

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

DANIEL CARSTENSEN,<sup>1\*</sup> JÜRGEN LAUDIEN,<sup>1,2</sup> WALTER SIELFELD,<sup>3</sup>  
MARCELO E. OLIVA<sup>4</sup> AND WOLF E. ARNTZ<sup>1</sup>

<sup>1</sup>Alfred Wegener Institute for Polar and Marine Research, PO Box 120161, D-27515 Bremerhaven, Germany; <sup>2</sup>Institute for Applied Ecology Ltd., Alte Dorfstrasse 11, 18184 Neu Broderstorf, Germany; <sup>3</sup>Departamento de Ciencias del Mar, Universidad Arturo Prat, Av. Arturo Prat 2120, Iquique, Chile; <sup>4</sup>Universidad de Antofagasta, Instituto de Investigaciones Oceanológicas, Avenida Angamos 601, Antofagasta, Chile

**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 *D. 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  $5 \pm 1$ ) at 2 different temperatures (NTC and ENTC). At both temperatures, larvae suffered no mortality at medium and low salinity (35, 25, and  $15 \pm 1$ ) but showed 100% mortality at very low salinity ( $5 \pm 1$ ) after 16 h at NTC and 32 h at ENTC. Activity of larvae was highest at medium salinity ( $25 \pm 1$ ) and lowest at normal salinity ( $35 \pm 1$ ). The results of this study indicate that early larval stages of *D. obesulus* can cope with temperature and salinity changes induced during EN. Only extremely low salinity ( $5 \pm 1$ ) such as that observed close to river mouths may cause high mortality rates in *D. obesulus* offspring.

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

### 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 intraannual temperature and oxygen conditions (e.g., Arntz et al. 1987, Camus 2001, Thiel et al. 2007). During strong El Niño (EN; warm phase of El Niño Southern Oscillation (ENSO)) parameters such as sea surface temperature and oxygen concentration may change drastically. Positive effects on macrobenthic communities have been recorded below 20–30 m water depth (oxygen minimum zone) as a result of flushing with oxygenated water (Arntz 1986). Conversely, the oxygen concentration of nearshore shallow waters (e.g., off sandy beaches) does not change during EN, because these habitats remain well oxygenated as a result of continued wave action. Changes in water temperature and salinity are the principal parameters modified during EN in these habitats (Arntz & Fahrback 1991, Thiel et al. 2007). Extremely arid areas of northern Chile and Peru are characterized by very low precipitation under normal conditions. During strong EN, high precipitation causing massive riverine runoff has been frequently documented (e.g., Waylen & Caviedes 1990, Houston 2006a, Houston 2006b, Romero et al. 2007). This can cause

dramatic drops in sea surface salinity near river mouths and can fundamentally affect benthic shallow-water communities (Arntz et al. 1987, Arntz et al. 1988, Arntz & Fahrback 1991). Surf clam species of the family Donacidae combine a highly mobile larval stage with a mostly sessile postmetamorphic stage during juvenile and adult life. After EN in shallow-water communities off Chile and Peru, successful larval settlement allows recolonization of vacant habitat (Arntz et al. 1987).

Typically for a member of the family Donacidae (Ansell 1983) *Donax obesulus* Reeve, 1854 (synonyms: *D. marincovichi* and *D. peruvianus* (Carstensen et al. 2009)) may exhibit very high abundances in beach communities and plays an important ecological role in beach ecosystems as primary consumer. *D. obesulus* colonizes intertidal sandy beaches from northern Chile ( $18^{\circ}27'S$ ,  $70^{\circ}18'W$ ) to the north of Peru ( $3^{\circ}30'S$ ,  $80^{\circ}29'W$ ) (Carstensen et al. submitted). The recent distribution center lies between central and northern Peru, overlapping with the impact zone of EN (Carstensen et al. in review). As a dominant (abundance and biomass) invertebrate species and as an active filter feeder, *D. obesulus* plays an important ecological role in beach ecosystems. This clam is exploited by fishermen and is regionally of notable economic importance for Peruvian benthic artisanal fisheries (Paredes & Cardoso 2001, Aguirre & Mendo 2008, Rey 2008).

As poikilothermic organisms, bivalves are primed and triggered to spawn by changes in sea surface temperature,

\*Corresponding author. E-mail: Daniel.Carstensen@awi.de

which stimulate gonad maturation and gamete release (Riascos 2006, Petes et al. 2007). Gamete development of *D. obesus* 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. obesus* 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. obesus*, 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. obesus* 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. obesus* 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. obesus* 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 ( $17.8 \pm 0.2^\circ\text{C}$ ) and salinity ( $35 \pm 1$ ) 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  $\mu\text{m}$ ), ultra-violet 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 annual mean sea surface temperature ( $17.8 \pm 0.2^\circ\text{C}$ ). The EN temperature condition (ENTC) was considered to be the highest monthly mean sea surface temperature registered by the Arica station (18°28' S, 70°19' W) during EN 1982 through

1983 ( $24.6 \pm 0.2^\circ\text{C}$ ). Likewise, ambient salinity of  $35 \pm 1$  was taken as normal, whereas 25, 15, and  $5 \pm 1$  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  $5^\circ\text{C}$  increase in water temperature ( $23 \pm 0.2^\circ\text{C}$ ) 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. obesus* 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 Sedgewick 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  $\mu\text{m}$ ) 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. obesus**

To test the combined effect of ENTC and reduced salinity, larvae (>48 h) were randomly assigned to a  $4 \times 2$  factor experimental design: 4 salinities (35, 25, 15, and  $5 \pm 1$ ) at NTC ( $17.8 \pm 0.2^\circ\text{C}$ ) and 4 salinities at ENTC ( $24.6 \pm 0.2^\circ\text{C}$ ) for 48 h. To obtain the exact prescribed salinity, seawater ( $35 \pm 1$ ) was diluted with the appropriate volume of distilled water. For each of the eight conditions, 20 replicates (4-mL 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 ( $LT_{50}$ ). This parameter was obtained by plotting the relationship between time and mortality, and extrapolating the time corresponding to 50% mortality. To estimate vitality 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. obesulus* 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. obesulus* 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 ( $5 \pm 1$ ), 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 ( $+5^{\circ}\text{C}$ ). Mature males ( $n = 17$ ) had a minimum shell length (SL, maximum anterior–posterior) of  $16.8 \pm 0.1$  mm and a maximum SL of  $24.7 \pm 0.1$  mm, whereas ripe females ( $n = 13$ ) exhibited a minimum SL of  $16.5 \pm 0.1$  mm and a maximum SL of  $23.6 \pm 0.1$  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,  $35 \pm 1$ ). 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  $5 \mu\text{m}$ , whereas the tail was approximately  $50 \mu\text{m}$  long. Unfertilized oocytes had a diameter of  $59.34 \pm 0.63 \mu\text{m}$  ( $n = 25$ ). 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 trochophore stage. After 24 h, a D-Veliger larva 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  $88.46 \pm 0.27$  ( $n = 90$ )– $160.85 \pm 1.95 \mu\text{m}$  ( $n = 90$ ) under NTC and from  $88.65 \pm 0.32 \mu\text{m}$  ( $n = 90$ )– $176.91 \pm 1.90 \mu\text{m}$  ( $n = 90$ ) at ENTIC (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 ENTIC was significantly different ( $F_1 = 358.0$ ,  $P = 0.00$ ). At 18 days postfertilization, larvae attained a maximum length of  $213.5 \mu\text{m}$  at NTC, whereas larvae under ENTIC reached a maximum length of  $240.75 \mu\text{m}$ . Overall mortality during the experimental period was very low:  $0.79 \pm 0.11\%$  at NTC, and  $1.25 \pm 0.37\%$  at ENTIC (Fig. 3). Rates of mortality did not differ significantly between treatments ( $F_1 = 2.82$ ,  $P = 0.36$ ).

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

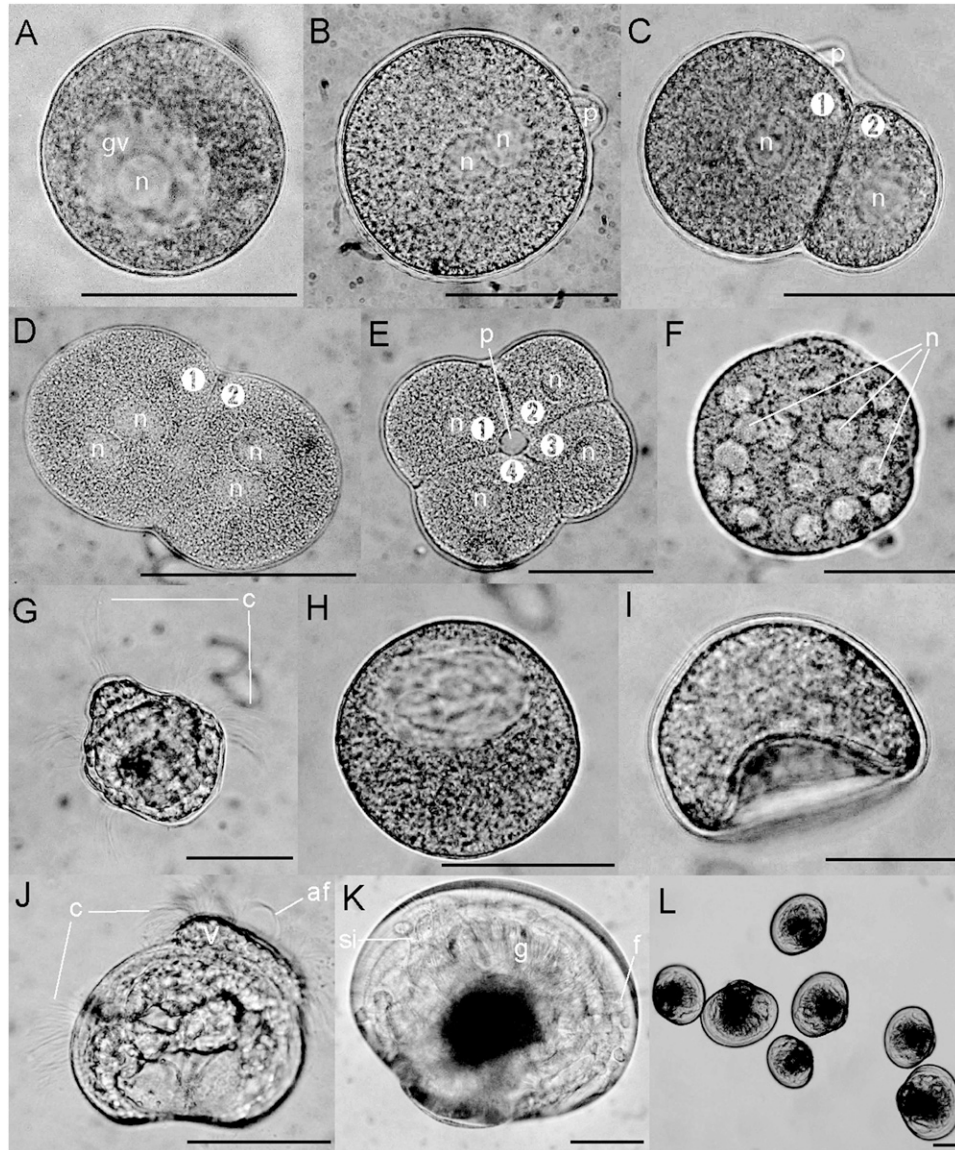
Exposure over a 48-h period to certain salinities ( $35$ ,  $25$ , and  $15 \pm 1$ ) at NTC and ENTIC, resulted in no larvae mortality. Larvae exposed to the lowest salinity ( $5 \pm 1$ ), however, suffered 100% mortality after 16 h (NTC) and 32 h (ENTIC; Fig. 4) at a significant difference between temperatures ( $F_1 = 4.87$ ,  $P = 0.03$ ). Under NTC,  $LT_{50}$  was reached after 4.3 h; under ENTIC,  $LT_{50}$  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  $25 \pm 1$  followed by  $15 \pm 1$ , whereas larvae at NTC and  $35 \pm 1$  showed the least activity (Fig. 5). Statistical analysis revealed no significant differences in the activity of larvae between test temperatures ( $F_1 = 0.308$ ,  $P = 0.59$ ). However, within different salinity levels ( $15$ ,  $25$ , and  $35 \pm 1$ ), significant differences in activity were apparent ( $F_2 = 18.29$ ,  $P < 0.00$ ). A Tukey post hoc comparison revealed significant differences in activity between salinities of  $25$  and  $35 \pm 1$  at both NTC and ENTIC ( $P = 0.01$  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



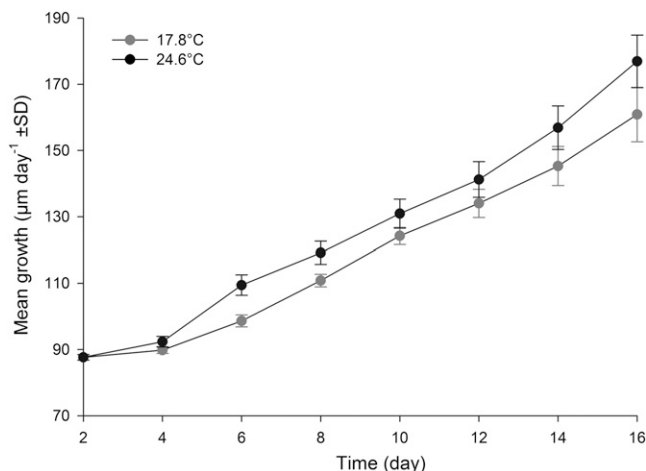


**Figure 1.** Light microphotograph (Leica DM LS2, Solms, Germany) taken at a magnification of  $400\times$  (except view L,  $100\times$ ) with a Canon Powershot S50 (Tokyo, Japan) digital camera. All scale bars are equivalent to  $50\ \mu\text{m}$ . (A) Spawned oocyte with large germinal vesicle (gv) and nucleolus (n), which indicates that the egg is still not fertilized ( $62.5\ \mu\text{m}$ ,  $<1\ \text{h}$ ). (B) Fertilized oocyte with first polar body (p) at the right side of the surface and divided nucleolus (n), which indicates commencing maturation (oocyte,  $75\ \mu\text{m}$ ; nucleolus,  $16\ \mu\text{m}$ ;  $4.5\ \text{h}$ ). (C) Two-cell embryo with unequal blastomeres (1 and 2). The polar body (p) is located in the cleavage plane ( $87.5 \times 62.5\ \mu\text{m}$ ; nucleolus,  $12.5\ \mu\text{m}$ ;  $5\ \text{h}$ ). (D) Two-cell embryo with equal blastomeres (1 and 2) in transition to a 4-cell embryo (nucleolus divided;  $100 \times 87.5\ \mu\text{m}$ ,  $6\ \text{h}$ ). (E) Four-cell embryo with 4 equal blastomeres (1, 2, 3, and 4;  $100 \times 95\ \mu\text{m}$ ,  $6\ \text{h}$ ). (F) Multicellular stage, nucleolus (n;  $82.5\ \mu\text{m}$ ,  $9.5\ \text{h}$ ). (G) Early trochophore, ciliate (c) indicated by lines ( $75\ \mu\text{m}$ ,  $<24\ \text{h}$ ). (H) Early gastrula stage ( $75\ \mu\text{m}$ ,  $9.5\ \text{h}$ ). (I) Late gastrula stage ( $80\ \mu\text{m}$ ,  $<24\ \text{h}$ ). (J) D-Veliger with velum (v), cilia (c), and apical flagellum (af;  $80 \times 67.5\ \mu\text{m}$ ,  $2.5\ \text{days}$ ). (K) Foot-stage larvae, siphon (si), gills (g), and foot (f;  $200 \times 170\ \mu\text{m}$ ,  $18\ \text{days}$ ). (L) Foot-stage larvae overview ( $\sim 187.5\text{--}250\ \mu\text{m}$ ,  $18\ \text{days}$ ).

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 ( $+5^\circ\text{C}$ ). 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

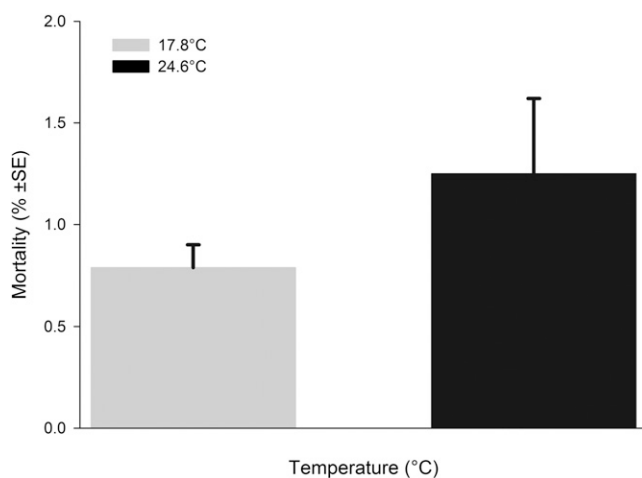
Early larval development under NTC and ENTC followed the typical pattern known for several marine bivalve species (Fig. 1) (Chanley 1969, Frenkiel & Moueza 1979). During the 16 days of the temperature-controlled experiment, larvae exposed to NTC exhibited lower growth and slower development compared with larvae reared under ENTC (Fig. 2). However, larvae maintained under NTC suffered a slightly lower mortality than those under ENTC (Fig. 3).



**Figure 2.** Mean growth rate of *Donax obesulus* larvae cultured at NTC ( $17.8 \pm 0.2^\circ\text{C}$ ) and ENTIC ( $24.6 \pm 0.2^\circ\text{C}$ ) for 16 days.

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 ENTIC, which must therefore not exceed the upper critical temperature limit for the species. Nevertheless, the slightly higher mortality observed under ENTIC compared with NTC



**Figure 3.** Total mortality of *Donax obesulus* larvae during a 16-day growth experiment carried out under NTC ( $17.8 \pm 0.2^\circ\text{C}$ ) and ENTIC ( $24.6 \pm 0.2^\circ\text{C}$ ).

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 ENTIC are highly tolerant of medium and low salinity ( $25$  and  $15 \pm 1$ , respectively). However, very low salinity ( $5 \pm 1$ ) resulted in 100% mortality within a short time period (16 h at NTC and 32 h at ENTIC).

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 ( $25 \pm 1$ ) and may be interpreted as a defense reaction by which larvae attempt to escape unfavorable conditions. At low salinity ( $15 \pm 1$ ), such a response may be hampered by the effects of osmotic stress (Fig. 4). Similarly reduced activity has been observed in tropical oyster (*Crassostrea iredalei*) larvae when salinity dropped below 15 (Devakie & Ali 2000). The  $LT_{50}$  indicates the point at which 50% of the larvae have died. Values for ENTIC are slightly lower than those for NTC (Fig. 4).

Larvae are clearly compromised by very low salinity ( $\geq 5$ ), 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 ENTIC exhibit significantly higher mortalities ( $\sim 15\%$ ) than their larvae ( $\sim 2\%$ ) 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

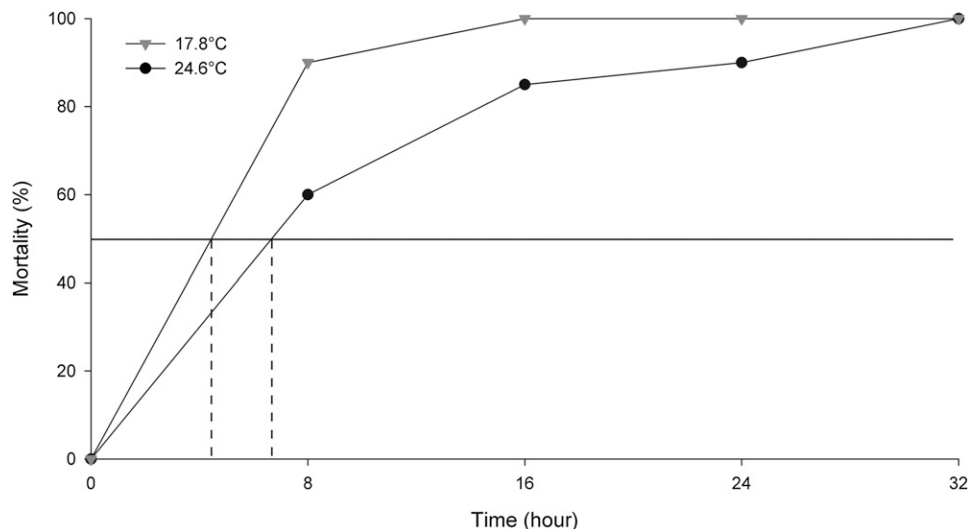


Figure 4. Mortality of *Donax obesulus* larvae ( $n = 20$ ) exposed for 48 h to a salinity of  $5 \pm 1$  under NTC ( $17.8 \pm 0.2^\circ\text{C}$ ) and ENT ( $24.6 \pm 0.2^\circ\text{C}$ ). Vertical dashed lines indicate the time at which 50% of the larvae had died ( $LT_{50}$ ) under NTC (left) and ENT (right).

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 ( $\geq 5$ ). 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.

#### ACKNOWLEDGMENTS

This study represents part of the first author's PhD dissertation and was financially supported by the University of Bremen, the German Academic Exchange Service (DAAD), and the International Bureau of the Federal Ministry of Education and Research. The authors are grateful to Mario Villegas, Roberto Vargas, Wolfgang Stotz, María Soledad Romero, Leonel Gonzalez, Pamela Chávez, Nik Probst, and José M. Riascos for their essential help. This work was conducted as part of the European Union-funded FP6-INCO-STREP project, Climate Variability and El Niño–Southern Oscillation: Implications for Natural Coastal Resources and Management (SENSOR-CT-2004-511071) and is SENSOR publication no. 387.

#### LITERATURE CITED

- Aguirre, A. & J. Mendo. 2008. Crecimiento y producción de *Donax obesulus* Reeve, 1854 (Bivalvia: Donacidae) en playa Sarapampa, Asia, Lima. *Ecol. Apl.* 7:63–70
- Ansell, A. D. 1983. The biology of the genus *Donax*. In: A. McLachlan, T. Erasmus, & W. E. Junk, editors. *Developments in hydrobiology*.

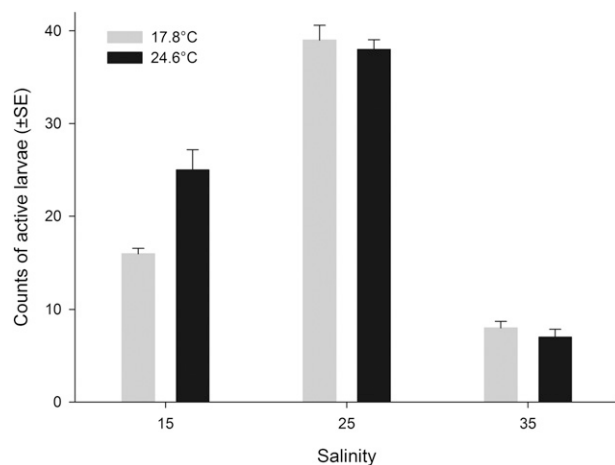


Figure 5. Activity of early larvae (24–48 h) recorded as counts of swimming larvae exposed to salinities of 15, 25, and 35  $\pm 1$  (each treatment,  $n = 20$ ) at NTC ( $17.8 \pm 0.2^\circ\text{C}$ ) and ENT ( $24.6 \pm 0.2^\circ\text{C}$ ).



- Vol. 19. Sandy beaches as ecosystems. The Hague, Netherlands: Dr. W. Junk. pp. 607–635
- Arntz, W. E. 1986. The two faces of El Niño 1982–83. *Arch. Fish. Mar. Res.* 31:1–46.
- Arntz, W. E., T. Brey, J. Tarazona & A. Robles. 1987. Changes in the structure of a shallow sandy-beach community in Peru during an El Niño event. *S. Afr. J. Mar. Sci.* 5:645–658.
- Arntz, W. E., & E. Fahrbach. 1991. El Niño-Klimaexperiment der Natur: Physikalische Ursachen und biologische Folgen. Basel: Birkhäuser. 264 pp.
- Arntz, W. E., E. Valdivia & J. Zeballos. 1988. Impact of El Niño 1982–83 on the commercially exploited invertebrates (mariscos) of the Peruvian shore. *Rep. Mar. Res.* 32:3–22.
- Baba, K., M. Tada, T. Kawajiri & Y. Kuwahara. 1999. Effects of temperature and salinity on spawning of the brackish water bivalve *Corbicula japonica* in Lake Abashiri, Hokkaido, Japan. *Mar. Ecol. Prog. Ser.* 180:213–221.
- Camus, P. A. 2001. Biogeografía marina de Chile continental. *Rev. Chil. Hist. Nat.* 74:587–617.
- Carstensen, D., J. Laudien, F. Leese, W. E. Arntz & C. Held. 2009. Genetic variability, shell and sperm morphology suggest that the surf clams *Donax marincovichii* and *D. obesulus* are one species. *J. Molluscan Stud.* 75:381–390.
- Carstensen, D., J. M. Riascos, O. Heilmayer, W. E. Arntz & L. Laudien. Recurrent, thermally-induced shifts of species distribution range: the fate of living in the Humboldt Current Upwelling System. *Mar. Environ. Res.* in review.
- Chanley, P. 1969. Larval development of the coquina clam, *Donax variabilis* Say, with a discussion of the structure of the larval hinge in the Tellinacea. *Bull. Mar. Sci.* 19:214–224.
- Devakie, M. N. & A. B. Ali. 2000. Salinity–temperature and nutritional effects on the setting rate of larvae of the tropical oyster, *Crassostrea iredalei* (Faustino). *Aquaculture* 184:105–114.
- Donn, T. E. J. 1987. Longshore distribution of *Donax serra* in two log-spiral bays in the eastern cape, South Africa. *Mar. Ecol. Prog. Ser.* 35:217–222.
- Dugan, J. E., E. Jaramillo, D. M. Hubbard, H. Contreras & C. Duarte. 2004. Competitive interactions in macroinfaunal animals of exposed sandy beaches. *Oecologia* 139:630–640.
- Dudas, S. E. & J. F. Dower. 2006. Reproductive ecology and dispersal potential of varnish clam *Nuttallia obscurata*, a recent invader in the Northeast Pacific Ocean. *Mar. Ecol. Prog. Ser.* 320:195–200.
- Frenkiel, L. & M. Moueza. 1979. Développement larvaire de deux Tellinacea, *Scrobicularia plana* (Semelidae) et *Donax vittatus* (Donacidae). *Mar. Biol.* 55:187–195.
- Goodbody, I. 1961. Mass mortality of a fauna following tropical rains. *Ecology* 42:150–155.
- He, Y.-C. & F.-S. Zhang. 1998. Effect of salinity on embryo and larval development of the southern bay scallop *Argopecten irradians concentricus* Say. *Chin. J. Oceanol. Limnol.* 16:91–96.
- Heilmayer, O., T. Brey & H. O. Pörtner. 2004. Growth efficiency and temperature in scallops: a comparative analysis of species adapted to different temperatures. *Funct. Ecol.* 18:641–647.
- Heilmayer, O., J. Digialleonardo, L. Qian & G. Roesijadi. 2008. Stress tolerance of a subtropical *Crassostrea virginica* population to the combined effects of temperature and salinity. *Estuar. Coast. Shelf Sci.* 79:179–185.
- Helm, M. M., N. Bourne, & A. Lovatelli. 2004. Hatchery culture of bivalves: a practical manual. Rome, Food and Agriculture Organization of the United Nations. FAO Fisheries technical paper no. 471. 177 pp
- His, E., R. Robert & A. Dinet. 1989. Combined effects of temperature and salinity on fed and starved larvae of the Mediterranean mussel *Mytilus galloprovincialis* and the Japanese oyster *Crassostrea gigas*. *Mar. Biol.* 100:455–463.
- Houston, J. 2006a. The great Atacama flood of 2001 and its implications for Andean hydrology. *Hydrol. Process.* 20:591–610.
- Houston, J. 2006b. Variability of precipitation in the Atacama Desert: its causes and hydrological impact. *Int. J. Climatol.* 26:2181–2198.
- Huaraz, F. & V. Ishiyama. 1980. Madurez sexual de la “concha mariposa” (*Donax peruvianus*) de la playa de Jahuary, Ica, Perú. *Rev. Cienc. U. N. M. SM* 72:47–56.
- Laudien, J., T. Brey & W. E. Arntz. 2001. Reproduction and recruitment patterns of the surf clam *Donax serra* (Bivalvia, Donacidae) on two Namibian sandy beaches. *S. Afr. J. Mar. Sci.* 23: 53–60.
- Laudien, J., D. Schiedek, T. Brey, H.-O. Pörtner & W. E. Arntz. 2002. Survivorship of juvenile surf clams *Donax serra* (Bivalvia, Donacidae) exposed to severe hypoxia and hydrogen sulphide. *J. Exp. Mar. Biol. Ecol.* 271:9–23.
- Mann, R., B. M. Campos & M. W. Luckenbach. 1991. Swimming rate and responses of larvae of three mactrid bivalves to salinity discontinuities. *Mar. Ecol. Prog. Ser.* 68:257–269.
- Miyaji, T., K. Tanabe & B. R. Schöne. 2007. Environmental controls on daily shell growth of *Phacosoma japonicum* (Bivalvia: Veneridae) from Japan. *Mar. Ecol. Prog. Ser.* 336:141–150.
- Paredes, C. & F. Cardoso. 2001. El género *Donax* en la costa peruana (Bivalvia: Tellinoidea). *Rev. Per. Biol.* 8:1–13
- Petes, L. E., B. A. Menge & G. D. Murphy. 2007. Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. *J. Exp. Mar. Biol. Ecol.* 351:83–91.
- Rey, R. 2008. Prohíben actividades de extracción, procesamiento, transporte y comercialización del recurso marucha o palabrillas en el litoral y ámbito del departamento de Lambayeque. Resolución ministerial no. 607-2008-Produce. 2. Informe no. 452-2008-PRODUCE/DGEPD-Dch. Perú: Instituto del Mar del Perú
- Riascos, J. M. 2002. Cambios en el macrobentos de playa arenosa durante “El Niño” 1997–98 en la Bahía de Málaga, Pacífico colombiano. *Cienc. Mar.* 28:13.
- Riascos, J. M. 2006. Effects of El Niño–Southern Oscillation on the population dynamics of the tropical bivalve *Donax dentifer* from Málaga Bay, Colombian Pacific. *Mar. Biol.* 148:1283–1293.
- Riascos, J. M., D. Carstensen, J. Laudien, W. Arntz, M. E. Oliva, A. Güntner & O. Heilmayer. 2009. Thriving and declining: temperature and salinity shaping life-history and population stability of *Mesodesma donacium* in the Humboldt Upwelling System. *Mar. Ecol. Prog. Ser.* 385:151–163.
- Riascos, J. M., and H.-J. Urban. 2002. Impact of El Niño/La Niña on the population dynamics of the tropical bivalve *Donax dentifer* in Bahía Málaga, *Colomb. Pac. Invest. Mar.* 30:152–154.
- Romero, C. C., G. A. Baigorria & L. Stroosnijder. 2007. Changes of erosive rainfall for El Niño and La Niña years in the northern Andean highlands of Peru. *Clim. Change* 85:343–356.
- Ruiz-Azcona, P., R. Rodríguez-Sierra & J. B. Martín. 1996. Culture of coquina clam, *Donax trunculus*, larvae. *Aquaculture* 139:151–155.
- Shanks, A. L. & L. Brink. 2005. Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. *Mar. Ecol. Prog. Ser.* 302:1–12.
- Tarifeño, E. 1980. Studies on the biology of the surf-clam *Mesodesma donacium* (Lamarck, 1818) (Bivalvia: Mesodesmatidae) from Chilean sandy beaches. Los Angeles, University of California: 203 pp.
- Tettelbach, S. T. & E. W. Rhodes. 1981. Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop *Argopecten irradians irradians*. *Mar. Biol.* 63:249–256.
- Thiel, M., E. C. Macaya, E. Acuña, W. Arntz, H. Bastias, K. Brokordt, P. A. Camus, J. C. Castilla, L. R. Castro, M. Cortés, C. P. Dumont, R. Escribano, M. Fernández, J. A. Gajardo, C. F. Gaymer, I. Gómez, A. E. González, H. E. González, P. A. Haye, J. E. Illanes, J. L. Iriarte, D. A. Lancellotti, G. Luna-Jorquera, C. Luxordo, P. H. Manríquez, V. Marin, P. Muñoz, S. A. Navarrete, E. Pérez, E. Poulin, J. Sellanes, H. H. Sepúlveda, W. Stotz, F. Tala, A. Thomas, C. A. Vargas, J. A. Vásquez & J. M. A. Vega. 2007. The Humboldt Current System of northern and central Chile: oceanographic

- processes, ecological interactions and socioeconomic feedback. *Oceanogr. Mar. Biol.* 45:195–345
- Urban, H. J. 1994. Upper temperature tolerance of ten bivalve species off Peru and Chile related to El Niño. *Mar. Ecol. Prog. Ser.* 107:139–145.
- Verween, A., M. Vincx & S. Degraer. 2007. The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae (Mollusca, Bivalvia): the search for environmental limits. *J. Exp. Mar. Biol. Ecol.* 348:111–120.
- Wade, B. A. 1968. Studies on the biology of the West Indian beach clam, *Donax denticulatus* L. 2. Life-history. *Bull. Mar. Sci.* 18:876–901.
- Waylen, P. R. & C. N. Caviedes. 1990. Annual and seasonal fluctuations of precipitation and streamflow in the Aconcagua river basin, Chile. *J. Hydrol. (Amst.)* 120:79–102.
- Yaroslavtseva, L. M. & E. P. Sergeeva. 2006. Adaptivity of the bivalve *Mytilus trossulus* larvae to short- and long-term changes in water temperature and salinity. *Russ. J. Mar. Biol.* 32:82–87.