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Response and condition of larvae of the scallops *Nodipecten subnodosus* and *Argopecten ventricosus* reared at the hatchery with different seawater sources

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ABSTRACT

Larvae of the scallops *Nodipecten subnodosus* and *Argopecten ventricosus* were reared at a hatchery under five seawater sources: (1) filtered seawater, as the control group; (2) pasteurized seawater; (3) seawater taken from a well; (4) synthetic seawater, and (5) seawater containing a commercial probiotic (Epicin). The quality of each seawater source was measured in terms of counts of *Vibrio* pathogenic bacteria, levels of nitrites, nitrates, and ammonium, and content of suspended and organic matter. Overall response of larvae under each treatment was measured in terms of growth, survival, biochemical composition, and recruitment rate of spat. Differences in all these parameters, as a function of the seawater source, were analyzed with one-way ANOVA. Larvae survived more, grew faster and larger, had higher protein levels, and recruited more in filtered seawater (in *N. subnodosus*) and pasteurized seawater (in *A. ventricosus*), but significant differences between treatments were slight. In *A. ventricosus* cultures, filtered seawater favored significantly higher *Vibrio* counts than pasteurized seawater, but this result did not affect the response and condition of larvae. The well seawater treatment ranked third in terms of low bacterial counts, high nitrate levels, larvae showing relatively high survival and growth rates, and spat reaching the settlement stage (only in *A. ventricosus*). The synthetic seawater and Epicin treatments did not yield satisfactory results for any of the indicators measured. In fact, in the Epicin treatment, larvae survived only the first days of hatchery cultivation and settlement occurred only in *A. ventricosus*. The results from these first set of trials are useful for planning additional experiments aimed to improve the protocol of seawater use at our research hatchery.
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1. Introduction

In bivalve hatcheries, the provision of seawater of the best quality is essential for maximizing survival and performance of larvae throughout all developmental stages (Helm, 1971; Waller, 1981; Helm et al., 2006). The term seawater quality' is relatively ambiguous and difficult to tag as good or bad without defining physical, chemical, and biological aspects of the source (Poxton, 2003; Simoes et al., 2002). These aspects, however, may be unique for a particular species and may also vary seasonally and in relation to the purpose of the study (Gretchen et al., 2004). In hatcheries, seawater quality also refers to the way incoming raw water is treated and mixed with growing larvae and cultured microalgae. Therefore, defining reliable criteria for assessing the quality of seawater is necessary, perhaps even critical, when rearing bivalve larvae.

Among many factors, one that strongly affects the quality of seawater used for rearing bivalves in hatcheries is the presence of bacterial communities, either pathogenic such as *Vibrio* and *Pseudomonas* (Riquelme et al., 1997; Luna-González et al., 2002; Saínz-Hernández and Maeda-Martínez, 2005) or beneficial such as bacilli, lactobacilli, or marine yeasts (Gómez-Gil et al., 2000; Balcázar et al., 2006). Other factor influencing seawater quality is the level of certain inorganic and organic compounds, among which ammonium, nitrates, and nitrites are particularly important (Colt and Armstrong, 1981; Weiling et al., 1995). All these factors affect overall development and condition of the larvae, including filtration and ingestion rates, growth and survival rates, and general vigor and viability to complete metamorphosis and settle (Pouvreau et al., 2000; Strugnell and Southgate, 2003; Sicard-González et al., 2006).

In hatcheries, raw seawater is usually treated to control the spread of pathogens and the increase of inorganic components. Traditionally, methods used to treat seawater include mechanic and chemical filtration, UV radiation, ozone disinfection, chlorination, and treatment with antibiotics or probiotics (Verschuere et al. 2000; Graeff and Mondardo, 2006; Helm et al., 2006). Although some of these methods work relatively well, they usually need to be combined for reducing or eliminating the load of harmful bacteria or suspended and dissolved materials. This strategy may, however, eliminate some important components from seawater, such as trace elements. In aquaculture, there are alternate seawater sources that, while scarcely explored, offer potential benefits for rearing larvae in hatcheries. For example, synthetic seawater has been successfully employed for completing larval development of crustaceans (Kanaujia et al., 1996; De Araujo et al.,

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2002) and the bivalves *Ostrea edulis* and *Crassotrea gigas* (Courtright et al., 1971; Helm, 1971). Underground seawater has also been used for larval rearing of some crustaceans (Weiling et al., 1995; Rahman et al., 2005) and fish (Scholes, 1980; Nakasone and Akea, 2000). The use of pasteurized seawater is rare, but recent studies suggest it contains ~95% less heterotrophic and TCBS + bacteria in cultures of *Argopecten ventricosus* larvae (Saínz-Hernández and Maeda-Martínez, 2005) and marine microalgae (Acosta-Galindo, 2006). Additionally, seawater treated with commercial probiotics (Bactosafe®, Epicin®, Estibion®) can reduce the level of harmful bacteria and organic matter, hence increase the quality of seawater used for cultivating fish larvae (Graeff and Mondardo, 2006) and shrimp larvae (Guerrero-Avalos, 2007). However, the value of these commercial probiotics remains unexplored with bivalve larvae.

This paper reports the results of a first set of trials evaluating different seawater sources with potential application for larval rearing of two scallops of economic value in Northwestern Mexico: the lions-paw scallop *Nodipecten subnodosus* (Sowerby, 1835) and the catarina scallop *A. ventricosus* (Sowerby II, 1835). These treatments of seawater were evaluated for levels of pathogenic microbes, some essential inorganic and organic compounds, and their effects on growth, survival, and biochemical composition of larvae, as well as total recruitment of spat.

2. Materials and methods

2.1. Origin of larvae

To obtain viable larvae from both species, broodstock were collected during their natural breeding seasons from two coastal sites of the Baja California Peninsula. This includes Laguna Ojo de Liebre (27°41′N, 113°59′W) in March–April for *N. subnodosus* and Bahía Madgalena (24°48′N, 112°05′W) in July–August for *A. ventricosus*. In both cases, broodstock were induced to spawn by thermal stimulation and the resulting gametes were separated by sex. Fertilized eggs were placed in two 1500-l fiberglass tanks at 40–50 embryos ml^{-1} and temperatures held at 25 ± 1 °C for the two species (Serrano-Guzmán et al., 1997; Mazón-Suástegui, 2005; Mazón-Suástegui et al., 2003). Both tanks were drained after 20–24 h and the early D-larvae were counted and used as follows.

2.2. Experimental design

One-day-old veliger larvae were stocked in 60-l conical tanks at densities of 5–6 larvae ml^{-1}. Triplicate tanks for raising the larvae were managed for each of the following seawater sources: (1) filtered seawater as the control group; (2) pasteurized seawater; (3) seawater taken from a beach well; (4) synthetic seawater (AZOO Reef Salt® AZ28001), and (5) filtered seawater containing a commercial probiotic (Epicin® Epicore Bionetworks, Mount Holly, NJ). Details of the routine treatment that each seawater source received before their use at the hatchery are presented in Table 1.

Maintenance during overall development of larvae followed procedures described by Serrano-Guzmán et al. (1997) for *N. subnodosus* and Mazón-Suástegui (2005) and Mazón-Suástegui et al. (2003) for *A. ventricosus*. In brief, larvae were fed once daily with a 1:1 ratio (by cell count) of *Isochrysis galbana* and *Pavlova salina* (from day 1 to umbo stage) and a 1:1:1 ratio of *I. galbana*, *P. salina*, and *Chaetoceros muelleri* (from umbo stage to settlement). Data of temperature, salinity, and food regimes used during the larval runs are shown in Table 2. Tanks were drained, washed, and refilled with fresh seawater every third day.

2.3. Survival, growth, and settlement of larvae

To estimate growth and survival, 1 ml samples of larvae were collected in triplicate during the tank draining procedure. Larvae were fixed in 3% formalin solution and counted under the microscope (10×) on a Sedgewick rafter cell to estimate mean survival percentages for each seawater treatment and species. Additionally, the relative mortality rate (*RMR*) was also calculated per treatment and species:

$$RMR = [(N_{final} / N_{initial})] / t,$$

where Ln = natural logarithm, N = number of live larvae, and t = time (days).

Samples were also used to estimate larval growth in shell height (μm). This was done by photographing with a digital camera 30 larvae from each of the triplicate sieve samples and transferring the resulting images to the computer for determining changes in size with Image Pro Plus (v. 5.1, Media Cybernetics, Bethesda, MD). Growth rate of larvae (*GR* in mm day^{-1}) was calculated for each treatment and species:

$$GR = (T_{final} - T_{initial}) / t,$$

where T = size of larvae at the start and end of the trials and t = time (days).

When 60% of the larval population reached the pediveliger stage (~150–180 μm height for both species), artificial collectors made from

Table 1
Routine treatment of raw seawater at CIBNOR's hatchery and its additional treatment to test each of the different seawater sources used during hatchery-rearing of *N. subnodosus* and *A. ventricosus* larvae.

Experimental source	Raw seawater treatment						Source treatment					
	Filtered (20 μm)	Sand filter	Cartridge filter (1 μm)	Cartridge filter (1 μm) (w/activated charcoal)	UV radiation	Gaff-filter bag (1 μm)	Filtered seawater	Pasteurized seawater	Well seawater	Synthetic seawater	Seawater with Epicin	Culturing tanks
Filtered seawater	×	×	×	×	×	×	×					×
Pasteurized seawater	×					×		*				×
Well seawater						×			**			×
Synthetic seawater						×				***		×
Seawater with Epicin	×	×	×	×	×	×					****	×

*Steps: (1) reservoir tank; (2) water heather with thermostat set at 54–60 °C; (3) water chillier set at 30 °C.
**Naturally filtered from a beach well at 10-m depth.
***Prepared according to the specifications of the supplier (AZOO® 200-l Reef Salt).
****Prepared according to the specifications of the supplier (Epicore Bionetworks Inc) at 0.1 g ml^{-1}.