the second delivery at 24 h. WFR also showed an overall increasing trend between days 12 and 55. High WFRs found in the group of scallops reared at 22°C can be attributed to a significant reduction in tissue weight shown by juveniles. In *Argopecten ventricosus-circularis* (Sowerby II, 1842) (SH, 10.5 mm), WFR did not differ

within the 16°C and 22°C, but was significantly depressed at higher temperatures (25°C and 28°C) (Sicard et al. 1999). Similar results were found in *Argopecten irradians* and *Chlamys opercularis* (Linnaeus, 1758) (Bricelj & Shumway 1991). It is possible that WFR reductions can mirror a detrimental effect as a result of higher temperature together with long-term exposure to TAN levels instead of an increased salinity effect. Also, at the end of the experiment, scallops reared at 22°C showed smaller shell sizes as well as a decreased survival rate (Soria et al. 2007). This undesirable outcome could be avoided by several methods (i.e., increasing water exchange, reducing scallop density and feed quantity, or proper biofilter design for RAS) to avoid detrimental levels of TAN. Information about TAN effect on filtration rate is not available for *A. purpuratus* so far, so we suggest further analysis of these aspects to understand its relationship. Nonetheless, the effect of TAN levels on scallop feeding behavior should be the subject of further studies to clarify in detail the physiological effects of short- and long-term exposure.

The amount of feed that scallops take up depends on the concentration of algae in the rearing media, and the corresponding filtration rate of the scallops, at the prevailing temperature. Laing (2004) reported that the king scallop (*P. maximus*) was able to maintain an adequate feed intake independent of algae concentration by regulating its filtration rate. Similar findings were described by Palmer (1980) for the bay scallop (*A. irradians*) for a feed suspension between 0.94–9.66 mg/L. In contrast, Rheault and Rice (1996), also working with bay scallops, found that clearance rates were constant over a wide range of chlorophyll concentrations. However, these results may have been obtained under algae-limited conditions. On the other hand, very high cell concentrations inhibit scallop filtration as a result of saturation of the alimentary canal, causing the animals to close their valves and restrict the filtration rate (Riisgård 2001). The filtration rates are depressed to such an extent that scallops obtain less feed. Therefore, filtration rate could be accelerated, kept constant, or inhibited, depending on algae concentration, which would have a direct impact on scallop growth rate (Laing 2004, Rheault & Rice 1996, Riisgård 2001, Martínez et al. 1995). Algae concentrations used in the current research were within the range studied by Palmer (1980) and Laing (2004), and therefore it could be assumed that *A. purpuratus* filtered algae continuously. Hence, differences in filtration rate reported here are related to the studied environmental factors. Martínez et al. (1995) reported that juveniles of *A. purpuratus* fed 1 or 2 portions per day will experience a higher cell density for an initial period of time than when the same total amount of algae is provided continuously, although in the latter case ammonia excretion increased at least 4 times. When cell density exceeds the upper critical level, the feed provided to *A. purpuratus* will not be filtered and ingested efficiently. In another study with *A. purpuratus,* no significant differences in clearance rates were observed between 16°C and 20°C (Navarro et al. 2000).

Soria et al. (2007) reported that high survival rates were observed at higher salinities, indicating that juvenile *A. purpuratus* are able to withstand increased salinities, although a considerable decrease in meat weight would be expected when reared for a longer time of period, as we observed in the current study. Given that *A. purpuratus* juveniles (SH, 6.5 mm) showed a different pattern in WFR (WFR was not significantly affected by salinity, temperature, or the interaction between factors) and experienced lower survival rates (Soria et al. 2007), the results presented here should be interpreted with caution outside the SH range described in this experiment.

Despite the rearing condition, the abrupt decrease in algae concentration 0.5 h after feeding suggests that juvenile scallops were able to filter. Even though it might be expected, a detrimental effect during algae digestion, and thus affecting the efficiency of nutrients absorption capabilities (see Fig. 10A in Soria et al., 2007), which could be related to the higher levels of TAN at lower salinities. However, the increment in salinity causes a reduction in NH_3-N proportion that, in turn, could reduce the toxicity of TAN levels (Lawson 1995). However, at salinities greater than 38 g/L, osmotic stress becomes more important, signifying an upper salinity limit (Soria et al. 2007). And of course, there is still the effect that as pH increases, a higher proportion of NH_3-N is present for a given TAN.

During the grow-out transferring processes (e.g., from mesh larvae collectors deployed in the sea to pearl net, and from the latter to lantern nets), juveniles are brought to the laboratory and maintained in CAS or flow-through systems (Merino et al. 2001). Sometimes, transferring scallops to the sea may be delayed because of weather or oceanographic constraints, as well as the fragility of the small scallops. A land-based nursery could be introduced as an intermediate step to bridge hatchery postlarval growth and grow-out in the sea to increase yield and to stabilize spat production (Magnesen & Christophersen 2007). Another strategy to solve this issue may be to keep juveniles in RAS until favorable conditions arise (Merino et al. 2009). An effective design of RAS requires knowledge about the physiological response of scallops to different physical–chemical water parameters, especially those that accumulate in aquaculture systems (i.e., TAN, suspended solids, CO_2) where large numbers of scallops are reared in relatively small water volumes.

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LITERATURE CITED

Avendaño, M., M. Le Pennec & M. Cantillanez. 2001. Anormalidades en larvas de *Argopecten purpuratus* (Lamarck, 1819) (Mollusca: Pectinidae), uno de los problemas en la producción artificial de semilla. *Estudios Oceanol.* 20:33–42.

Bricelj, V. M. & S. E. Shumway. 1991. Physiology: energy acquisition and utilization. In: S. E. Shumway, editor. Scallops: biology, ecology and aquaculture. Amsterdam: Elsevier. pp. 305–337.

Christophersen, G. & O. Strand. 2003. Effect of reduced salinity on the great scallop, *Pecten maximus* spat at two rearing temperatures. *Aquaculture* 215:79–92.

Christophersen, G., L. Torkildsen & T. van der Meeren. 2006. Effect of increased water recirculation rate on algal supply and post-larval performance of scallop (*Pecten maximus*) reared in a partial open and continuous feeding system. *Aquacult. Eng.* 3:271–282.

Colt, J. E. & D. A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish and molluscs. In: L. J. Allen & E. C. Kinney, editors. Proceedings of the Bio-Engineering Symposium for Fish Culture. Bethesda, MD: Fish Culture Section of the American Fisheries Society. pp. 34–47.

Coughlan, J. 1964. The estimation of filtering rate from the clearance of suspensions. *Mar. Biol.* 2:356–358.

DiSalvo, L.H., E. Alarcón, E. Martinez & E. Uribe. 1984. Progress in mass culture of *Chlamys* (*Argopecten*) *purpuruta* (*1819*) with notes on its natural history. *Rev. Chil. Hist. Nat.* 57:3545.

Epifanio, C. E. & R. F. Srna. 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. *Mar. Biol.* 33:241–246.

Fernández-Reiriz, M. J., J. M. Navarro & U. Labarta. 2005. Enzymatic and feeding behaviour of *Argopecten purpuratus* under variation in salinity and food supply. *Comp. Biochem. Physiol.* 141:153–163.

Fivelstad, S. 1988. Waterflow requirements for salmonids in single-pass and semi-closed land-based seawater and fresh-water systems. *Aquacult. Eng.* 7:183–200.

González, M. L., D. A. López, M. C. Pérez & J. M. Castro. 2002. Effect of temperature on the scope for growth in juvenile scallops *Argopecten purpuratus* (Lamarck, 1819). *Aquacult. Int.* 10:339–349.

Harris, J. O., G. B. Maguire, S. Edwards & S. M. Hindrum. 1998. Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, *Haliotis laevigata* Donovan. *Aquaculture* 160:259–272.

Huchette, S. M. H., C. S. Koh & R. W. Day. 2003. Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tanks: effects of density and ammonia. *Aquaculture* 219:457–470.

Laing, I. 2002. Effect of salinity on growth and survival of king scallop spat (*Pecten maximus*). *Aquaculture* 205:171–181.

Laing, I. 2004. Filtration of king scallops (*Pecten maximus*). *Aquaculture* 240:369–384.

Lawson, T. B. 1995. Water quality and environmental requirements: fundamental of aquacultural engineering. London: Chapman & Hall. pp. 12–39. 355 pp.

MacDonald, B. A., V. M. Bricelj & S. E. Shumway. 2006. Physiology: energy acquisition and utilisation. In: S. E. Shumway & G. J. Parsons, editors. Scallops: biology, ecology and aquaculture. Amsterdam: Elsevier. 417–492 pp.

MacMillan, R., R. Cawthorn, S. Whyte & E. Lyon. 1994. Design and maintenance of a closed artificial seawater system for long-term holding of bivalve shellfish. *Aquacult. Eng.* 13:241–250.

Magnesen, T. & G. Christophersen. 2007. Large-scale raceway nursery for improved scallop (*Pecten maximus*) spat production. *Aquacult. Eng.* 36:149–158.

Martínez, G., L. Caceres, E. Uribe & M. Diaz. 1995. Effects of different feeding regimens on larval growth and the energy budget of juvenile Chilean scallops, *Argopecten purpuratus* Lamarck. *Aquaculture* 132: 313–323.

Merino, G. 2005. Balance de masas: principios de ingeniería en diseño y operación de sistemas de recirculación para el cultivo de moluscos. In: E. von Brand, editor. Curso de tecnología de recirculación de agua aplicada al cultivo de moluscos. Coquimbo: Universidad Católica del Norte. pp. 9–20.

Merino, G., A. Cortés-Monroy & J. Barraza. 2001. Diseño y operación de sistemas de cultivo. In: A. N. Maeda-Martínez, editor. Los moluscos pectínidos de Iberoamérica: ciencia y acuicultura. Mexico City, Mexico: Limusa. pp. 375–404.

Merino, G., E. Uribe, G. Soria & E. von Brand. 2009. A comparison of larval production of the northern scallop, *Argopecten purpuratus*, in closed and recirculating culture systems. *Aquacult. Eng.* 40:95–103.

Navarro, J. M. & C. M. González. 1998. Physiological responses of the Chilean scallop, *Argopecten purpuratus* to decreasing salinities. *Aquaculture* 167:315–327.

Navarro, J. M., G. E. Leiva, G. Martínez & C. Aguilera. 2000. Interactive effects of diet and temperature on the scope of growth of the scallop *Argopecten purpuratus* during reproductive conditioning. *J. Exp. Mar. Biol. Ecol.* 247:67–83.

Palmer, R. E. 1980. Behavioral and rhythmic aspects of filtration in the bay scallop, *Argopecten irradians concentricus* (Say), and the oyster, *Crassostrea virginica* (Gmelin). *J. Exp. Mar. Biol. Ecol.* 45:273–295.

Paul, J. D. 1980. Salinity and temperature in the queen scallop *Chlamys opercularis*. *Mar. Biol.* 56:295–300.

Reddy-Lopata, K., L. Auerswald & P. Cook. 2006. Ammonia toxicity and its effect on the growth of the South African abalone *Haliotis midae* Linnaeus. *Aquaculture* 261:678–687.

Rheault, R. B. & M. A. Rice. 1996. Food-limited growth and condition index in the eastern oyster, *Crassostrea virginica* (Gmelin 1791), and the bay scallop, *Argopecten irradians irradians* (Lamarck 1819). *J. Shellfish Res.* 15:271–283.

Riquelme, C. E., M. A. Jorquera, A. I. Rojas, R. E. Avendaño & N. Reyes. 2001. Addition of inhibitor-producing bacteria to mass cultures of *Argopecten purpuratus* larvae (Lamarck, 1819). *Aquaculture* 192:111–119.

Riisgård, H. U. 2001. Physiological regulation versus autonomous filtration in filter-feeding bivalves: starting points for progress. *Ophelia* 54:193–209.

Rupp, & J. Parson. 2004. Effects of salinity and temperature on the survival and byssal attachment of the lion's paw scallop *Nodipecten nodosus* at its southern distribution limit. *J. Exp. Mar. Biol. Ecol.* 309:173–198.

Shumway, S. E. 1977. Effect of salinity fluctuation on the osmotic pressure and Na^{+}, Ca^{2+} and Mg^{2+} ion concentrations in the hemolymph of bivalve molluscs. *Mar. Biol.* 41:153–177.

Sicard, M. T., A. Maeda-Martínez, P. Ormart, T. Reynoso-Granados & L. Carvalho. 1999. Optimum temperature for growth in the Catarina scallop, *Argopecten ventricosus-circularis*, Sowerby II, 1842. *J. Shellfish Res.* 18:385–392.

Singnoret-Brailowski, G., A. Maeda-Martínez, T. Reynoso-Granados, E. Soto-Galera, P. Monsalvo-Spencer & G. Valle-Meza. 1996. Salinity tolerance of the Catarina scallop *Argopecten ventricosus-circularis* (Sowerby II, 1842). *J. Shellfish Res.* 15:623–626.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14:799–801.

Soria, R. G., G. Merino & E. von Brand. 2007. Effect of increasing salinity on physiological response in juvenile scallops *Argopecten purpuratus* at two rearing temperatures. *Aquaculture* 270:451–463.

Strand, O., P. T. Solberg, K. K. Andersen & T. Magnesen. 1993. Salinity tolerance of juvenile scallops (*Pecten maximus* L.) at low temperature. *Aquaculture* 115:169–179.

Tettelbach, S. T. & E. W. Rhodes. 1981. Combined effects of temperature and salinity and embryo and larvae of the Northern bay Scallop *Argopecten irradians irradians*. *Mar. Biol.* 63:249–256.

Uriarte, I., G. Rupp & A. Abarca. 2001. Producción de juveniles de pectínidos Iberoamericanos bajo condiciones controladas. In: A. N. Maeda-Martínez, editor. Los moluscos pectínidos de Iberoamérica: ciencia y acuicultura. México: Editorial Limusa. pp. 147–171.

von Brand, E., G. Merino, A. Abarca & W. Stotz. 2006. Scallop fishery and aquaculture in Chile. In: J. Parsons, editor. Scallop, biology, ecology and aquaculture: developments in aquaculture and fisheries sciences. The Netherlands: Elsevier, pp. 1293–1311. 1460 pp.

Widman, J. C. 1998. Design of a recirculating nursery culture system for the bay scallop, *Argopecten irradians irradians*. *J. Shellfish Res.* 17:364. (abstract).

Widman, J., S. Meseck, G. Sennefelder & D. Veilleux. 2008. Toxicity of un-ionized ammonia, nitrite, and nitrate to juvenile bay scallops, *Argopecten irradians irradians*. *Arch. Environ. Contam. Toxicol.* 54:460–465.