Early Larval Development of *Donax obesuslus*: Response to el Niño Temperature and Salinity Conditions

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INTRODUCTION

Environmental conditions of the Humboldt Current System (HCS) are quite stable compared with those of other coastal ecosystems at similar latitudes, in terms of primary production and fluctuations of intrannual temperature and oxygen conditions (e.g., Arntz et al. 1987, Camus 2001, Thiel et al. 2007). However, the mechanisms by which these changes effect change on the structure of coastal populations remains largely unknown. The surf clam Donax obesulus is dominant on large sandy beaches of the Humboldt Current System. Its biogeographical distribution is largely influenced by EN-induced environmental changes. Despite the species’ key role in the beach ecosystem, the effects of modified abiotic conditions on the meroplanktonic larval stages and threshold temperatures involved have not yet been investigated.

After EN episodes, meroplanktonic larval stages play a crucial role in the medium- and long-term stability of shallow-water species. Thus, this study makes a first attempt to describe the ontogeny of Donax obesulus and examines the effects on development of EN temperature conditions (ENTC) in comparison with normal temperature conditions (NTC). Results indicate that early life history follows a pattern previously described for other donacid bivalves. Development, growth, and mortality of larvae were assessed during a 3-wk in vitro experiment, indicating that larvae reared under ENTC grew and developed faster in comparison with those reared under NTC; mortality was slightly higher under ENTC. During a 2nd experiment, larvae were exposed for 48 h to a distinct range of different salinities (35, 25, 15, and 5 ± 1) at 2 different temperatures (NTC and ENTC). At both temperatures, larvae suffered no mortality at medium and low salinity (35, 25, and 15 ± 1) but showed 100% mortality at very low salinity (5 ± 1) after 16 h at NTC and 32 h at ENTC. Activity of larvae was highest at medium salinity (25 ± 1) and lowest at normal salinity (35 ± 1). The results of this study indicate that early larval stages of Donax obesulus can cope with temperature and salinity changes induced during EN. Only extremely low salinity (5 ± 1) such as that observed close to river mouths may cause high mortality rates in Donax obesulus offspring.

KEY WORDS: Bivalvia, Chile, early life history, Humboldt Current System, Peru, Donax

**EARLY LARVAL DEVELOPMENT OF DONAX OBESULUS: RESPONSE TO EL NIÑO TEMPERATURE AND SALINITY CONDITIONS**

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**ABSTRACT** The Humboldt Current System is a highly productive ecosystem that is subject to the dynamics of the El Niño Southern Oscillation (ENSO). El Niño (EN, the warm phase of ENSO) causes vital changes in surface water temperature, oxygen levels, and salinity conditions, which are reflected in various responses of coastal pelagic and benthic organisms. For very shallow habitats such as sandy beaches, temperature and salinity are considered the principal parameters changing during strong EN. However, the mechanisms by which these changes effect change on the structure of coastal populations remains largely unknown. The surf clam Donax obesulus is dominant on large sandy beaches of the Humboldt Current System. Its biogeographical distribution is largely influenced by EN-induced environmental changes. Despite the species’ key role in the beach ecosystem, the effects of modified abiotic conditions on the meroplanktonic larval stages and threshold temperatures involved have not yet been investigated. After EN episodes, meroplanktonic larval stages play a crucial role in the medium- and long-term stability of shallow-water species. Thus, this study makes a first attempt to describe the ontogeny of Donax obesulus and examines the effects on development of EN temperature conditions (ENTC) in comparison with normal temperature conditions (NTC). Results indicate that early life history follows a pattern previously described for other donacid bivalves. Development, growth, and mortality of larvae were assessed during a 3-wk in vitro experiment, indicating that larvae reared under ENTC grew and developed faster in comparison with those reared under NTC; mortality was slightly higher under ENTC. During a 2nd experiment, larvae were exposed for 48 h to a distinct range of different salinities (35, 25, 15, and 5 ± 1) at 2 different temperatures (NTC and ENTC). At both temperatures, larvae suffered no mortality at medium and low salinity (35, 25, and 15 ± 1) but showed 100% mortality at very low salinity (5 ± 1) after 16 h at NTC and 32 h at ENTC. Activity of larvae was highest at medium salinity (25 ± 1) and lowest at normal salinity (35 ± 1). The results of this study indicate that early larval stages of Donax obesulus can cope with temperature and salinity changes induced during EN. Only extremely low salinity (5 ± 1) such as that observed close to river mouths may cause high mortality rates in Donax obesulus offspring.

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which stimulate gonad maturation and gamete release (Riascos 2006, Petes et al. 2007). Gamete development of *D. obesulus* occurs mainly when water temperatures rise during the austral summer, and the spawning season takes place from April to July (Huaraz & Ishiyama 1980, Aguirre & Mendo 2008). The reproductive period of this species coincides with the period when water anomalies peak during EN, as recorded in 1982 through 1983 (Arntz et al. 1987). However, to achieve a better understanding of the population and distributional changes of *D. obesulus* during strong EN, it is important to assess further the early life history stages of the species. Neither the effects of environmental changes on the meroplanktonic larval stages of *D. obesulus*, nor the temperature thresholds involved have so far been investigated (Thiel et al. 2007).

Although the influence of temperature and salinity changes on adult surf clam species of the HCS have been examined before, the impact of the modified conditions on early larval stages of *D. obesulus* remains unknown (Riascos & Urban 2002, Riascos 2006, Riascos et al. 2009, Carstensen et al. in review). Therefore, the objectives of this study were (1) to describe early life stages of *D. obesulus* and (2) to analyze the effects of higher temperature and reduced salinity conditions, such as those recorded during EN, on the larval development of the species.

**MATERIALS AND METHODS**

**Sampling: Broodstock Conditioning and Spawning**

Adult specimens (>15 mm, n = 100) of *D. obesulus* were collected by hand from 18°27’S, 70°18’W, Chinchorro Beach, Arica, northern Chile, in October 2007. To minimize stress, clams were transported immediately to the Marine Laboratory of the University of Antofagasta and acclimatized in a temperature chamber for at least 2 wk at temperature (17.8 ± 0.2°C) and salinity (35 ± 1) reflecting ambient levels in the natural habitat. This broodstock was maintained in a 200-L tank filled with 13 cm sterilized sand. Seawater was filtered (1 μm), ultraviolet light treated, and aerated. Twenty percent of the seawater was exchanged weekly to prevent contamination with excreted waste. Animals were exposed to a 12-h day/night cycle. Dead clams were removed daily (mortality, <5%). Because multispecies diets of microalgae are known to enhance larval survival (Ruiz-Azcona et al. 1996, Helm et al. 2004), the broodstock was fed *ad libitum* with a mixture (1:1) of living *Chaetoceros calcitrans* and *Isochrysis galbana*. The maturation of the initially inactive gonads was monitored weekly by dissecting five individuals taken at random from the maintenance tank. Gonad ripeness was monitored by observations of gonad smear preparations; small portions of excised tissue were microscopically observed on an object slide. Gonads were found to be fully mature after 4 wk of conditioning.

Experimental temperatures were defined according to the analysis of a long-term (1980 to 2006) sea surface temperature database compiled by the Servicio Hidrográfico y Oceanográfico de la Armada de Chile (http://www.shoa.cl). The normal temperature condition (NTC) was defined as the long-term average sea water temperature (17.8 ± 0.2°C). The EN temperature condition (ENTC) was considered to be the highest monthly mean sea water temperature registered by the Arica station (18°28’S, 70°19’W) during EN 1982 through 1983 (24.6 ± 0.2°C). Likewise, ambient salinity of 35 ± 1 was taken as normal, whereas 25, 15, and 5 ± 1 were chosen arbitrarily to represent medium, low, and very low levels of salinity.

To induce spawning, mature specimens of the broodstock were exposed to a 5°C increase in water temperature (23 ± 0.2°C) until gamete release stopped (<3 h). Preliminary experiments elicited a weaker spawning response in specimens exposed to lower temperature increases. Female individuals released fluffy batches of eggs a few millimeters in size, which quickly sank to the bottom after spawning, whereas males released a milky liquid substance. To prevent uncontrolled intermixture of germ cells, spawning specimens were separated according to sex as soon as visual identification of germ cells could be made. Released oocytes and sperms were carefully extracted from extended siphons using a Pasteur pipette, and stored in separate glass beakers. Finally, the number of oocytes and sperm cells produced was estimated microscopically. Thereafter, germ cells were mixed under sterile conditions in a ratio of 1:10 (oocyte to sperm).

**Early Larval Development Under Normal and El Niño Temperatures**

The early larval stages of *D. obesulus* were described using light microscopy (Leica DM LS2, Solms, Germany) and documented with photography (Canon Powershot S50, Tokyo, Japan) over an 18-day period after fertilization. For the first two days, embryonic stages were observed hourly; thereafter, samples of larvae from both treatments were taken daily with a Pasteur pipette.

To describe the effects of ENTC on development, growth, and mortality, early larvae (D-Veliger, >48 h) were exposed to NTC and ENTC for a period of 16 days. Larvae were cultured under the conditions described earlier for the broodstock, with the exception that 3 replicate 1-L glass beakers were used for each temperature condition instead of 1 large tank. Using a Sedgekew Rafter counting cell slide (PYSER-SGI, England), the total number of live (abundance) and dead (mortality) larvae was determined. To compensate for increasing larval size, the density of each culture was reduced from ~50 larvae/mL to ~25 larvae/mL on the 10th day. To minimize contamination by bacteria and ensure good water quality, each replicate was sieved (mesh size, 100 μm) and passed into a sterile glass beaker with new water and microalgae daily. Samples of 1 mL were taken from each of the 3 replicates to determine daily abundance and mortality. Mortality was estimated by counting empty larval shells in each sample. To determine larval growth (maximum posterior–anterior length), 30 individuals of each replicate were evaluated daily.

**Impact of El Niño Temperature and Lower Salinity on Early Larvae of *D. obesulus***

To test the combined effect of ENTC and reduced salinity, larvae (>48 h) were randomly assigned to a 4 × 2 factor experimental design: 4 salinities (35, 25, 15, and 5 ± 1) at NTC (17.8 ± 0.2°C) and 4 salinities at ENTC (24.6 ± 0.2°C) for 48 h. To obtain the exact prescribed salinity, seawater (35 ±1) was diluted with the appropriate volume of distilled water. For each of the eight conditions, 20 replicates (4-L, plastic beakers) were examined, each containing 1 larva. Larvae were not fed during the experiment to avoid changes in experimental parameters. Dead larvae (defined as inactive, larval shell open, velum
extended) were registered every 8 h. A dosage–mortality approach (Urban 1994, Laudien et al. 2002) was used to determine the time after which 50% of the experimental population had died (LT50). This parameter was obtained by plotting the relationship between time and mortality, and extrapolating the time corresponding to 50% mortality. To estimate viability of larvae, specimens were recorded as swimming actively or not. Observations were carried out every 8 h between 24 h and 48 h after the experiment started.

**Statistical Analysis**

**Development and Growth of Larvae Under Normal and El Niño Conditions**

To evaluate the effect of two different temperature conditions on the growth of *D. obtusulus* larvae, a 2-way analysis of covariance (ANCOVA) was performed using time (day) as a steady factor, and temperature and replication as categorical factors. To apply the ANCOVA, length was log-transformed to ensure a linear correlation between time and length. To evaluate differences in mortality, a 1-way analysis of variance (ANOVA) model was applied. The model treated mortality as a dependent variable and temperature as an independent variable.

**Activity and Mortality of Larvae Under El Niño Temperature and Lower Salinity**

To test for significant differences in the activity of *D. obtusulus* larvae between treatments of different temperature and salinity, a 2-way ANOVA model was used. The model treated activity of larvae as a dependent variable, and temperature and salinity as independent variables. Significant differences between levels were tested using the Tukey HSD post hoc test. To assess significant differences in mortality at the lowest salinity (5 ± 1), a 1-way ANOVA model was used. The model treated mortality (hours of survival) as a dependent variable and temperature as an independent variable.

**RESULTS**

**Broodstock: Conditioning and Spawning**

Immature specimens of the broodstock were successfully conditioned within 4 wk under *ad libitum* nutrition conditions. Spawning was induced by temperature shock treatment (+5°C). Mature males (n = 17) had a minimum shell length (SL, maximum anterior–posterior) of 16.8 ± 0.1 mm and a maximum SL of 24.7 ± 0.1 mm, whereas ripe females (n = 13) exhibited a minimum SL of 16.5 ± 0.1 mm and a maximum SL of 23.6 ± 0.1 mm. When exposed to the increased temperature, the majority of specimens released their germ cells after 1.5–2.5 h, but a proportion remained inactive. Oocytes were ejected in batches and sunk immediately after spawning to the bottom of the spawning jar (salinity, 35 ± 1). Milky sperm was released in single jets. Releases occurred in intervals of 1–5 min over a period of 15–30 min.

**Early Larval Development Under Normal and El Niño Temperatures**

Measured under a light microscope, the spermatozoid head exhibited a length of approximately 5 μm, whereas the tail was approximately 50 μm long. Unfertilized oocytes had a diameter of 59.34 ± 0.63 μm (n = 25). Larval development followed the typical sequence of successive stages for bivalve species and for Donacidae in particular. During the first 24 h, different stages of cell division were observed, followed by a ciliated blastula, a gastrula, and a trophophore stage. After 24 h, a D-Veliger larvae was formed (Fig. 1). The D-Veliger larva develops a rudimentary foot first, and later a probing foot that is characteristic of larvae ready to settle before metamorphosis takes place (Fig. 1).

Larval length (maximum anterior–posterior) increased during day 3 after fertilization, from 88.46 ± 0.27 (n = 90)–160.85 ± 1.95 μm (n = 90) under NTC and from 88.65 ± 0.32 μm (n = 90)–176.91 ± 1.90 μm (n = 90) at ENTC (Fig. 2). The increase in larval height (dorsal–ventral) was less than the increase in length at both temperatures. The growth of larvae under NTC and ENTC was significantly different (F1 = 358.0, P = 0.00). At 18 days postfertilization, larvae attained a maximum length of 213.5 μm at NTC, whereas larvae under ENTC reached a maximum length of 240.75 μm. Overall mortality during the experimental period was very low: 0.79 ± 0.11% at NTC, and 1.25 ± 0.37% at ENTC (Fig. 3). Rates of mortality did not differ significantly between treatments (F1 = 2.82, P = 0.36).

**Early Larvae Exposed to El Niño Temperature and Lower Salinity**

Exposure over a 48-h period to certain salinities (35, 25, and 15 ± 1) at NTC and ENTC, resulted in no larvae mortality. Larvae exposed to the lowest salinity (5 ± 1), however, suffered 100% mortality after 16 h (NTC) and 32 h (ENTC; Fig. 4) at a significant difference between temperatures (F1 = 4.87, P = 0.03). Under NTC, LT50 was reached after 4.3 h; under ENTC, LT50 occurred 6.5 h into the experiment (Fig. 4). Activity of larvae, assessed as counts of actively swimming individuals taken every 8 h between 24 h and 48 h into the experiment, showed similar tendencies at different salinities: For both temperature treatments, highest activity was observed at 25 ± 1 followed by 15 ± 1, whereas larvae at NTC and 35 ± 1 showed the least activity (Fig. 5). Statistical analysis revealed no significant differences in the activity of larvae between test temperatures (F1 = 0.308, P = 0.59). However, within different salinity levels (15, 25, and 35 ± 1), significant differences in activity were apparent (F2 = 18.29, P < 0.00). A Tukey post hoc comparison revealed significant differences in activity between salinities of 25 and 35 ± 1 at both NTC and ENTC (P = 0.01 and P = 0.01, respectively).

**DISCUSSION**

**Broodstock: Conditioning and Spawning**

Temperature shock treatment is a common method of inducing spawning in marine bivalve species, in which the temperature gradient applied depends on the habitat conditions of the species. To increase stimulation, it is common practice to add gametes from stripped or dissected individuals to the seawater (His et al. 1989, Ruiz-Azcona et al. 1996, Baba et al. 1999, Dudas & Dower 2006). Alternatively, spawning may be triggered artificially by the addition of chemicals such as ammonium hydroxide or the hormone serotonin. Regardless of the techniques used, relatively few studies have documented successful spawning and larval culture of Donacidae (e.g., Chanley 1969, Ruiz-Azcona et al. 1996), because specimens
often show resistance to the aforementioned methods (Ansell (1983) and references herein). In the current study, unripe broodstock was conditioned to ripeness within approximately 1 mo. To induce spawning, D. obesus was exposed to a temperature shock treatment (+5°C). No gametes or chemicals were added to the seawater. Interbreeding was successful and culture conditions were deemed favorable as a result of (1) a very low mortality rate among larvae (<2%) and (2) very few deformed stages of larvae (Tettelbach & Rhodes 1981, Helm et al. 2004).

Early Larval Development Under Normal and El Niño Temperatures

Early larval development under NTC and ENTC followed the typical pattern known for several marine bivalve species (Fig. 1) (Chanley 1969, Frenkiel & Moueza 1979). During the 16 days of the temperature-controlled experiment, larvae exposed to NTC exhibited lower growth and slower development compared with larvae reared under ENTC (Fig. 2). However, larvae maintained under NTC suffered a slightly lower mortality than those under ENTC (Fig. 3).
Temperature has been considered to be the most significant abiotic factor controlling growth and nutrition, triggering reproduction and regulating other physiological processes in marine bivalves (Laudien et al. 2001, Heilmayer et al. 2004, Miyaji et al. 2007, Riascos et al. 2009, Carstensen et al. submitted). Temperature may also be considered one of the main abiotic factors affecting larvae, because it influences larval growth (Tettelbach & Rhodes 1981, Devakie & Ali 2000). Increases in temperature can intensify metabolic processes, as long as the critical upper temperature limit is not exceeded (Heilmayer et al. 2008). Thus, larvae of different species require different optimal temperature conditions for maximal growth. Under unfavorable environmental conditions, growth may be reduced and mortality increased (Tettelbach & Rhodes 1981, His et al. 1989, Baba et al. 1999).

Results show that *D. obesulus* larvae are able to cope with ENTC, which must therefore not exceed the upper critical temperature limit for the species. Nevertheless, the slightly higher mortality observed under ENTC compared with NTC may be interpreted as an early indicator of metabolic stress resulting from higher temperature.

### Early Larvae Exposed to El Niño Temperature and Reduced Salinity

The results of the current 48-h *in vitro* experiment testing mortality under modulated salinity indicate that larvae reared under NTC and ENTC are highly tolerant of medium and low salinity (25 and 15 ± 1, respectively). However, very low salinity (5 ± 1) resulted in 100% mortality within a short time period (16 h at NTC and 32 h at ENTC).

Changes in salinity may occur along the coastal HCS as a result of high rainfall within a short time period. Highest rainfall intensities tend to coincide with EN events (Waylen & Caviedes 1990, Romero et al. 2007). Data regarding sea surface salinity changes during EN episodes off northern Chile and Peru are scarce (Riascos et al. 2009); however, in the tropics in general, strong salinity changes occur annually and may be intensified during EN (Goodbody 1961, Wade 1968, Riascos 2002, Riascos 2006). In Jamaica, Goodbody (1961) described massive drops in salinity (down to 5) close to river mouths during 3 rainy seasons, causing mass mortality events in the neighboring benthic community. Recovery to normal salinity conditions took around 2 mo (Goodbody 1961). Wade (1968) documented high mortality rates for adult *D. denticulatus* at salinities below 10, along with a strong reduction in the number of larvae and spat. As a result of heavy precipitation off northern Chile, frequent strong salinity decreases are expected during EN years, especially close to river mouths (Waylen & Caviedes 1990, Houston 2006a). As seen in this study, Goodbody (1961) also documented rapid increases in mortality when the salinity tolerance limit was surpassed.

Highest activity of *D. obesulus* larvae was recorded under conditions of medium salinity (25 ± 1) and may be interpreted as a defense reaction by which larvae attempt to escape unfavorable conditions. At low salinity (15 ± 1), such a response may be hampered by the effects of osmotic stress (Fig. 4). Similarly reduced activity has been observed in tropical oyster (*Crassostrea iridea*) larvae when salinity dropped below 15 (Devakie & Ali 2000). The LT₅₀ indicates the point at which 50% of the larvae have died. Values for ENTC are slightly lower than those for NTC (Fig. 4).

Larvae are clearly compromised by very low salinity (<5), thus it can be expected that massive salinity drops may hamper subpopulations inhabiting areas close to river mouths, such as the population at Chinchorro Beach, Arica (Carstensen et al. in review). The annual swelling of rivers during the wet Bolivian summers since 2005 may be an explanation for the scattered population of *D. obesulus* (pers. obs.).

High tolerance to abnormal temperature and salinity is a frequently observed in marine larvae (His et al. 1989, He & Zhang 1998, Devakie and Ali 2000). Adult *D. obesulus* at ENTC exhibit significantly higher mortalities (~15%) than their larvae (~2%) at the same temperature (Carstensen et al. in review). No data concerning salinity tolerance are available for adult *D. obesulus*.

Depending on environmental conditions, the meroplanktonic larval period may last several weeks or months, during which the distribution of individuals is mainly steered by the prevailing currents. This passive latitudinal and vertical migration may imply constant changes in abiotic parameters such as temperature and salinity (Yaroslavtseva & Sergeeva 2006). Conversely, the adult life span is characterized by a mainly sessile lifestyle, which
implies more stable conditions. Early life stages (juveniles) of the sympatric surf clam Mesodesma donacium revealed higher resistance to low salinity (10) than adults (Riascos et al. 2009). For D. serra, it was documented that juvenile species are able to survive closer to river mouths than adults, implying higher tolerance to low salinity (Donn 1987). This adaptation may be justified by nutrition gains to be made feeding in an area of higher primary production and by avoiding predation of larvae by adult specimens. Finally, early life stages are the means by which populations colonize vacant habitat areas (e.g., Mann et al. 1991, Shanks & Brink 2005). By dispersing, specimens are reducing intra- and interspecific competition for food and habitat quality (Tarifeno 1980, Dugan et al. 2004). Another factor that may favor the ability of D. obesulus larvae to resist higher temperature and reduced salinity may be the tropical origin of Donacidae. Tropical species may encounter consistently high temperatures and strong salinity changes caused by the large annual fluctuations in precipitation common to tropical regions (Riascos 2006, Carstensen et al. in review).

In conclusion, the results of this study reveal early larvae to be highly resistant to EN conditions (higher temperature and lower salinity) except for very low salinities (≤5). Nevertheless, the influence of changing environmental conditions on early larval stages of bivalves remains poorly understood. Therefore, further studies should focus on early life stage development to get a better understanding of species reproduction and distribution. Early embryonic stages in particular (<48 h) are highly sensitive to changing environments, and better knowledge will improve our understanding of the dynamics of populations (He & Zhang 1998, Verween et al. 2007). Furthermore, given the influence of spontaneous temperature increases on spawning, the effect of sudden EN-induced temperature changes on adult specimens should be investigated.

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LITERATURE CITED

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