Berichte

zur Polar- und Meeresforschung

Reports on Polar and Marine Research

Environmentally induced responses of *Donax obesulus* and *Mesodesma donacium* (Bivalvia) inhabiting the Humboldt Current System

Umweltinduzierte Resonanz von *Donax obesulus* und *Mesodesma donacium* (Bivalvia) aus dem Humboldt Auftriebssystem

Daniel Carstensen

Environmentally induced responses of *Donax obesulus* and *Mesodesma donacium* (Bivalvia) inhabiting the Humboldt Current System

Umweltinduzierte Resonanz von *Donax obesulus* und *Mesodesma donacium* (Bivalvia) aus dem Humboldt Auftriebssystem

Daniel Carstensen

dedicado a Sofía y

dedicado a Sudamérica y su gente

gracias por conocer, por aprender y por compartir

Daniel Carstensen

Alfred Wegener Institute for Polar and Marine Research (AWI)
Section of Marine Animal Ecology
P.O. Box 120161
D-27515 Bremerhaven (Germany)
✉ Daniel.Carstensen@awi.de

Contents Page

Summary

The Humboldt Current System (HCS), which spans from southern Chile (~42°S) to northern Ecuador (~0°S), is one of the most productive ecosystems on earth. It is characterized by very stable temperature conditions compared to other systems at comparable latitudes. However, the system is deeply modified by the El Niño Southern Oscillation (ENSO). El Niño (EN, the warm phase of ENSO) and La Niña (LN, the cold phase of ENSO) cause changes in vitally important environmental parameters including water temperature, salinity and oxygen concentration. The changes elicit distinct responses in coastal populations and communities. However, the mechanisms underlying the observed changes remain largely unknown. Species of the intertidal and upper sublittoral, such as surf clams, have a limited capacity to migrate compared to pelagic species and are thus particularly vulnerable to such effects.

The use of shell morphological characteristics to separate the surf clams *Donax obesulus* Reeve, 1854 and *D. marincovichi* according to Coan, 1983 frequently revealed incongruences in taxonomically important morphometric parameters. Since both species are exploited by artisanal fisheries, verification of species taxonomy was essential. The taxonomy of the two sympatric surf clams inhabiting the coastal HCS was revised by genetics (cytochrome c oxidase subunit 1 gene, CO1) and sperm morphology. The aim was to (i) clarify phylogenetic relationships, (ii) analyse intraspecific relatedness between single populations within the distribution area, and (iii) estimate larval dispersal between regions and assess the dependency of local stocks on recruitment. Genetic analyses showed no significant mitochondrial differentiation within or among populations, a conclusion supported by observations of sperm ultrastructure. The latter, described here for the first time, proved indistinguishable between morphotypes. Thus it is concluded that the two morphotypes do not represent distinct species and should be included together under *D. obesulus.*

The surf clams *D. obesulus* and *Mesodesma donacium* Lamarck, 1818 dominate the benthic communities of sandy beaches of the HCS in terms of both

abundance and biomass. While *D. obesulus* inhabits mainly subtropical beach communities, *M. donacium* dominates on temperate beaches. Both are of commercial importance for artisanal fisheries. They are sold at local markets and fetch high values in European markets. Existing knowledge gleaned from field data indicates that EN and LN events affect populations of each species differently, but understanding of the mechanisms underlying the effects are lacking. It is documented that strong EN episodes along the HCS lead to mass mortality of *M. donacium*, whereas the same events enable *D. obesulus* to temporarily extend its distributional range. The life cycle of bivalve species is characterized by a highly mobile meroplanktonic larval stage, lasting from a few weeks to several months. In the wake of EN events, meroplanktonic larval stages may define the medium- and long-term stability of shallow water-species. Unfortunately, the effects of environmental changes and temperature thresholds on both species and the larval stages of *D. obesulus* have not yet been addressed.

Specimens of *D. obesulus* were collected from the southernmost limit (18°27`S; 70°18`W) of the species' distribution range, whereas those of *M. donacium* were collected from that species' northernmost locale (22°54`S; 70°17`W). In order to assess cause and effect relationships that might explain field observations made during strong EN and LN events, we tested the effect of salinity (on *M. donacium* and larvae of *D. obesulus*) and temperature on growth and mortality of these clams under controlled experimental conditions. For this, both species were exposed to reduced salinity under normal (NTC), El Niño (ENTC) and La Niña (LNTC) temperature conditions. Growth, mortality and development of *D. obesulus* larvae were recorded under the different temperature regimes. During a second 48 hour *in vitro*-experiment at NTC and ENTC, activity and mortality of larvae under reduced salinities (25, 15 and 5 ±1) was documented. Early larval development of *D. obesulus* and *M. donacium* was studied and found to follow the successive stages commonly observed for bivalve species.

Results of the *in vitro*-experiments with adult and larval *D. obesulus* indicated that compared with NTC, adults showed lower tolerance to LNTC and higher tolerance to ENTC. Similarly, *D. obesulus* larvae reared under ENTC grew

and developed faster compared to those reared under NTC. Larvae were highly tolerant of salinity changes (5-35). In contrast, *M. donacium* showed lower tolerance to ENTC, although juveniles are more tolerant than adults when exposed to ENTC and lower salinity. The distinct responses observed to environmental factors may be related to the different evolutionary origins of the two families, which are tropical for the Donacidae and temperate for the Mesodesmatidae, supporting previous hypotheses suggesting that thermal tolerance is a factor in the observed distributional changes of both species during warm and cold episodes of ENSO. The enhanced osmotic tolerance of larvae and juveniles compared to adult specimens may represent an adaptation in early life stages permitting colonization of vacant areas close to outlets of estuaries. Results of this thesis and data concerning the former and current distributions of each species clearly indicate that *D. obesulus* is better adapted to ENTC than to LNTC. *M. donacium* on the other hand, appears better adapted to normal temperate conditions of the HCS than to ENTC. The origins and thermal sensitivity of *M. donacium* suggest its biogeographical expansion and dominance from the Pliocene/Pleistocene transition until the present is closely linked to the establishment and development of the cold HCS. The recurrence of warming events (particularly EN, since at least the Holocene) has therefore subjected this cold-water species to a continuing cycle of local extinction and recolonization. Due to its tropical origin, *D. obesulus* seems able to cope with these changes better than *M. donacium*. Having improved knowledge about the responses of both species to temperature and salinity-induced changes during EN and LN, it remains important to gain a better understanding of further biotic and abiotic parameters driving these population and community changes along the HCS (e.g. beach profile, competition, nutrition, oxygen and parasitism). Such progress will help us to understand, predict and cope with the widely-felt socio economic impacts of the environmental changes during ENSO.

Management and conservation strategies should become an important part of future exploitation of for both surf clam species. Greater emphasis should be given to indentifing sink and source populations of both species. The early

embryonic and larval stages play a crucial role in population stability, and future studies should therefore focus in particular on early life stages and successful settlement and recruitment of these economically important invertebrates. The description and the general requirements of these species should be clarified and their responses to changing abiotic and biotic parameters should be identified.

Resumen

El sistema de la corriente de Humboldt (SCH) que se extiende desde el sur de Chile (~42°S) hasta el norte del Ecuador (~0°S) es uno de los ecosistemas más productivos del planeta. Este sistema se caracteriza por presentar condiciones de temperatura relativamente estables en comparación a otros sistemas en la misma latitud. Sin embargo, sus características son modificadas drásticamente durante El Niño- Oscilación del Sur (ENOS). El Niño (EN, la fase cálida del ENOS) y La Niña (LN, la fase fría del ENOS) causan cambios vitales en la temperatura, salinidad y oxigeno del agua, entre otros parámetros. Estos cambios producen distintos efectos a nivel de las comunidades y poblaciones costeras. Sin embargo, los mecanismos subyacentes responsables de los cambios descritos siguen siendo en gran parte desconocidos. En particular especies del intermareal y la zona superior del sublitoral (i.e. almejas) son afectadas por dichos cambios por su capacidad limitada de migrar compeardo a especies del piélago.

La identificación taxonómica de las almejas *Donax obesulus* Reeve, 1854 y *D. marincovichi* Coan, 1983 basada en las características morfológicas de la valva es frecuentemente incongruente para los parámetros morfométricos mas importantes. Ambas especies son explotadas por la pesquería artesanal, por lo que es esencial verificar su estatus taxonómico. La taxonomía de estas especies simpátricas de almejas que habitan las costa del SCH fue revisada mediante análisis genético (gen de la enzima citocromo c oxidasa subunidad 1, CO1) y la morfología espermática. Los objetivos fueron (i) clarificar las relaciones filogenéticas, (ii) analizar la relación intraespecífica entre poblaciones a lo largo del área de distribución, y (iii) estimar la dispersión larval entre regiones y la dependencia del reclutamiento de los stocks locales. El análisis genético (CO1) no mostró una diferenciación mitocondrial significativa entre poblaciones y la ultraestructura del espermatozoide no fue diferente en ambas especies. Por lo tanto, se concluye que los dos morfotipos no representan dos especies distintas y deben ser integrados dentro de *D. obesulus.*

Las almejas *D. obesulus* y *Mesodesma donacium* Lamarck, 1818 dominan (en abundancia y biomasa) las comunidades bentónicas de playas de arena en el SCH. Mientras que *D. obesulus* habita principalmente en comunidades de playas subtropicales, *M. donacium* domina en comunidades de playas temperadas. Ambas especies son de importancia comercial para las pesquerías artesanales en Perú y Chile (solo *M. donacium*), se venden en los mercados locales, e incluso pueden alcanzar precios altos en los mercados europeos. El conocimiento actual obtenido a través de datos de campo indica que EN y LN afectan las poblaciones de ambas especies diferencialmente. Sin embargo, es necesario conocer los mecanismos para entender los procesos. Se sabe que eventos fuertes de EN a lo largo del SCH produce eventos de mortalidad masiva en *M. donacium*, mientras que *D. obesulus* extiende temporalmente su rango de distribución. El ciclo de vida de los bivalvos se caracteriza por una etapa larval meroplanctónica altamente móvil, que dura entre unas pocas semanas hasta unos meses. Especialmente después del EN esta etapa puede determinar la estabilidad de mediano y largo plazo de las especies de aguas someras. Desafortunadamente, los efectos de los cambios ambientales y los niveles de tolerancia a la temperatura en ambas especies y los estadios larvales de *D. obesulus* no han sido estudiados detalladamente.

Se colectaron especímenes de *D. obesulus* en el límite sur (18°27`S; 70°18`W) del rango de distribución, mientras que los de *M. donacium* se colectaron en el límite norte del rango (22°54`S; 70°17`W). Para determinar relaciones causa-efecto que expliquen las observaciones existentes durante eventos EN y LN fuertes, se evaluó el efecto de la salinidad y la temperatura sobre el crecimiento y la mortalidad de estas almejas en condiciones experimentales controladas. Para esto, ambas especies fueron expuestas a una reducción de salinidad (solo *M. donacium* y larvas de *D. obesulus*) bajo temperatura normal (NTC), temperatura El Niño (ENTC) y temperatura La Niña (LNTC). El crecimiento, mortalidad y desarrollo larvario de *D. obesulus* se registró a NTC y ENTC. Durante un segundo experimento de 48 horas in vitro se registró la actividad y mortalidad de las larvas a NTC y ENTC y con bajas salinidades (25, 15 y 5 ±1). Se describió

también el desarrollo de los diferentes estadios larvales de *D. obesulus* y *M. donacium*, los cuales mostraron las etapas sucesivas comunes de los bivalvos.

Los resultados de los experimentos *in vitro* con larvas e individuos adultos de *D. obesulus* indican que los adultos presentan poca tolerancia a LNTC y mejor tolerancia a ENTC. Así mismo, las larvas de *D. obesulus* mantenidas en ENTC se desarrollaron más rápidamente en comparación con las larvas sometidas a NTC. Las larvas fueron altamente tolerantes a los cambios de salinidad (5-35). Por el contrario, *M. donacium* mostró una baja tolerancia en ENTC, aunque los juveniles de *M. donacium* fueron más tolerantes que los adultos cuando fueron expuestos a ENTC y baja salinidad. Estas distintas respuestas a factores ambientales pueden deberse a los diferentes orígenes evolutivos de las familias Donacidae (tropical) y Mesodesmatidae (temperado), respaldando la hipótesis previa que sugiere que la tolerancia térmica es el factor clave que causa los cambios en la distribución de ambas especies, durante los episodios cálidos y fríos del ENOS. La alta tolerancia osmótica de las larvas y juveniles comparada con los especimenes adultos, representan una adaptación de los estadios de vida tempranos que permitirían el asentamiento en áreas vacantes en desembocaduras de ríos y estuarios. Los resultados de esta tesis y la distribución pasada y actual indican claramente que *D. obesulus* está más adaptada a ENTC y menos a LNTC. Por el contrario *M. donacium*, de acuerdo a su distribución actual y pasada, está más adaptada a las condiciones temperadas del SCH más que a ENTC. Dado el origen y la sensibilidad térmica de *M. donacium*, su expansión biogeográfica y dominancia desde la transición Plioceno/Pleistoceno hasta el presente parece estar fuertemente ligada al establecimiento y desarrollo del SCH.

Por lo tanto, la ocurrencia de eventos cálidos (particularmente EN, desde por lo menos el Holoceno) ha sometido a esta especie de aguas frías a procesos continuos de extinción y recolonización local. Dado su origen tropical, *D. obesulus* parece resistir esos cambios mejor que *M. donacium*. Además del conocimiento obtenido sobre las respuestas de ambas espcecies a los cambios inducidos por la temperatura y la salinidad durante EN y LN, es crucial obtener un mejor entendimiento de otros parámetros bióticos y abióticos (por ej. perfil de playa,

competencia, nutrición, oxigeno, y parasitismo) que condicionan cambios poblacionales y comunitarios a lo largo del SCH. Esto puede ayudar a entender los los impactos socio económicos causados por los cambios ambientales durante ENOS. Para ambas especies de almejas, las estrategias de conservación y manejo deben ser atendidas en el futuro, e.g. se debe poner más énfasis en la identificación de las poblaciones fuente y sumidero de ambas especies. El desarrollo embrionario y los estadios larvales cumplen una función muy importante en la estabilidad poblacional.

Por lo tanto, estudios a futuro deben enfocarse los estadios tempranos de vida y el éxito en el asentamiento y el reclutamiento de los invertebrados de importancia económica. La descripción y en general las demandas de estas especies deben ser clarificadas y las respuestas a los cambios en los parámetros abióticos y bióticos deben ser identificadas.

Zusammenfassung

Der kalte Humboldtstrom zählt zu den produktivsten Ökosystemen der Erde. Er erstreckt sich vom Süden Chiles (~42°S) bis in den Norden Ecuadors (~0°S). Verglichen mit auf der gleichen geographischen Breite gelegenen Auftriebsgebieten ist der Humboldtstrom durch sehr konstante Temperaturbedingungen charakterisiert, gleichwohl unterliegt er der Dynamik von El Niño und der südlichen Oszillation (ENSO). El Niño (EN, warme Phase der ENSO) und La Niña (LN, kalte Phase der ENSO) haben einen grundlegenden Einfluss auf die Wassertemperatur, die Salinität und den Sauerstoffgehalt neben weiteren Parametern. Die Änderung dieser Parameter beeinflusst die marine Organismengemeinschaft in unterschiedlichster Form, wobei die eigentlichen Mechanismen die diese Änderungen hervorrufen, überwiegend unbekannt sind. Speziell die Arten der Gezeitenzone und des oberen Sublitorals (z.B. Brandungsmuscheln) werden durch die Änderungen abiotischer Parameter beeinflusst, da sie in ihrem Migrationsvermögen verglichen mit pelagischen Arten äußert eingeschränkt sind.

Die Identifikation der Brandungsmuscheln *Donax obesulus* Reeve, 1854 und *D. marincovichi* Coan, 1983 an Hand wichtiger morphometrischer Bestimmungsmerkmale führte häufig zu Inkongruenzen. Da es sich um wichtige Ressourcen für die Küstenfischerei handelt, ist eine exakte taxonomische Bestimmung von grundlegender Bedeutung. Die Taxonomie der entlang des Humboldtstroms sympatrisch auftretenden Brandungsmuschelarten wurde unter Anwendung genetischer Methoden und eines Vergleichs der Spermienmorphologie untersucht. Ziel war es dabei (i) den phylogenetischen Verwandtschaftsgrad zu definieren, (ii) den intraspezifischen Verwandtschaftsgrad zwischen den einzelnen Populationen entlang des Verbreitungsgebiets zu ermitteln und (iii) die Larvalverbreitung zwischen den Regionen und die Abhängigkeit der Rekruten von den lokalen Beständen abzuschätzen. Ergebnisse der genetischen Studie (CO1) zeigten keine signifikante mitochondrielle Differenzierung innerhalb oder zwischen den Populationen. Die Spermienultrastruktur wurde erstmals beschrieben und wies

ebenfalls keinerlei Unterschiede zwischen den Arten auf. Abschließend kann gesagt werden, dass beide Morphotypen unter dem Artnamen *D. obesulus* geführt werden sollten.

Die Brandungsmuscheln *D. obesulus* und *Mesodesma donacium* Lamarck, 1818 dominieren benthische Sandstrandgemeinschaften entlang des Humboldtstroms in Abundanz und Biomasse. *D. obesulus* überwiegt hauptsächlich in subtropischen Gemeinschaften, wohingegen *M. donacium* vornehmlich temperierte Gemeinschaften dominiert. Beide Arten sind für die Küstenfischerei in Peru und Chile (nur *M. donacium*) von kommerzieller Wichtigkeit und finden sowohl auf dem nationalen, als auch auf dem europäischen Markt Absatz. Der an Hand von Felddaten gewonnene aktuelle Wissensstand belegt, dass EN und LN die Populationen beider Arten unterschiedlich beeinflussen, wobei die genauen Mechanismen dieser Prozesse unbekannt sind. Es ist dokumentiert, dass starke EN entlang des Humboldtstroms zu Massenmortalitäten von *M. donacium* führen, wohingegen *D. obesulus* zeitweise das Verbreitungsgebiet erweitert. Der Reproduktionszyklus von Bivalven ist über wenige Wochen bis Monate geprägt von hochmobilen meroplanktonischen Larven. Diesen kommt speziell nach EN eine wichtige Rolle bezüglich der mittel- bis längerfristigen Bestandsstabilität innerhalb des Flachwassers zu. Die Einflüsse von Umweltveränderungen und Temperaturtoleranzen beider Arten und die Larvalentwicklung von *D. obesulus* sind bis dato unbekannt.

D. obesulus wurde im südlichsten Verbreitungsgebiet (18°27`S; 70°18`W), *M. donacium* im nördlichsten Verbreitungsgebiet (22°54`S; 70°17`W) gesammelt. Ziel dieser Arbeit war es, die während starker ENs und LNs beobachteten Phänomene diverser Feldstudien durch kontrollierte experimentelle Rahmenbedingungen zu erklären. Beide Arten (*M. donacium* und *D. obesulus*-Larven) wurden bei reduzierter Salinität unter normalen (NTC), EN (ENTC) und LN (LNTC) Temperaturbedingungen gehältert sowie Wachstum und Mortalität registriert. Zudem wurden Wachstum, Mortalität und Larvalentwicklung von D. obesulus unter NTC und ENTC ermittelt. Während eines zweiten 48 Stunden in vitro-Experiments unter NTC und ENTC wurden das Aktivitätsniveau und die

Mortalität der Larven unter reduzierter Salinität (25, 15 und 5 ±1) dokumentiert. Die frühe Larvalentwicklung von *D. obesulus* und *M. donacium* wurde beschrieben. Es zeigte sich, dass diese der für Muschelarten bekannten Entwicklungsreihe folgte.

Die Ergebnisse der *in vitro*-Experimente mit adulten Individuen und Larven von *D. obesulus* zeigten eine geringe Toleranz gegenüber LNTC und eine höhere gegenüber ENTC. Entsprechend wiesen Larven unter ENTC ein größeres Wachstum und eine schnellere Entwicklung auf als unter NTC. Insgesamt zeigten die Larven eine hohe Toleranz gegenüber geringer Salinität (bis 5). Andererseits war *M. donacium* gegenüber ENTC wenig tolerant. Dennoch zeigten juvenile verglichen mit adulten *M. donacium* eine höhere Toleranz bei ENTC und geringerer Salinität. Die verschiedenen Ansprüche an die Umwelt können durch den unterschiedlichen evolutionären Ursprung von Donaciden (tropisch) und Mesodesmatiden (temperiert) erklärt werden. Darüber hinaus stützen sie die Hypothese, dass Temperaturtoleranz der entscheidende Faktor bei den beobachteten Verschiebungen der Verbreitungsgrenzen beider Arten während der warmen und kalten Phasen der ENSO ist. Die erhöhte osmotische Toleranz der Larven und der juvenilen Stadien, verglichen mit den Adulten, könnte als eine mögliche Übertragung früher Lebensstadien interpretiert werden, die es ermöglicht, neue Gebiete oder auch Ästuare zu besiedeln. Die Ergebnisse dieser Arbeit und die ehemaligen und aktuellen Verbreitungsgebiete zeigen eindeutig, dass *D. obesulus* besser an ENTC adaptiert ist als an LNTC. *M. donacium* ist hingegen bezüglich der ehemaligen und aktuellen Ausbreitung besser an die temperierten Bedingungen des Humboldtstroms angepasst als an ENTC. Bezüglich der Herkunft und der Temperaturempfindlichkeit von *M. donacium* und der biogeographischen Ausbreitung und Dominanz während des Übergangs vom Pliozän zum Pleistozän bis heute scheint die Art eng mit der Entstehung und Entwicklung des Humboldtstroms verknüpft zu sein. Das wiederholte Auftreten warmer Phasen (EN mindestens seit dem Holozän) führte zu einem kontinuierlichen Dezimierungs- und Wiederansiedlungsprozess. Aufgrund ihres tropischen Ursprungs scheint *D. obesulus* diese Wechsel besser zu verkraften als *M. donacium*.

Neben dem gewonnenen Wissen über die Reaktion beider Arten auf unter EN und LN induzierte Temperatur- und Salinitätswechsel ist es von fundamentaler Wichtigkeit weitere, die küstennahe Organismengemeinschaft des Humboldtstrom beeinflussende biotische und abiotische Parameter (z.B. Strandprofil, Ernährung, Sauerstoff, Konkurrenz und Parasitismus) zu untersuchen. Dies wird zu einem besseren Verständnis der durch ENSO verursachten Umweltschwankungen und daraus resultierenden sozioökonomischen Konsequenzen beitragen.

Management- und Artenschutzpläne beider Brandungsmuschelarten sollten in der Zukunft eine stärkere Beachtung erfahren, dies gilt insbesondere für die Gebiete der Quell-Populationen und des Larvenfalls beider Arten. Die frühen Embryonal- und Larvalstadien haben eine entscheidende Funktion hinsichtlich der Populationsstabilität einer Art. Daher sollten sich zukünftige Studien speziell auf die frühen Lebensstadien und erfolgreicher Ansiedlung und Rekrutierung wirtschaftlich wichtiger Evertebraten konzentrieren. Hierbei sollten die Biologie und die Ansprüche einer Art sowie die Reaktion auf sich ändernde biotische und abiotische Faktoren im Vordergrund stehen.

1 Introduction

1.1 The Humboldt Current Upwelling System and its biogeographic units

Of the world's four Eastern Boundary Currents (California Current, Canary Current, Benguela Current and Humboldt Current), the Humboldt Current System (HCS) is considered to be the most productive (e.g. Halpin *et al.* 2004; Arntz *et al.* 2006). It extends from southern Chile (~42°S) to the equator. The high productivity of the system results from trade wind-driven coastal upwelling, bringing deep nutrient rich water from approximately 50 to 150 m depth into the euphotic zone. The nutrient rich water fuels a vast phytoplankton production. The latter forms the basis of one of the world's largest fisheries (e.g. Arntz & Fahrbach 1991; Hill *et al.* 1998; Daneri *et al.* 2000; Thiel *et al.* 2007). The Peruvian fisheries deliver approximately 50% of the world's fish meal production, with the catches comprising mainly zooplankton-feeding Peruvian anchovy *Engraulis ringens* (approx. 5-10 million tons annually) (Jacquet *et al.* 2009).

Upwelling is highly variable within the HCS, but mainly occurs inshore (Marin *et al.* 2003; Thiel *et al.* 2007). Off the coast of Peru, relatively strong winds and a reduced Coriolis force enhance offshore Ekman transport and generate stronger upwelling throughout the year. Further south off Chile, Ekman transport is weaker and upwelling of the subantarctic water of the equatorward-directed coastal current is less intense (Thiel *et al.* 2007). A temperature gradient can be observed along the HCS, declining from north to south (Thiel *et al.* 2007). However, the constant upwelling leads to a weakened oscillation in seasonal temperature over a wide distance, an unusual phenomenon in temperate systems at this latitude (Brattström & Johanssen 1983; Urban 1994).

The temperature gradient in the HCS has lead to a separation of biota of tropical and temperate origins (for review: Brattström & Johanssen 1983; Camus 2001) resulting in three biogeographical units (Fig. 1). The northernmost unit, dominated by subtropical and temperate biota and extending from northern Peru to northern Chile is known as the 'Peruvian Province' (~4-36°S). The southernmost

unit, the 'Magellan Province' is dominated by subantarctic and temperate biota and extends from the Chilean archipelago to the Magellan Province (~41-54°S). Between the two lies a transitional unit (~30-43°S) characterized by a strong numerical reduction in subantarctic and subtropical species at its northern and southern borders respectively (Thiel *et al.* 2007). Despite these taxonomic gradients, a number of species of subantarctic affinity inhabiting the transition zone are widely distributed in northern Chile and Peru, perhaps as a result of the cool northward flow of the HCS (Thiel *et al.* 2007).

Sea surface salinity (SSS) along the southern Peruvian and northern Chilean coast of the HCS (~5-30°S) is very stable, and especially so alongside the super-arid Atacama Desert, where very low precipitation (Rutllant *et al.* 2003; Thiel *et al.* 2007) means very low riverine runoff under normal conditions.

In all three biogeographical zones, the coasts of Peru and Chile are characterized by rocky shorelines and extensive sandy beaches with varying morphodynamics (e.g. Jaramillo 1994; Jaramillo *et al.* 2001; McLachlan & Brown 2006). These beaches are highly dynamic habitats subject to constantly changing physical conditions dictated mainly by wave energy, tides and winds. Depending on their morphodynamics they are classified as dissipative (Dean's parameter 5-10, for details see Defeo & McLachlan 2005), intermediate (Dean's parameter 2-5) and reflective types (Dean's parameter 0-2) (Short & Wright 1983). The distinct morphodynamics of the different sandy beach types are reflected in different species richness, biomass and diversity (Jaramillo *et al.* 2001; Soares 2003; McLachlan & Dorvlo 2005). Surf clams of the families Donacidae and Mesodesmatidae are the main primary consumers of beaches of the HCS. Supported by the high levels of phytoplankton production due to the upwelling, these taxa may contribute up to 95% of beach biomass (McLachlan *et al.* 1981; Ansell 1983; Arntz *et al.* 1987; Herrmann 2008). They play an important ecological role, both as dominant primary feeders and as an essential food source themselves for a diverse range of invertebrates, demersal fish, seabirds and mammals (Ansell 1983; Riascos 2008).

Bivalves are poikilotherms. The temperature of the organism is directly linked to the ambient temperature (Pörtner *et al.* 2005; Wang & Overgaard 2007). Thus, they are closely adapted to fairly constant water temperature conditions and exhibit a high sensitivity to anomalous temperature changes (e.g. Urban 1994; Camus 2001; Riascos *et al.* 2009).
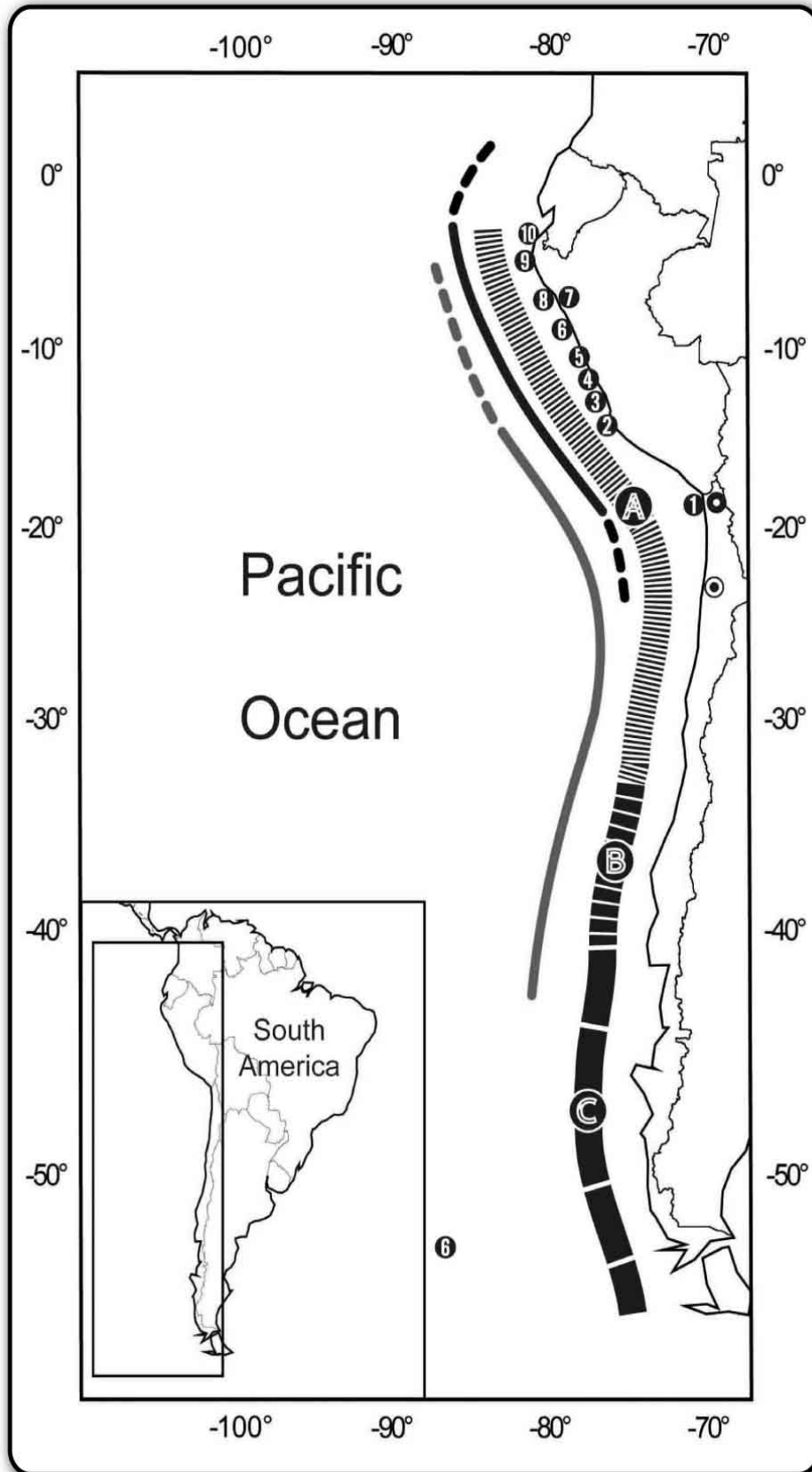
Fig. 1: Biogeographical units of the Humboldt Current System after Thiel *et al*. (2007): A: Peruvian Province, B: transition zone and C: Magellanic Province. Sample location of *Donax obesulus* (18°27'53.8`S; 70°18'24.3`W) ◖ Chinchorro beach, Arica, Chile (Publication I to III) and *Mesodesma donacium* (22°54.99`S; 70°17.42`W) ◉. Hornitos beach, northern Antofagasta, Chile (Publi-cation III and IV). Sampling stations for genetic analysis of *Donax* sp. in Chile: 1. Arica, Chinchorro (18°27'53`S; 70°18'24`W) and Peru: 2. Chincha, Violetas (13°29'5`S; W76°11'25`W), 3. San Bartolo, Silencio (13°24'33`S; 76°11'49`W), 4. Bujama, Sarapampa (12°43'20`S; 76°37'42`W), 5. Asia, Asia (12°48'04`S; 76°33'56`W), 6. Chimbote, El Dorado (9°10'24`S; 78°32'12`W), 7 and 8. Chiclayo, La Laguna (7°4'8`S, 79°44'00`W and 7°4'23`S; 79°43'47`W), 9. Sechura, San Pedro (5°29'49`S; 80°53'53`W), 10. Tumbes, Hueso de Ballena (3°30'20`S; 80°29'4`W); outgroups not included (for details see Publication I). Continuous and dashed black line indicates historic distri-bution of *D. obesulus* after Coan (1983), while the continuous part of the black line indicates the current distribution after an ample sampling. The continuous and dashed grey line indicates the historic distribution of *M. donacium* after Tarifeño (1980), while the continuous part of the grey line indicates the actual distribution (for details see Publication III).

Key Points

● The HCS is one of the world's most productive coastal systems with fairly constant temperature conditions compared to other systems at the same latitude.

● The HCS is characterized by three biogeographic provinces (Peruvian Province, Magellan Province and transitional zone).

● The coast of the HCS is dominated by rocky shore and sandy beaches, the latter are classified as dissipative, intermediate and reflective.

● The beach type influences abundance, biomass and species diversity.

● The main primary consumers of this highly dynamic habitat are surf clams of the families Donacidae and Mesodesmatidae.

1.2 El Niño Southern Oscillation and its impacts on the coastal ecosystem

Alexander von Humboldt was the first to describe temperature anomalies occurring along the East Pacific (Kortum 2002), thought at that time science was far from understanding the global consequences of the phenomenon (NOAA 2008). The El Niño Southern Oscillation (ENSO) reflects the periodical atmospheric changes occurring in the Pacific region and ranks among the strongest signals in the interannual variation of the ocean-atmosphere system (Wang *et al.* 1999). The complex mechanisms of the ENSO remain the subject of scientific research.
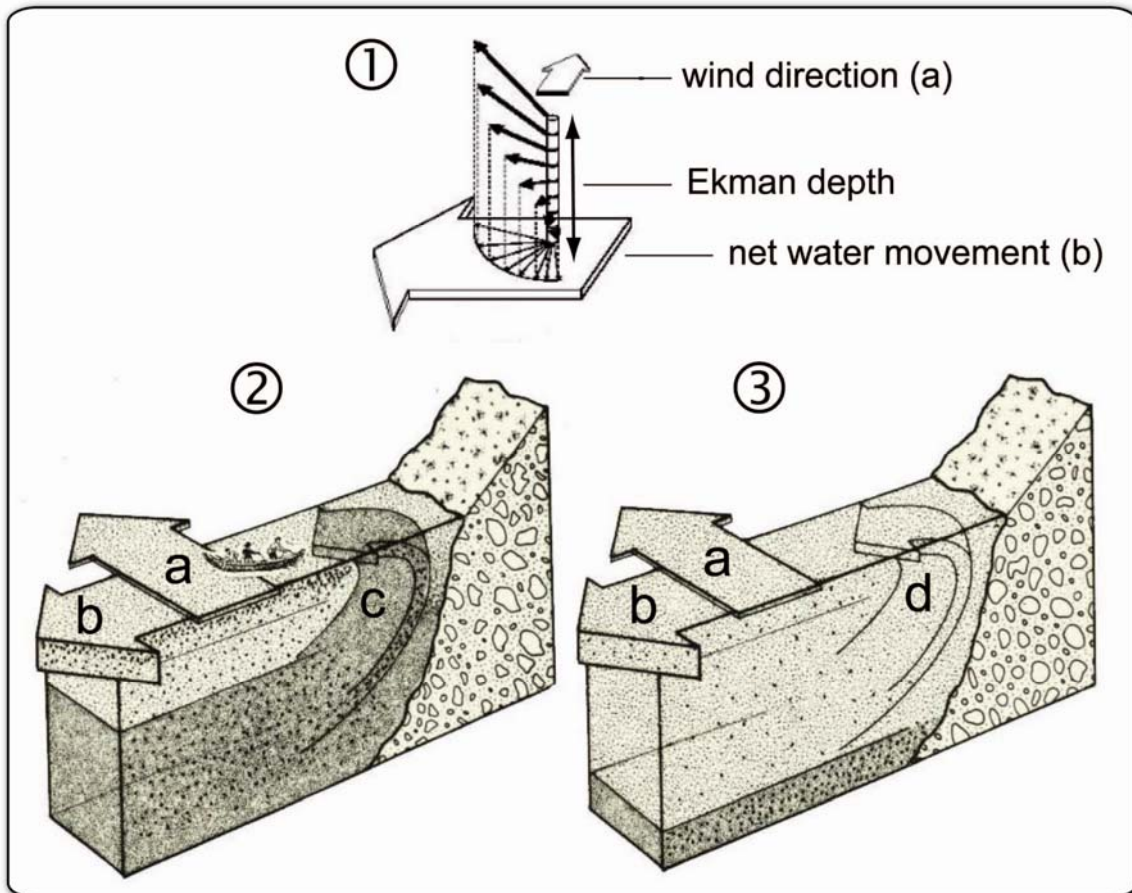
Fig. 2: (1) The Ekman Spiral illustrated for the southern hemisphere: The combined effect of alongshore winds (a) and the Coriolis force in the southern hemisphere result in an offshore Ekman transport of coastal waters (b) westwards. (2) Under normal conditions, sea surface waters are replaced by deeper nutrient rich waters (c). (3) Due to the sinking of the thermocline during El Niño, upwelling occurs from above the pycnocline, and the transport of nutrient rich waters to the surface layers is strongly reduced (d). Figures 2 and 3 from Canby (1984) redrawn after an original diagram by R. Barber.

In the southern hemisphere, alongshore winds and the Coriolis force combine to generate an offshore Ekman transport of coastal waters (Fig. 2). The westward water transport results in an aggregation of warm water and an enhanced water level (approx. 50 cm) in the western Pacific compared to the eastern Pacific region. These processes depress the thermocline in the western Pacific and force the thermocline in the eastern Pacific to rise closer to the surface. Thus, deep nutrient-rich water is upwelled to replace the surface waters being drawn offshore. The upwelling supports the region's extraordinary phytoplankton productivity. If the trade winds are suppressed, as during El Niño (EN, the warm phase of ENSO,

Fig. 3), weakening of offshore transport follows and the upwelling of cold nutrient-rich waters can slow down or stop. Conversely, strengthening of the winds increases the upwelling during La Niña (LN, the cold phase of ENSO, Fig. 3). The different phases of ENSO are defined by the so called Southern Oscillation Index (SOI), which is calculated from the pressure gradient between the western and eastern Pacific (i.e. Tahiti and Easter Island). Strong EN episodes, such as observed in 1982-1983 and 1997-1998, are classified by an SOI >2.
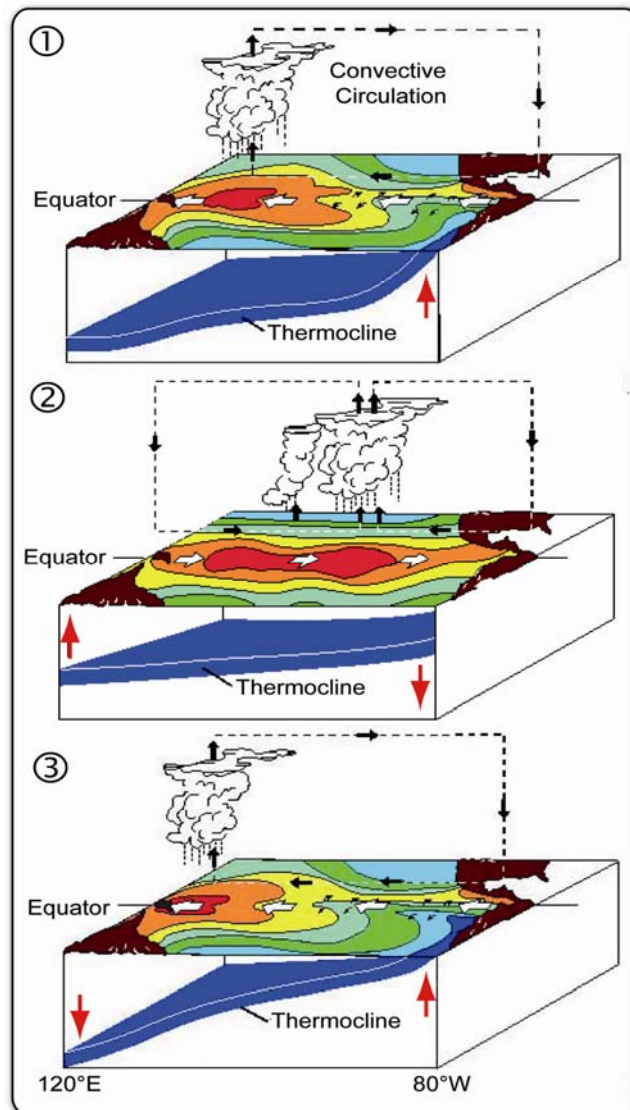
Fig. 3: Schematic diagram: The blue water layer represents the relative depth of the thermocline. (1) During normal conditions southeast trade winds (Convective Circulation) depress the thermocline in the western Pacific whereas it is pushed closer to the surface in the eastern Pacific. (2) During El Niño, trade winds are suppressed and the thermocline in the eastern Pacific sinks. (3) During La Niña a strengthening of the trade winds causes an increased upwelling in the eastern Pacific (figure modified after NOAA 2008).

Strong ENSO events have drastic effects on the marine and terrestrial environment (Arntz & Fahrbach 1991; Arntz *et al.* 2006; Thiel *et al.* 2007). For the marine environment strong EN episodes induce changes in important parameters such as sea surface temperature (SST), oxygen and salinity. In 1983, the temporary but complete suppression of upwelling of cold, nutrient rich waters at some nearshore localities off the Peruvian coast lead to positive anomalies in SST of up to 11°C (Arntz 1986). The effects on nearshore coastal communities were drastic: kelp forests and mussel banks were destroyed, depriving other invertebrate taxa of their shelter (Arntz 1986).

Another crucial parameter determining macrobenthic biomass and species composition in the upwelling zone of the HCS is oxygen concentration, and this too is modified during strong EN events (Rosenberg *et al.* 1983). Under normal conditions off Peru, the oxygen concentration of areas below 20-30 m water depth (oxygen minimum zone) is very low (usually <0.5 ml^{-1}). Most of the product of the euphotic zone sinks to the sea floor. The limited oxygen means that on its short journey to the sediment of the continental shelf there is insufficient time for this quantity of organic matter to be worked up. During strong EN events such as 1982-1983 and 1997-1998, oxygen concentration in these waters may be increased by three to seven times due to the inflow of oxygen-richer waters brought by the Kelvin waves (Arntz 1986; Arntz *et al.* 2006). In such circumstances, oxygen deficient grounds may become much more hospitable environments. In the very shallow, consistently well oxygenated surface layers, however, the effects of EN-enhanced oxygen concentration are negligible (Rosenberg *et al.* 1983; Arntz 1986).

In the constantly well oxygenated intertdial and shallow subtidal areas inhabited by Donacidae and Mesodesmatidae, changing salinity during strong EN events has to be regarded as important factor. The advection of equatorial (tropical and subtropical) waters southward from the equator brings water of lower salinity (27-31) compared to the normal (35) (Arntz 1986). Salinity changes may also be caused by massive rainfalls increasing riverine runoff, especially in the arid areas of northern Chile and southern Peru. Surf clams settled close to river outlets are

particularly vulnerable to strong salinity changes (Riascos 2008). Increased river volumes can also carry huge terrigenous sediment loads and cause strong impacts on benthic communities close to river mouths (Waylen & Caviedes 1990; Dávila *et al.* 2002; Stotz *et al.* 2003; Meza 2005; Thiel *et al.* 2007).

During strong EN events these multiple and contrasting abiotic variables trigger strong biotic changes, including fluctuations in species composition, abundance and biomass along the different biogeographical provinces (Fig. 1) of the HCS (e.g. Tarazona *et al.* 1985; Arntz 1986; Arntz & Fahrbach 1991; Castilla & Camus 1992; Riascos 2006; Thatje *et al.* 2008). Tropical species may respond by migrating southward, whereas temperate species may move into cooler zones (Arntz *et al.* 2006). Beside the north-south migrations, movements of marine species of coastal waters into deeper offshore waters have also been observed (Arntz & Fahrbach 1991).

Taking into account the fairly stable thermal regime of the HCS and the strong thermal anomalies associated with the different episodes of ENSO, temperature, oxygen and salinity have been proposed as key factors influencing latitudinal distributions of marine benthic organisms and community structure within the HCS (e.g. Rosenberg *et al.* 1983; Soto 1985; Tomicic 1985; Arntz 1986; Arntz & Fahrbach 1991; Díaz & Ortlieb 1993; Urban 1994; Laudien *et al.* 2007). As surf clams of sandy beaches inhabit very shallow habitats, temperature and salinity are supposed to be the main factors affecting them during strong EN events. However these impacts have yet to be fully assessed. Specific information about salinity changes during EN and LN off northern Chile and Peru is scarce, but in the tropics, strong salinity changes occur annually and are known to intensify during EN phases (Goodbody 1961; Wade 1968; Riascos 2002; 2006; 2008).

Studying the effects of variable temperature and salinity on surf clam species (Donacidae and Mesodesmatidae) along their southern (Donacidae) and northern (Mesodesmatidae) distribution range will give a better understanding of species responses to the changing environment. Field studies conducted during the strong EN of 1982-1983 clearly indicated an influence on mortality and distribution of Donacidae and Mesodesmatidae stocks along the HCS (Arntz & Fahrbach 1991;

Riascos 2008), but experimental investigations of the underlying mechanisms are lacking (Arntz *et al.* 2006).

In this study, the clouded taxonomy of the locally dominant donacid clam types was clarified by applying genetic analysis and sperm morphological approaches (Publication I). Furthermore, the study also set out to describe the early larval development (Donacidae) and evaluate the effects on increased temperature (as during EN) and reduced salinity on the larvae (Publication II).

Finally, in this study the dominant representatives from both surf clam families inhabiting the HCS were chosen to evaluate the effects of temperature and salinity changes on a broad experimental base. The aim was to identify single effects of increased and decreased temperature conditions (as experienced during EN and LN) and the combined effect of higher temperature and reduced salinity on growth and mortality of locally dominant species of Donacidae and Mesodesmatidae (Publication III and IV). In so doing, the author aims to shed light on the capacity of both species to cope with extreme conditions and to supply a broader understanding of ENSO induced changes on their distribution.

Key Points

● Under EN conditions, crucial parameters such as temperature, oxygen and salinity are changing; surf clams inhabiting shallow coastal oxygenated waters are mainly influenced by temperature and salinity.

● EN induced changes in abiotic parameters cause alterations in species composition, abundance and biomass within the HCS.

● Experimental investigations should reveal mechanisms explaining field observations concerning the two most abundant surf clam species along the HCS, made during a strong EN episode.

1.3 *Donax obesulus* (Donacidae)

1.3.1 Origin and distribution

The family Donacidae are likely to have originated in the tropics and more than 75% of the living species of the family currently inhabit tropical waters (where they are often dominant), with a strongly decreasing proportion occurring towards temperate zones (5%) (Ansell 1983). The same general pattern holds true along the HCS: in Ecuador the family is represented by 12 species, decreasing to nine species in Peru and with just two species previously recorded off far northern coasts of Chile (Coan 1983). As a dominant invertebrate taxon (in terms of both abundance and biomass) and as active filter feeders, the Donacidae occupy a major role in nearshore trophic webs: they feed on phytoplankton, and in turn become prey for gastropods, demersal fish, birds and mammals (Ansell 1983). Species of this family live in very dense populations in the intertidal and shallow subtidal and are often found close to river mouths (Wade 1967b; 1968; Donn 1987). During this study, abundances of up to 1900 individuals per square metre (Jahuay beach, Peru) were observed. However, abundances > 20,000 individuals per square metre have been previously documented for some species of the family (Coe 1953; Peres 1982).

Fig. 3: *Donax obesulus* collected from Chinchorro beach, Arica, northern Chile (18°27'53.8`S; 70°18'24.3`W), scale bar: 1 cm.

Donax obesulus Reeve, 1854 (Fig. 3) is known informally in Chile as 'machilla' and in Peru as 'mariposa', 'palabrita', 'concha blanca' or 'marucha' (Huaraz & Ishiyama 1980; Soto 1985; Paredes & Cardoso 2001; Rey 2006; Aguirre & Mendo 2008). The historic species distribution area (23°28`S; 70°31`W;0°27`S; 80°7`W, Fig. 1) was reconstructed by Coan (1983).

The current species distribution of *D. obesulus* - confirmed during this study - ranges from northern Peru (Tumbes) to northern Chile (Arica) (Fig. 1). The southernmost extent of the distribution is represented by a small population located at Chinchorro beach, Arica, Chile, where no changes in population density were observed between 2005 and 2008. Evidence of former populations of *D. obesulus*, in the form of empty shells, is found up to ~800 km south of Arica at Hornitos beach, Antofagasta (personal communication J. M. Riascos). The distribution centre (defined as populations with the highest abundance) of *D. obesulus* is actually located on the coast of northern Peru, decreasing off central Peru (personal observation and communication with fishermen). Thus *D. obesulus* belongs exclusively to the first biogeographical unit of the HCS, the Peruvian Province (Thiel *et al.* 2007, Fig. 1), but the species' distribution is known to fluctuate during strong EN phases (Arntz *et al.* 1987).

Key Points

● Donacidae originated in tropical waters.

● The resent distribution centre of *D. obesulus* is northern central Peru.

● Populations are often located close to river mouths.

● The distribution of the species is affected by strong EN events.

1.3.2 Reproduction

D. obesulus is a dioecious species with a sex ratio close to 1:1, with no hermaphrodite specimens reported (Huaraz & Ishiyama 1980). The main period of gamete maturation occurs between January and March, followed by spawning between April and July (Huaraz & Ishiyama 1980; Aguirre & Mendo 2008). Thus the reproductive cycle of *D. obesulus* coincides with the main impact time of EN (Arntz *et al.* 1987). Maturation and spawning are triggered by temperature and salinity (Ansell 1983; Riascos 2006).

1.3.3 Fisheries

The small size of the population of *D. obesulus* in the Chilean part of the species' range means that no fishery is established here. On Peruvian beaches, however *D. obesulus* is extracted all year round by hand dredge (Zeichen *et al.* 2002). Various types of hand dredges are in use, hauled by man power at low tide (Fig. 4). Overall, stocks of *D. obesulus* off central to northern Peru are stable, but local abundances are highly variable. Access to the stocks off central to northern Peru was open until drastic declines in the northern population caused by high fishing pressure (e.g. at Lambayeque and Piura) lead to government restrictions being established in 2006 and in following years (Rey 2006; 2007; 2008). In Peru as a whole the species is protected by a minimum catch size of 22 mm (Rey 2006;

2007) and at the time of writing is subject to complete protection in the north (Lambayeque, Rey 2008).
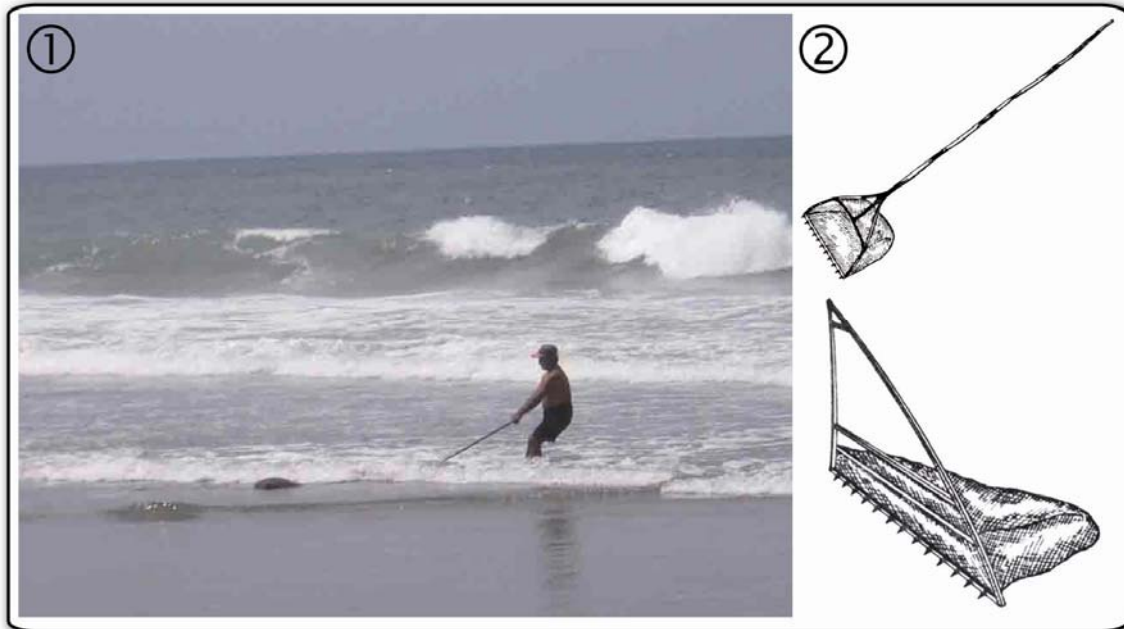
Fig. 4: (1) Peruvian fisherman collecting *Donax obesulus* at Jahuay beach, Peru, using a hand dredge (picture: R. Soto). (2) Different hand dredge types (figure: FAO).

Locally *D. obesulus* represents an important species for the artisanal fisheries. It is exploited from natural banks and sold at the local and international markets. In Europe it is sold frozen or tinned. In the year 2005 a total of 2650 t were exported (Aguirre & Mendo 2008). Whole frozen clams reach prices of up to 3.50 €/500g at European markets (Fig. 5). Shell accumulations at a northern Peruvian beach bear witness to the high abundances this species can attain; shell residuals are now collected and processed for use as fertilizer (Fig. 5).

Fig. 5: (1) Whole frozen Peruvian *D. obesulus* sold in European markets (Carrefour, Málaga, Spain) as 'coquina entera' at 3.50 €/500g. (2) Beach close to Chimbote in northern Peru, with ancient shell accumulations of *Donax obesulus* and other species, scale bar: 2 cm. Shells are sieved (far right of picture) and further processed to make fertilizer.

Key Points

● The reproduction cycle of *D. obesulus* coincides with the main impact time of EN.

● Maturation and spawning are processes triggered by temperature and salinity.

● *D. obesulus* is locally an important species for the artisanal fisheries of Peru.

1.3.4 Taxonomy of Donacidae

The taxonomic status of several members of the Donacidae is subject to debate (Loesch 1957; Wade 1967a; b; Chanley 1969a; Morrison 1971; Narchi 1983; Bonsdorff & Nelson 1992; Nelson *et al.* 1993; Guzmán *et al.* 1998; Paredes & Cardoso 2001; Laudien *et al.* 2003). Discrepancies have arisen in part from the former practice of identifying species according to shell features and the development during the last decades of newer genetic techniques (Olsson 1961; Keen 1971; Coan 1983). However, to verify management strategies and to optimize sustainable exploitation it is essential to know if species can be treated as one or if they have to be managed separately.

The extreme diversity of shell characters and the dearth of clear diagnostic features for Donacidae is apparent from various studies (Wade 1967a; b; Adamkewicz 1989; Donn 1990; Adamkewicz & Haraseewych 1994; McLachlan *et al.* 1995; Laudien *et al.* 2003). Past difficulties in the recognition of the Donacidae are demonstrated by the reduction in the number of 'valid' species from 64 (Ansell 1983) to 45 in a recent taxonomic revision of the family Donacidae (Coan *et al.* 2000). The taxonomy of *D. obesulus* and *D. marincovichi* (Coan, 1983) is uncertain (Olsson 1961; Keen 1971; Coan 1983; Guzmán *et al.* 1998; Paredes & Cardoso 2001). Further synonyms exists as a result of taxonomic confusion, for example *D. peruvianus* Deshayes, 1855 (e.g. Arntz *et al.* 1987; Carbajal *et al.* 1995; Ortlieb *et al.* 1996). With regard to *D. obesulus* and *D. marincovichi*, in the course of this

study we began to suspect that on the Pacific coast of the HCS we are dealing in reality with several isolated units, Evolutionary Significant Units (ESU) or even cryptic species. In this thesis, genetic analysis and sperm morphology are used to verify the status of this taxon. Genetic tools have often proved suitable for resolving taxonomic and systematic problems in other marine invertebrates (e.g. Kimura & Weiss 1964; Levinton & Suchanek 1978; Koehn *et al.* 1984; Utter 1991; Held 2000; Hebert *et al.* 2003; Held & Wägele 2005; Witt *et al.* 2006; Coghlan & Gosling 2007). By applying phylogenetic analyses to DNA sequences the taxonomy of *D. marincovichi* and *D. obesulus* (inter- and intraspecific variability) was tested. In preliminary studies sequence data from the gene CO1, which encodes the last enzyme (ATPase) in the respiratory electron transport chain of mitochondria (Fig. 6), proved to be useful for species discrimination within the genus.
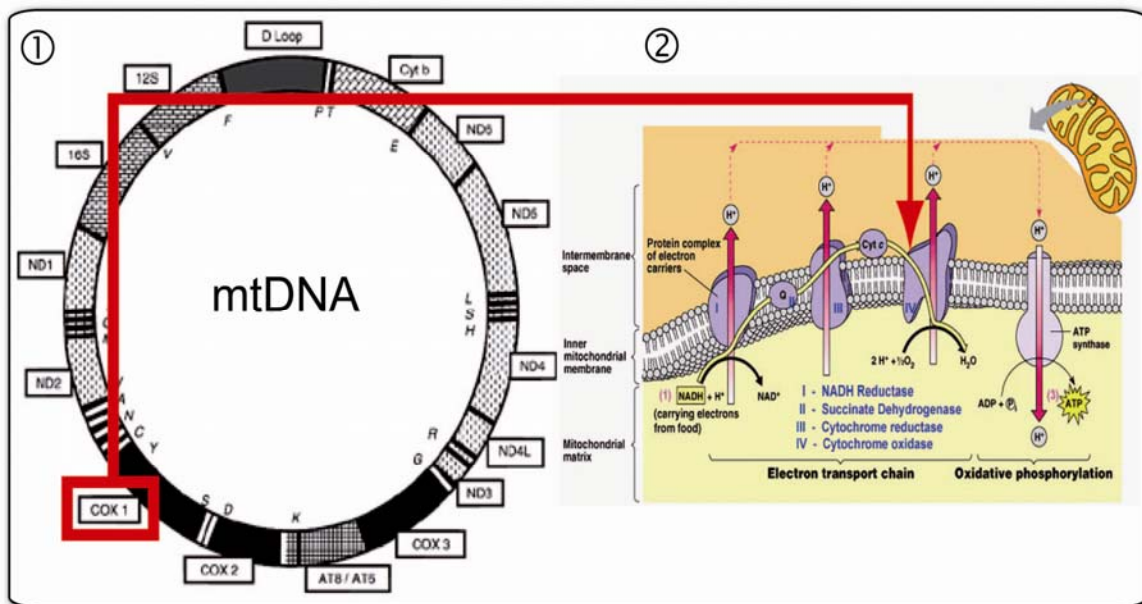
Fig. 6: (1) Mitochondrial DNA, cytochrome oxidase subunit 1 gene (red square) (figure: http://www.hbcg.utmb.edu/faculty). (2) Scheme of the membrane processes of the respiration chain, steered by the CO1 coded enzyme (figure: http://fig.cox.miami.edu).

Sperm ultrastructure studies provide a useful additional tool in taxonomic investigations and have been successfully applied in elucidating phylogenetic relationships of bivalves for more than 45 years (Galtsoff & Gallardo 1960). To this end, transmission electron microscopy (TEM) is especially valuable in providing high resolution pictures of microscopic patterns (Healy *et al.* 2000).

Key Points

● A high degree of taxonomic uncertainty exists within the Donacidae.

● The taxonomy of *D. obesulus* and *D. marincovichi* is controversial.

● Genetic analysis (CO1) and the comparative sperm morphology are useful tools in clarifying the phylogenetic relationships of bivalve species.

● Taxonomic determination of species is important for management purposes.

1.4 *Mesodesma donacium* (Mesodesmatidae)

1.4.1 Origin and distribution

The family Mesodesmatidae comprises a few genera inhabiting highly dynamic sandy beaches from temperate to subtropical regions. Unlike the donacid clams, which dominate tropical beaches, members of the Mesodesmatidae mostly inhabit sandy beaches at higher latitudes (Davis 1967; Arntz *et al.* 1987; McLachlan *et al.* 1996; Marins & Levy 1999). Within the Mesodesmatidae, the genus *Mesodesma* is one of the most abundant and most exploited (McLachlan *et al.* 1996; Beu 2006). The genus comprises four species, two of them endemic in South America. The Atlantic coast is populated by *Mesodesma mactroides* Reeve, 1854, whereas the HCS is inhabited by *Mesodesma donacium* Lamarck, 1818 (e.g Herrmann 2008; Riascos 2008, Fig. 7).

Fig. 7: *Mesodesma donacium* extracted from Hornitos beach, northern Antofagasta, Chile (22°54.99`S; 70°17.42`W), scale bar: 2 cm.

M. donacium is one of the best studied species and has been commercially exploited for more than half a century (Defeo & McLachlan 2005). In Chile and Peru *M. donacium* is known as 'macha'. Historically, its distribution extended from Chiloé in southern Chile (~16°S) to Sechura in northern Peru (~42°S) (Tarifeño 1980, Fig. 1). These days, however it decreases strongly in biomass from southern Chile to central Peru and, the most northerly population to contain a full range of size classes is found at Hornitos beach in Chile (Fig. 1). In terms of the biogeographical regions defined by Thiel *et al.* (2007) *M. donacium* is therefore associated predominantly with the Magellanic Province (~54-43°S) and the transition zone (~30-43°S) and less common in the Peruvian Province (~4-36°S). It inhabits the intertidal and subtidal zones and may colonize sandy areas down to 20 m water depth. Populations of *M. donacium*, especially juveniles, are often located close to river mouths (Tarifeño 1980; Jaramillo *et al.* 1994; Riascos *et al.* 2008). This species is affected by strong EN events, which case mass mortalities in the northern part of the species' geographical range (Tomicic 1985; Arntz *et al.* 1987).

> **Key Points**
> ● Mesodesmatidae originated in temperate waters.
> ● The distribution centre of *M. donacium* is southern Chile.
> ● Populations are often located close to river mouths.
> ● *M. donacium* is strongly affected by EN.

1.4.2 Reproduction

M. donacium is a dioecious species with a sex ratio close to 1:1; no hermaphrodites are reported. Generally a single spawning event occurs during the year, starting in late austral winter or spring and extending into summer. Biannual spawning (in spring and autumn) has been documented as an exception (Tarifeño 1980; Riascos *et al.* 2009). These local exceptions may be due to changes in water temperature and food availability, however the exact reason remains unknown (Tarifeño 1980; Peredo *et al.* 1987; McLachlan *et al.* 1996). *M. donacium* is a fast-growing species, which may attain very high abundances and may contribute up to 95% of the biomass of shallow soft bottom communities (Arntz *et al.* 1987; McLachlan *et al.* 1996).

> **Key Points**
> ● The reproductive season of *M. donacium* (active and ripe stage of gonads) partly overlaps with the main impact time of EN.
> ● *M. donacium* contributes up to 95% of biomass of shallow soft bottom communities.

1.4.3 Fisheries

Archaeological studies show that *M. donacium* was one of the most important food sources of shellfish species long before the first Hispanic settlers arrived in South

America (Tarifeño 1980). Nowadays the artisanal fishermen, so called 'macheros', still catch the species by stamping with their feet in the intertidal and upper subtidal. The movement dislodges individuals from the sand, and they are then collected by hand into mesh bags. In the deeper subtidal zone, animals are collected by hooker diving. *M. donacium* ranks among the most important invertebrates species for Chilean and Peruvian benthic fisheries (Defeo *et al.* 1993; McLachlan *et al.* 1996). The fishery exhibits cycles of 'boom-and-bust', where dense local stocks are quickly depleted and fishermen move on to other regions (Thiel *et al.* 2007). At the present time the northern Chilean stock are collapsed. At La Macha beach in Arica, northern Chile, former dense populations are documented only by old shells still covering the beach (Fig. 8).

Fig. 8: (1) Old shells of *Mesodesma donacium*, relicts of former dense populations at La Macha beach in Arica, northern Chile, scale bar: 2 cm. (2) Picture of the a Peruvian tidal zone before EN 1982-1983 giving evidence of the exceptionally high abundances which *M. donacium* can attain. Arrows indicating specimens of *Semimytilus algosus* using *M. donacium* as substrate (Villegas *et al.* 2005), scale bar: 4 cm (picture by Wolf E. Arntz).

Since 1997, the Chilean government has established marine management areas, so called 'Areas de Manejo y Explotación de Recursos Bentónicos' (AMERB), which protect exclusive user rights in defined localities. Implementation of AMERBs has had a positive effect on the local fisheries (Aburto & Stotz 2003). *M. donacium* is sold at local markets, so called 'caletas', and offered fresh, frozen and tinned at national super markets. The species is also exported to European markets were it is sold tinned for prices up to 33.00€/kg (Fig. 9).

Fig. 9: (1 and 2) Tinned and fresh Chilean *Mesodesma donacium* sold at a local supermarket (Lider, Antofagasta, Chile) at prices of approximately 3000 $/kg (~3.70 €/kg). (3) *M. donacium* sold tinned at a European supermarket (Carrefour, Mallorca, Spain) as 'macha' for approximately 1.94 €/100g (~25.00€/kg).

Key Points

● *M. donacium* is the most commercially important benthic invertebrate in the HCS, reaching high prices at national and international markets.

● AMERBs were established to stabilize declining stocks of marine living resources.

1.5 Aims of this study

The first part of this thesis addresses the controversial taxonomic status of *D. obesulus*. The sympatric surf clam species *D. obesulus* and *M. donacium* have shown to be strongly influenced by changes associated with EN and LN events. This study aims to supplement known field observation (e.g. Arntz & Fahrbach 1991) with an experimental approach examining the consequences of EN- and LN-type changes for the two species. The responses of early larval, juvenile and adult stages of *D. obesulus* and juvenile and adult stages of *M. donacium* to EN- and LN-type changes in temperature and salinity conditions are described. Observed changes in geographical distributions in response to strong climate variation, evolutionary pathways, and further abiotic parameters are discussed. A summary

of the objectives and methods applied in this thesis is given in Figure 10. This thesis incorporates four publications addressing the aims outlined above as follows:

(I) To determine the taxonomic state of the two species *D. marincovichi* and *D. obesulus* inhabiting the HCS coast and to clarify their distribution.

⇒ Hypothesis: the historic use of shell characteristics as main identification feature and the high phenotypic plasticity of Donacidae have resulted in several synonyms describing the same species **(Publication I)**.

(II) To describe the embryonic early larval stages of *D. obesulus* and to determine the effects of increased water temperature and reduced salinity, as observed during typical EN, on development, activity, growth and mortality of larvae.

⇒ Hypothesis: periods of anomalous temperature and salinity have distinct, measurable effects on development, activity, growth and mortality of early larvae of *D. obesulus* **(Publication II)**.

(III) To determine the effects of increased and decreased water temperature, as observed during typical EN and LN, on the growth rate and mortality of adult *D. obesulus* and *M. donacium*.

⇒ Hypothesis: periods anomalous temperatures have distinct, measurable effects on growth rate and mortality of *D. obesulus* and *M. donacium* **(Publication III)**.

(IV) To determine the effects of increased temperature and reduced salinity, as observed during typical EN, on growth rate and mortality of *M. donacium*.

⇒ Hypothesis: periods of anomalous temperature and salinity have distinct, measurable effects on *M. donacium* growth and mortality **(Publication IV)**.
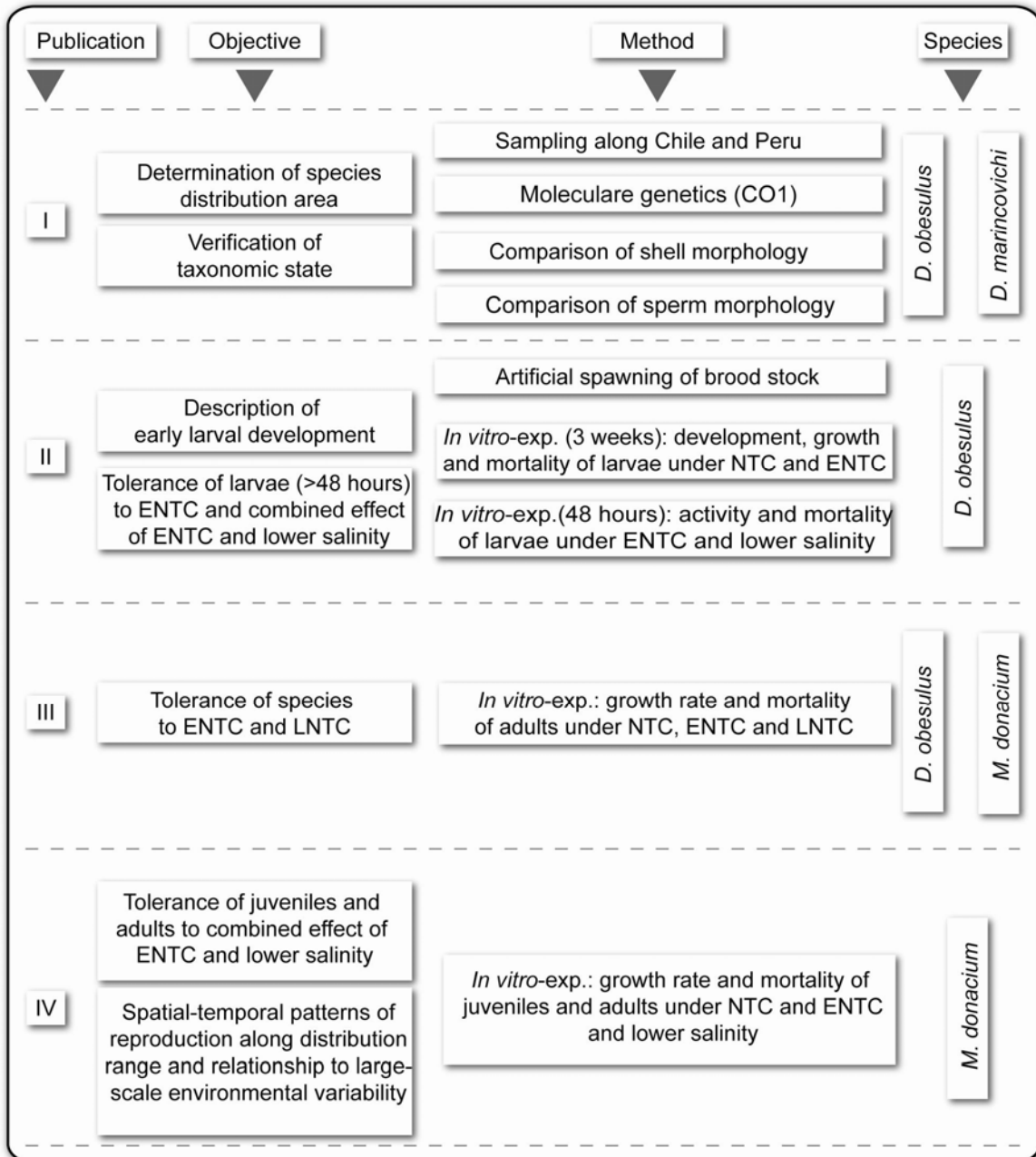
Fig. 10: Summary of objectives and methods applied in Publications I to IV. Abbreviations: Cytochrome oxidase 1 (CO1), El Niño temperature conditions (ENTC), La Niña temperature conditions (LNTC) and normal temperature conditions (NTC).

2 Material and Methods

Investigations on *D. obesulus* D and *M. donacium* M

2.1 Study sites and sampling

The coast of Chile and Peru is characterized by rocky shoreline frequently interrupted by extensive sandy beaches varying from the dissipative to the reflected type (e.g. Jaramillo 1994; Villegas *et al.* 2005). Given that past episodes of EN and LN have modified the historical distribution of *D. obesulus* and *M. donacium*, the recent southern limit of *D. obesulus* and the corresponding recent northern limit of *M. donacium* were redetermined by ample sampling. The southernmost population of *D. obesulus* was located at Chinchorro beach in Arica, northern Chile (18°27'53.8`S; 70°18'24.3`W; Fig. 1) and the northernmost population comprising the whole range of size classes of *M. donacium* was found at Hornitos beach, northern Antofagasta, also in northern Chile (22°54.99`S; 70°17.42`W). For *in vitro*-experiments brood stocks were collected from beaches in Antofagasta and Arica in order to produce larvae for the description of larval development. Specimens were also collected for use in the temperature and salinity experiments (Publication II to IV). For the genetic analysis specimens of *Donax sp.* were sampled at ten locations along the species' distributional range (Fig. 1, Station 1-10). Specimens of two further species, *D. asper* and *D. hanleyanus* were obtained from Tumbes in northern Peru and Villa Gesell in Argentina respectively, and were included in the analysis as outgroups (for details see Publication I).

D

2.2 Taxonomy of *Donax* sp. – shell morphology

Based on shell characteristics (following Coan 1983) all individuals collected were identified as either *D. marincovichi* or *D. obesulus* (for details see Publication I). Specimens were measured with a vernier caliper (±0.01 mm) to record length (maximum anterior-posterior distance), height (maximum ventro-dorsal distance) and width (maximum left-right distance) (Fig. 11).
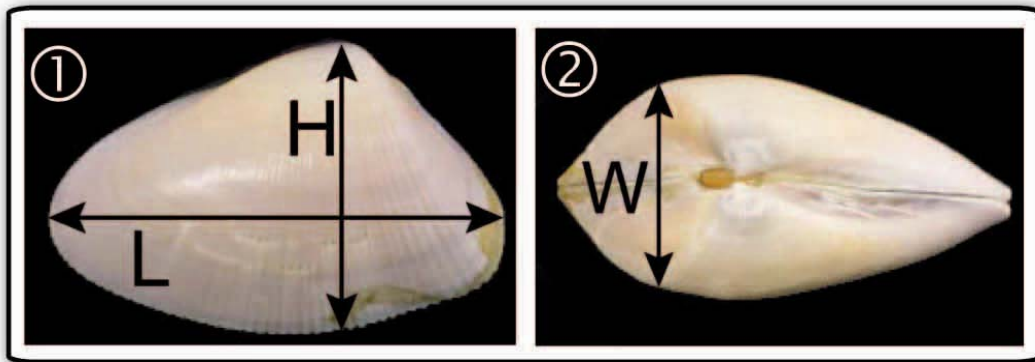
Fig. 11: Distances measured to describe shell morphological parameters of *Donax* sp. (Coan 1983): (1) length (maximum anterior-posterior), height (maximum ventro-dorsal). (2) Width (maximum left-right).

Width:height (W:H) and height:length (H:L) ratios were calculated and the nonparametric Kruskal-Wallis test was used to evaluate statistical differences in each ratio among all stations. When the Kruskal-Wallis test revealed significant differences, post hoc comparisons were performed using the Dunn test at a level of 5% significance to evaluate paired (i.e. between every pair) differences (Zar 1999). The 'maximum anterior-posterior SL' limits specified by Coan (1983) as 32 mm for *D. marincovichi* and 38 mm for *D. obesulus* was not applied in this study as all specimens were smaller than this (*D. marincovichi*, SL $\leq$ 29.75 mm; *D. obesulus*, SL $\leq$ 27.83 mm).

D

2.3 Taxonomy of *Donax* sp. – genetic analysis

2.3.1 DNA extraction

For a subset of 79 *Donax* specimens (*D. asper*, n = 9; *D. hanleyanus* n = 9; *D. marincovichi*, n = 25 and *D. obesulus*, n = 36) a fragment of the mitochondrial gene CO1 was sequenced as follows. Specimens with opened and closed shells were collected from each station (Fig. 1) and preserved in 80% ethanol. A sample of muscle tissue (1 mm^2) was extracted from the middle and apex region of the foot and cleaned with ethanol (75%) to remove sand, detritus or external organic matter. Each tissue sample was cut into yet smaller pieces to decrease tissue lysis time. To avoid contamination of DNA, extraction was carried out under sterile conditions. DNA extraction was performed with the Qiagen DNA Mini kit (Fig. 12). The standard tissue protocol was modified such that only 150 µl of elution buffer was used in order to increase the concentration of DNA.
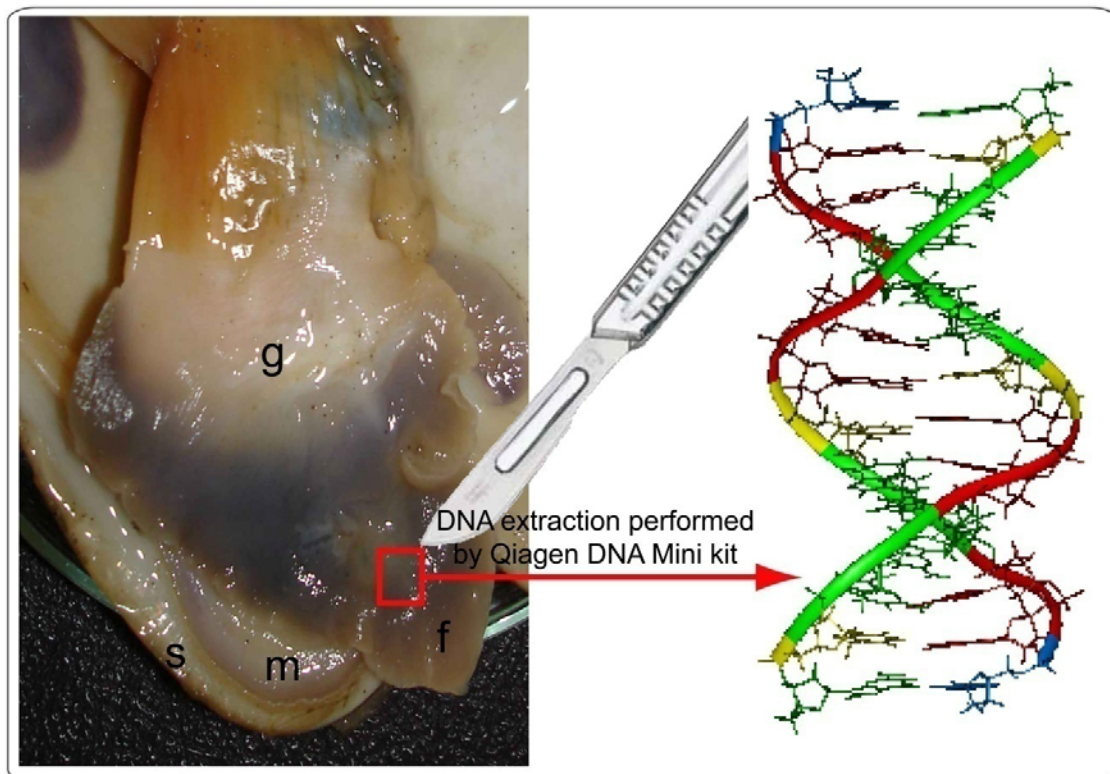
Fig. 12: Shells (s) of specimens were opened and approximately 1 mm^2 of muscle tissue was excised from the middle and apex region of the foot (f). Extraction of DNA from cleaned tissue was performed using a Quiagen DNA Mini kit. Other abbreviations: Gonad (g) and mantle (m).

2.3.2 Polymerase chain reaction

Initially, DNA amplification of two mitochondrial genes (CO1, 16S) was attempted using universal primers (Palumbi *et al.* 1991; Folmer *et al.* 1994). However the ribosomal large subunit gene (16S) failed to amplify in many specimens and further analyses therefore focused solely on the CO1 gene, which amplified successfully using the primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994) (Fig. 13). Amplification of the CO1 fragment was carried out in 25 µl reactions containing $1 \times$ HotMaster buffer, 0.2 µM dNTPs, 0.5 µM of each primer, 1–3 µl of DNA template, 0.02 U/µl HotMaster Taq polymerase (5 Prime, Hamburg, Germany), topped up to 25 µl with sterile H_2O. The PCR temperature profile for the CO1 amplification was: 94°C for 2 min, 38 cycles of 20 s at 94°C, 15 s at 42°C, 80 s at 65°C, followed by a final extension step of 7 min at 65°C. PCR products were checked on a 1.5% TAE agarose gel and purified using a Qiagen QIAquick PCR purification kit according to the manufacturer's recommendations. Cycle-sequencing of PCR products was carried on an ABI 3130xl automated sequencer using the terminal primers given above and a BigDye Terminator v3.1 kit from Applied Biosystems (ABI), purified using DyeEx-Kit (Qiagen) according to the modified protocol.
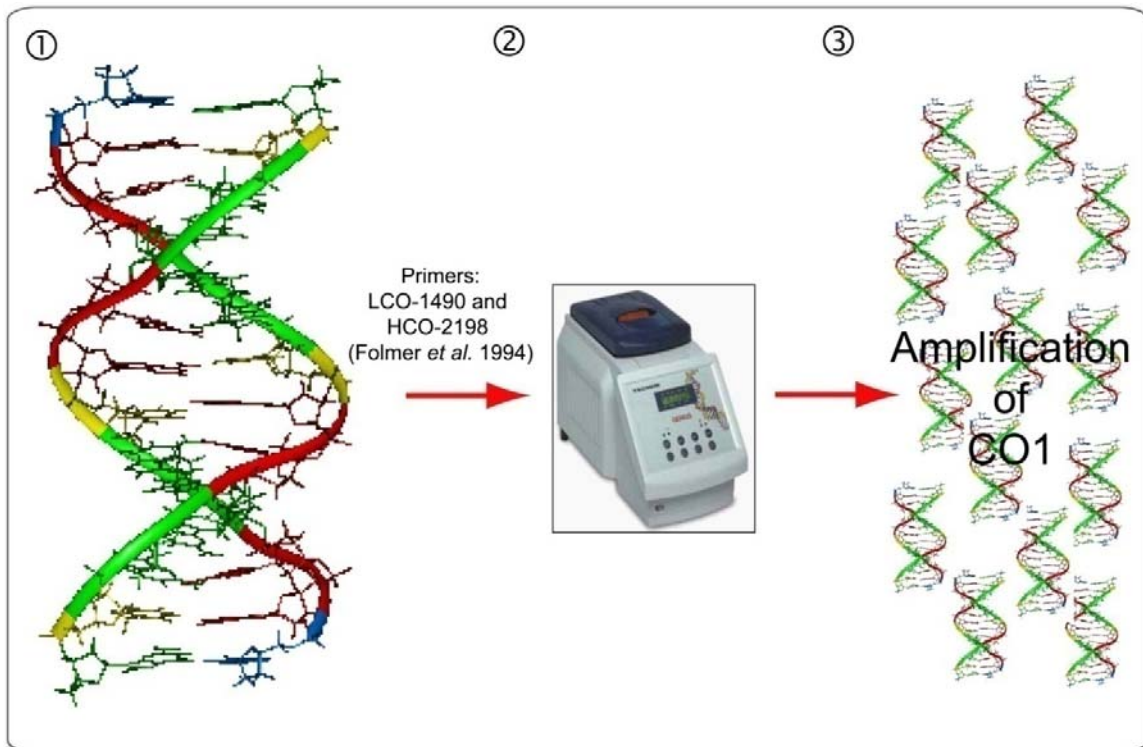
Fig. 13: (1) Small cytochrome oxidase subunit of extracted DNA was amplified by (2) polymerase chain reaction under the application of the primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994) (3) resulting in a millionfold duplication of the CO1 subunit.

2.3.3 DNA sequencing and data analysis

Sequences were aligned using the software MUSCLE (Edgar 2004). As an outgroup for the alignment, a sequence from the tellinid bivalve *Macoma balthica* was used (GenBank accession number EF044136). Phylogenetic analyses were performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The optimal model of sequence evolution was identified using hierarchical likelihood ratio tests and the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.2 (Nylander 2004) (Fig. 14). The MCMC analysis was performed using two independent runs with 10 million generations each and sampling every 100th tree. Convergence was reached after 60000 generations (discarded as burn-in). A statistical parsimony network was calculated from the *D. marincovichi* and

D. obesulus data set using TCS 1.21 (Clement *et al.* 2000) and a 95% connection limit.

Fig. 14: (1) Cycle-sequencing of PCR products was carried out using terminal primers and the BigDye Terminator v3.1 kit of Applied Biosystems (ABI), purified using DyeEx-Kit (Qiagen) on an ABI 3130xl automated sequencer. (2) An alignment was created from the sequences obtained whereas phylogenetic analyses were performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Hierarchical likelihood ratio tests and the Akaike Information Criterion (AIC) model was applied (Nylander 2004).

D

2.4 Taxonomy of *Donax* sp. – sperm morphology

In order to compare sperm morphology, gonads of *D. marincovichi* and *D. obesulus* were dissected 1 mm^3 of tissue (always from the same area) was fixed in 4% glutaraldehyde buffered with 0.1 M disodium phosphate buffer (pH 7.4) for 4 hours at 48°C and then thoroughly rinsed in phosphate buffer. Tissue samples were dehydrated using an ascending alcohol series (20% to absolute ethanol), then embedded in Araldite resin. Ultra-thin sections were produced using a LKB 2128 ultramicrotome and mounted on 200 mm mesh uncoated copper grids. The

sections were stained with uranyl acetate (10-20 min) and lead citrate (10-20 min). For further details of preparation see Giménez *et al.* (2008). Ultrastructural sperm morphology was examined and photographed using a Philips EM 301 TEM (Philips, Netherlands) at 60 kV and a Jeol (Jeol, USA) 1200 EX II at 80 kV. All measurements of sperm structures are based on TEM images. As there are several important changes during sperm maturation, such as changes in the acrosome and the positioning of the mitochondria (Pal 1996), only mature sperm were included in the analysis. Sperm morphology could then be characterized using precise descriptions of the general components (acrosome, nucleus, midpiece and flagellum).

DM

2.5 *D. obesulus* and *M. donacium*: *in vitro*-experiments on larvae

2.5.1 Photographic documentation of embryonic and larval development

During the *in vitro*-experiments, the embryonic and larval development of *D. obesulus* and *M. donacium* was documented using light microscopy and photography (Leica DM LS2, Germany and Canon Powershot S50, Japan). Larval samples were taken hourly during the first day and daily thereafter so that the progression of developmental stages could be followed.

2.5.2 Definition of temperature and salinity

The sites from which specimens for *in vitro*-experiments were collected, Arica for *D. obesulus* and Hornitos for *M. donacium,* are approximately 800 km apart from each other (Fig. 1), explaining the slightly different temperature regimes observed. Experimental temperatures were set to mimic the normal temperature conditions (NTC), El Niño temperature conditions (ENTC) and La Niña temperature conditions (LNTC) typically recorded at each sampling site. These were defined according to

the analysis of a long-term (1980-2006) SST data series archived in the database of the Servicio Hidrográfico y Oceanográfico de la Armada de Chile (available from http://www.shoa.cl/). For *D. obesulus*, NTC was defined as the long-term annual mean SST (17.8°C), ENTC reflected the highest monthly mean SST registered during EN 1982-1983 (24.6°C), and the LNTC resembled the lowest monthly mean SST reported for the Arica station (18°28`S, 70°19`W) during LN 1990 (14.9°C). Similarly, for *M. donacium,* NTC was set to 17.4°C, ENTC was 24.2°C, and LNTC was 14.5°C, based on records for the Antofagasta station (22°54`S; 70°17`W). The *in vitro*-experiments, were performed in temperature chambers with computer-controlled systems to maintain precise temperature control (±0.2°C) (Publication II to IV).

The combined effect of ENTC and reduced salinity (Publication II and IV) was tested on larval *D. obesulus*, and on juvenile and adult specimens of *M. donacium*. The ambient salinity level of 35 ±1 was designated as normal. Lower salinity levels of 25, 15 and 5 ±1 (Publication II - larvae) and to 20 and 10 ±1 (Publication IV - juveniles and adults) were achieved by diluting ambient sea water with distilled water.

D

2.5.3 *D. obesulus*: brood stock conditioning and temperature induced spawning

Adult specimens (>15 mm; n = 100) of *D. obesulus* were collected from Chinchorro beach (18°27'S/70°18'W) in Arica, northern Chile. The population is located at the southern end of the species range, a few hundred metres north of a river outlet. To minimize stress, clams were immediately transported to the laboratory and acclimatized to NTC (17.8°C for this species) and ambient salinity (35 ±1) for at least two weeks. This brood stock was maintained in a 200 l tank filled with 13 cm of sterilized sand from the collection site. Sea water was constantly aerated, filtered (1 µm), and UV-light treated. To prevent accumulation of excreted wastes, 20% of the sea water was exchanged weekly. Animals were exposed to a 12 hour day-night cycle. As multi-species diets of micro-algae enhance survival of larvae

(Ruiz-Azcona *et al.* 1996; Helm *et al.* 2004) specimens of the brood stock were fed *ad libitum* with a mixture (1:1) of living *Chaetoceros calcitrans* and *Isochrysis galbana*. The maturation of initially inactive gonads was monitored weekly by dissecting five individuals taken at random from the maintenance tank. Gonad ripeness was monitored by light-microscopical observations on smear preparations of small pieces of excised of tissue. Gonads were judged to be fully mature after four weeks of conditioning.

To induce spawning, mature specimens of the brood stock were exposed to a temperature shock treatment, being placed in water at 23°C ±0.2°C, an increase of 5°C over NTC. Preliminary experiments had revealed a weaker spawning response of specimens exposed to smaller temperature increases. Female individuals released fluffy egg batches of a few millimetres in size, which sank to the bottom, whereas male individuals released a milky liquid (Fig. 15). To prevent uncontrolled intermixture of gametes, spawning specimens were separated according to their sex as soon as visual identification of germ cells was obtained. Oocytes and sperm cells were carefully extracted using a Pasteur pipette and stored separately in glass beakers. The higher temperature was maintained until gamete release finished (<3 h). The number of oocytes and sperm cells was determined microscopically before the germ cells were brought together under sterile conditions in a ratio of 1:10 (oocytes:sperm).
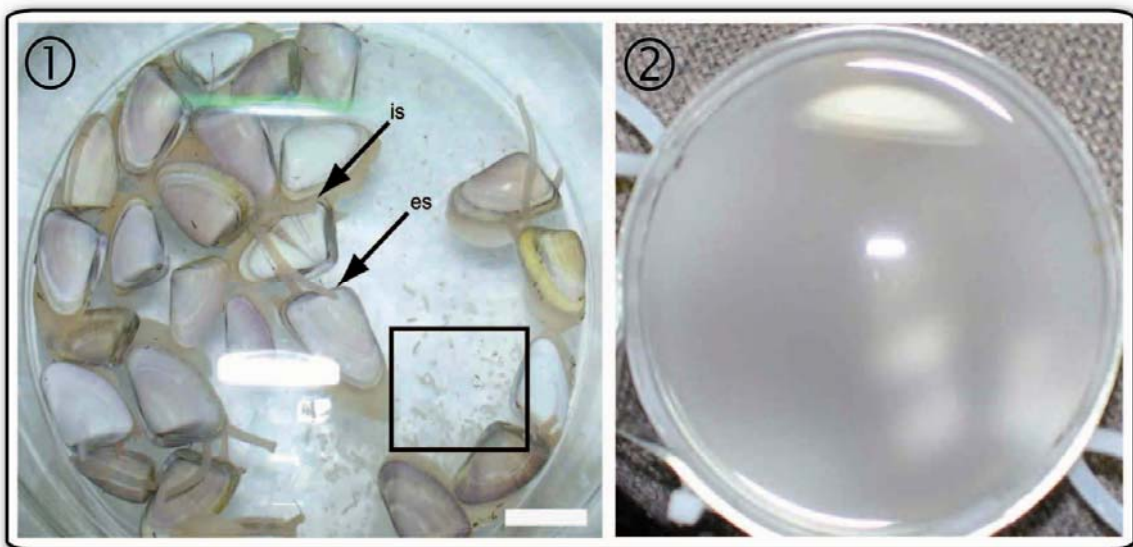
Fig. 15: (1) Female specimens of *Donax obesulus* during spawning induced by temperature shock treatment (increase of 5°C). During ejection of oocytes the clams extended their inhalant (is) and exhalant (es) siphons. Oocytes were ejected in visible 1 to 5 mm clusters (black square), which sank immediately at a salinity of 35; scale bar: 2 cm. (2) Male specimens of *D. obesulus* after sperm ejection, specimens are poorly visible through the milky solution within the glass beaker.

D

2.5.4 *D. obesulus*: early larval development under normal and El Niño temperatures

During a 16 day *in vitro*-experiment, early larval development stages of *D. obesulus* were monitored as described in Chapter 2.5.1. During the first two days embryonic stages were observed hourly (<48 h), thereafter samples of larvae from both treatments were taken daily.
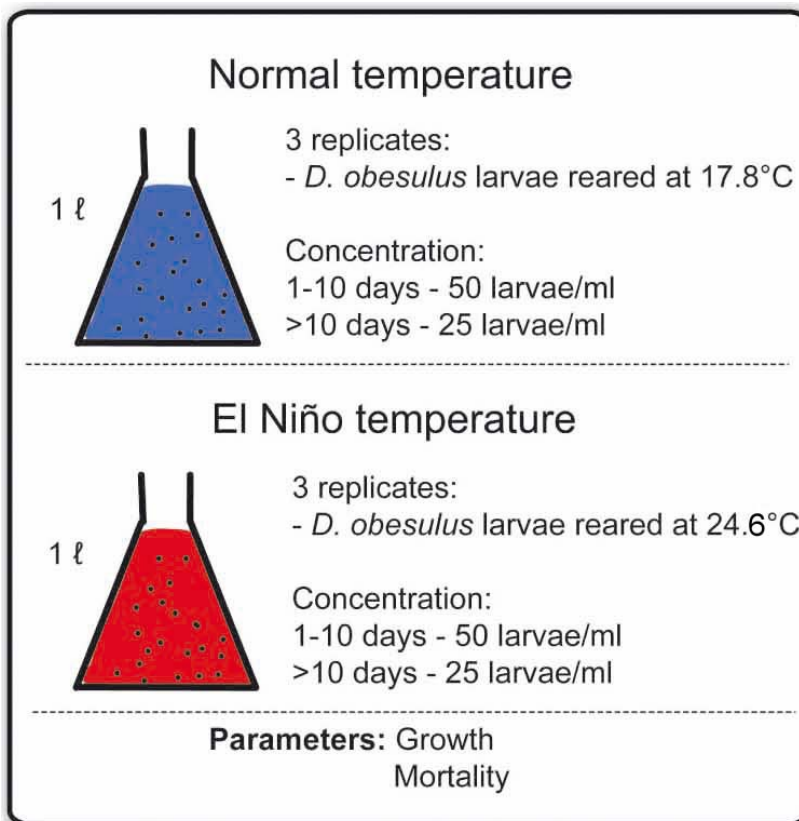
Fig. 16: Larvae of *Donax obesulus* maintained for 16 days under NTC (17.8°C) and ENTC (24.6°C). Growth and mortality larval development progress under the two different temperature regimes was recorded.

In order to describe the effect of ENTC on larval development, growth and mortality, early stage larvae (D-Veliger, >48 h) were exposed to ENTC and NTC for a period of 16 days (Fig. 16): Larvae were cultured in 1 litre glass beakers under the conditions described in Chapter 2.5.2 and 2.5.3. The ENTC and NTC treatments were replicated three times. With the growth of larvae, density was reduced from ~50 larvae/ml to ~25 larvae/ml at day 10 (counted in a Sedgewick Rafter counting cell, slide PYSER-SGI, England). To minimize contamination by bacteria and to ensure good water quality, each replicate was filtered (mesh size 100 µm), and transferred into a sterile glass beaker with new water and microalgae daily. Samples of 1 ml were taken from each of the three replicates daily and a Sedgewick Rafter counting cell slide (PYSER-SGI, England) was used to determine abundance (number live larvae) and mortality (estimated by counting empty larval shells). Body length (maximum posterior-anterior distance) of 30 larvae from each replicate was evaluated daily. For details of statistical analysis see Material and Methods section of Publication II.

D

2.5.5 *D. obesulus*: early larvae exposed to El Niño temperature and reduced salinity

To test the combined effect of reduced salinity and the ENTC, larvae were randomly assigned to treatments within a 4×2 factor experimental design: four salinities (5, 15, 25 and 35 ±1) at NTC and ENTC (Fig. 17). The *in vitro*-experiment was continued until one treatment induced 100% mortality, after 48 h. To obtain the exact salinity prescribed for each treatment, ambient sea water (35 ±1) was diluted with distilled water to the required value (Chapter 2.5.2). For each of the eight treatments, 20 replicates (4 ml plastic beakers) were run, containing one larva each. Larvae were not fed during the experiment to avoid changes in experimental parameters.
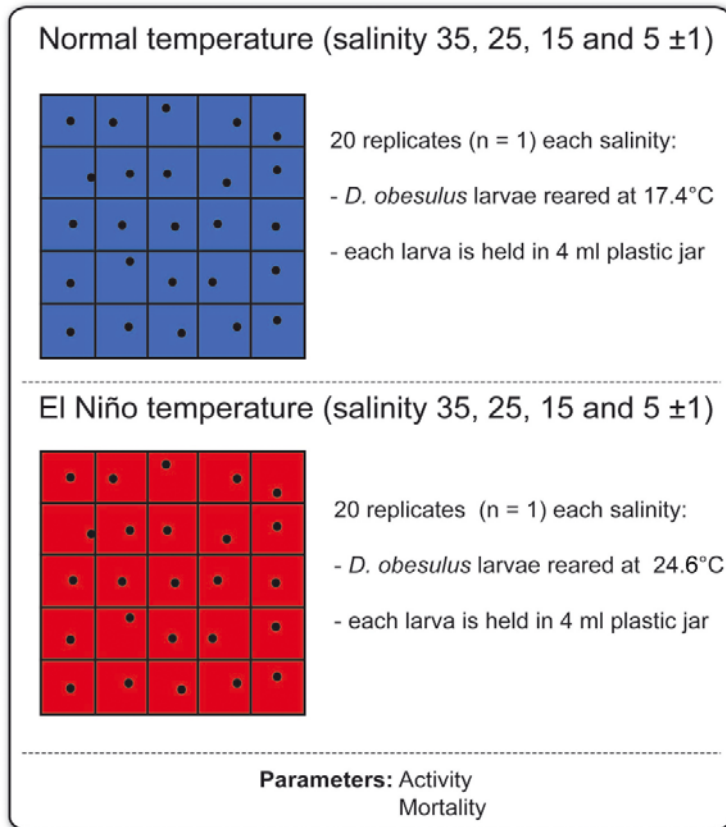
Fig. 17: Larvae of *Donax obesulus* were maintained for 48 hours at four different salinities (35, 25, 15, and 5 ±1) under NTC (17.8°C) and ENTC (24.6°C), respectively. Activity and mortality of larvae were determined.

Dead larvae (characterized by inactivity, opened shells, extended velum) were recorded every eight hours. Mortality was plotted against time such that the time at which 50% of the experimental population had died (LT_{50}) could be extrapolated (Urban 1994; Laudien *et al.* 2002). To assess larval vitality, activity was recorded as swimming or not swimming every eight hours between 24 and 48 hours from the start of the experiment. For statistical analysis see Material and methods section of Publication II.

DM

2.6 *D. obesulus* and *M. donacium*: *in vitro*-experiments on juveniles and adults

2.6.1 Estimation of growth rate

Certain stains are known to label internal calcified structures of mollusc shells. The quality of the labeling depends on the stain applied, the exposure time and the concentration of the stain. Best results for *Donax* sp. and *M. donacium* are achieved using Calcein (Riascos *et al.* 2007; Herrmann *et al.* 2009b). To estimate microgrowth rates of the clams in the temperature and salinity experiments (Publication III and IV), specimens were incubated for three hours in moderately aerated, filtered seawater containing 50 mg l^{-1} Calcein (Sigma, CAS 1461-15-0). Clams treated in this way incorporate a non-deleterious fluorescent mark in their shell, allowing the measurement of short-term (i.e. daily) growth increments (Riascos *et al.* 2007).

Fig. 19: Digital image (fluorescence microscopy) of Calcein mark, used to determine microgrowth of *Donax obesulus* and *Mesodesma donacium* (Publication III and IV).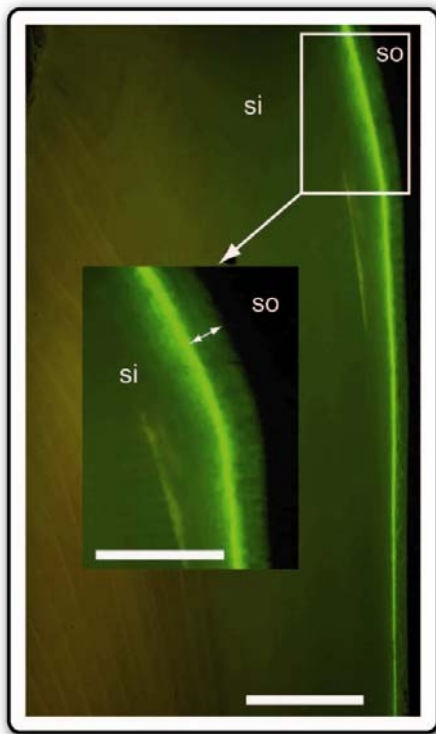 Double headed- arrow indicates growth increment of specimens produced during the experiment. Scale bar: 200 µm and 100 µm (detail), shell inside (si) and shell outside (so).

At the end of the temperature and salinity experiments, all animals were sacrificed, soft parts were removed and the shells oven-dried at 65°C for 24 hours. The right valve of each clam was embedded in epoxy-resin. A transverse section of each valve was obtained by cutting across the longest growth axis. In order to clearly identify the Calcein marker incorporated into the shell, the cut surface of the section was polished on a speed grinder-polisher (Buehler, Alpha, 2 Speed Grinder-Polisher, USA) using grinding paper (200 µm to 5 µm) and a polycrystalline diamond suspension (1 µm to 0.05 µm). Calcein markings were visualised using fluorescence microscopy and appeared as a bright band, which could be measured in order to evaluate the individual growth increment (Fig. 19). Microgrowth was measured (to the nearest µm) in digital fluorescence micrographs (blue light, 460-490 nm) from the Calcein mark to the shell edge. A daily growth rate (µm day^{-1}) was calculated for each specimen.

DM

2.6.2 Experimental setup for *in vitro*-experiments on juveniles and adults

In order to evaluate the response of clam specimens to abnormal temperature and salinity, daily growth rate and mortality of specimens maintained in closed circulation systems was estimated (Publication III and IV). SL (maximal anterior-posterior distance) of each specimen was measured to the nearest ±0.01 mm with a vernier caliper and the clam tagged with a consecutive code to allow individual identification. Sea water was UV-filtered (1 µm) and each 35 l tank was filled with an adequate quantity of clean sand (~13 cm depth) to provide substrate for complete retraction of the surf clams. Experimental temperature was adjusted as described in Chapter 2.5.2 and specimens were exposed to a 12:12 hour day-night light regime. Sand was aerated and ~15% of the water was exchanged daily to avoid problems associated with water pollution. Animals were fed as described in Chapter 2.5.3, and any dead animals (open shell, foot and siphons extended) were removed on a daily basis. At the end of the *in vitro*-experiments all shells were processed as described in Chapter 2.6.2. Data on growth was evaluated as

described in Chapter 2.6.1. Mortality of each species was calculated from the daily percentage of surviving clams throughout the experimental period. A dosage-mortality approach (Urban 1994) was used as described in Chapter 2.5.4.

<mark>DM</mark>

2.6.3 *D. obesulus* and *M. donacium*: El Niño and La Niña temperature tolerance

Specimens of both species were collected (*D. obesulus*, n = 104; *M. donacium*, n = 122, Fig. 1). To minimize stress, clams were transported within 12 hours of collection and immediately transferred to laboratory conditions. Clams were randomly assigned to a single factor (temperature), three-level (NTC, ENTC, LNTC) *in vitro* experiment, with three replicate aquaria per level, each containing 12 individuals of *D. obesulus* and 16 individuals of *M. donacium* (Fig. 20). The experimental duration was 31 days. For details of statistical analysis see the Materials and Methods section of Publication III.
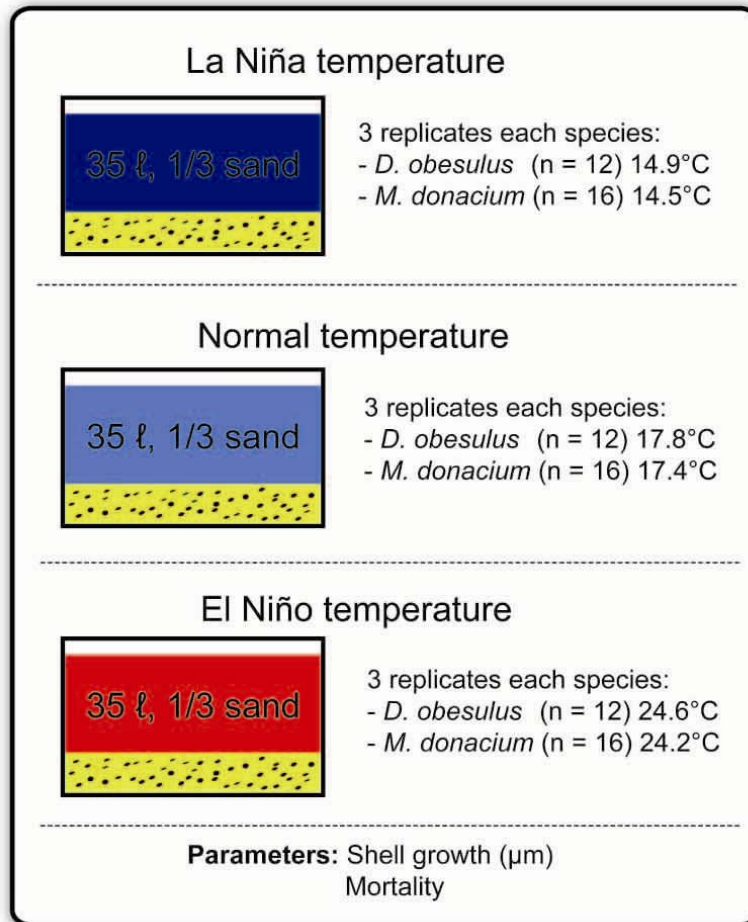
Fig. 20: Adult specimens of *Donax obesulus* and *Mesodesma donacium* were maintained under LNTC, NTC and ENTC for one month. Shell growth and mortality were monitored. System: 35 l plastic tanks, containing approximately 13 cm depth sterilized sand; the sea water passed UV radiation and a1 µm filter); approximately 15% of water was exchanged each day. Bivalves were fed *ad libitum* with microalgae daily.

M

2.6.4 *M. donacium*: El Niño temperature and low salinity

Juvenile and adult specimens of *M. donacium* (n = 109) were collected (Fig. 1) and acclimatized to laboratory conditions as described in Chapter 2.5.2. In order to test the temperature and salinity tolerance of juvenile and adult *M. donacium,* clams were exposed to ENTC and reduced salinity for 43 days (Fig. 21). Technical aspects of experimental design were as described in Chapter 2.6.3. For details of statistical analysis see Materials and Methods of Publication IV.

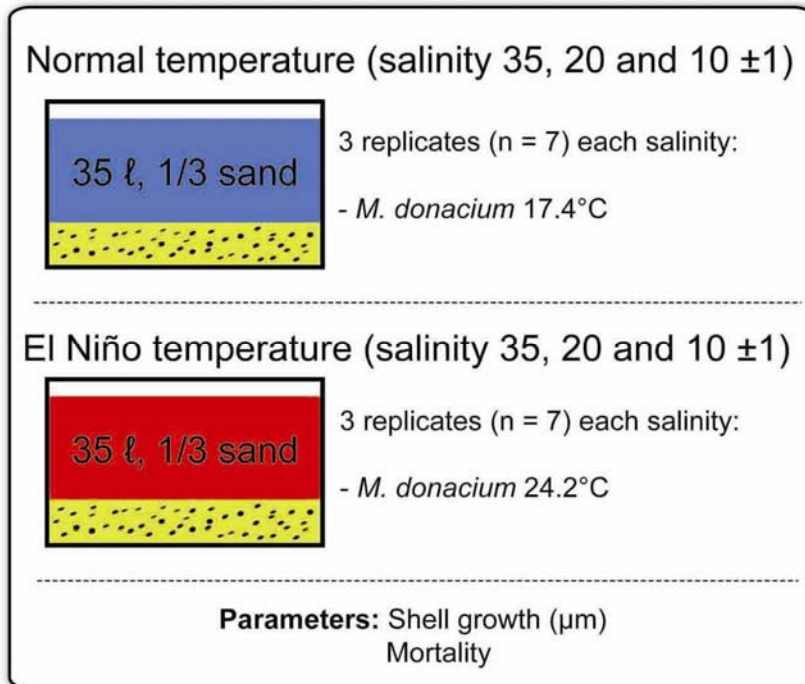**Fig. 21:** Juvenile and adult specimens of *Mesodesma donacium* were maintained at different salinities (35, 20 and 10 ±1) under NTC and ENTC. Shell growth and mortality were monitored. System: 35 l plastic tanks, containing approximately 13 cm depth of sterilized sand, UV treated filtered (1 µm) sea water, approximately 15% of water was exchanged each day. Bivalves were fed *ad libitum* with microalgae daily.

3   Summary of the Results and Publications

In this chapter the results of the thesis are summarized. More detailed results are provided in the attached publications.

D

3.1 Taxonomy of *Donax* sp.

3.1.1 Shell morphology

Specimens were identified as *D. marincovichi* or *D. obesulus* according to the morphological characters specified by Coan (1983) (Publication I Fig. 2 and Tab. 2). Of 109 specimens, only 20% were a perfect match for the morphotype expected from the species description. The other 80% of specimens each exhibited at least one character state expected for the other species. The percentage of mismatches for each morphological character in both species is presented in Publication I Table 3. Of all the morphological characters used for identification in this study, only 'punctation' appeared to be reliable, while 'shape of beak' was shown to be most ambiguous (Publication I, Tab. 2). Within the nine characters applied, specimens allocated to the morphotype *D. marincovichi* (n = 56) presented 133 mismatches, while those identified as morphotype *D. obesulus* (n = 53) exhibited 58 mismatches (Publication I, Tab. 3).

The W:H and H:L ratios of all specimens were tested and revealed significant differences (p = 0.001). Further comparison of W:H and H:L between stations revealed significant differences (Publication I Tab. 4), indicating that stations from northern Chile and northern Peru (Publication I Fig. 1, Tab. 4, Stations 1, 7, 9 and 10) which were dominated by the *D. obesulus* type differed significantly from central Peruvian stations (Fig. 1 and Publication I Tab. 4, Stations 2-6 and 8), which are dominated by the *D. marincovichi* type. Specimens from the former stations are more wedge-shaped (shorter and thicker) indicating a compacter shape than clams from the other sites (Publication I Fig. 2). Recent

results of this study increased the maximum value of the H:L ratio (Coan 1983) (Publication I Tab. 2) for *D. marincovichi* to 0.62-0.73 and for *D. obesulus* to 0.66-0.85. Further sampling at Jahuay beach (Publication I Fig. 1, Station 3) revealed that the *D. marincovichi* type reaches a maximum length up to 35 mm, not 32 mm as reported by Coan (1983).

3.1.2 Genetic analysis

Neither species showed any significant mitochondrial genetic differentiation within or among populations. The alignment of the CO1 gene sequences yielded a 567-bp alignment. Analyses of uncorrected P-distances and haplotype sharing in the statistical parsimony network revealed no consistent differences between *D. marincovichi* and *D. obesulus* (Publication I Figs. 5 and 6). However strong differences were recorded between *D. marincovichi* and *D. obesulus* and the two *Donax* outgroup species (15% P-distance, Publication I Fig. 5). The pairwise genetic difference calculated for *D. obesulus-D. obesulus* or *D. marincovichi-D. marincovichi* pairs was equal to within-species pairwise comparisons, i.e. *D. marincovichi-D. obesulus* pairs and *vice versa* (0–1.2%). Furthermore, no genetic differentiation was observed between geographically separated populations.

3.1.3 Sperm morphology

Sperm studies of *D. marincovichi* and *D. obesulus* revealed that both species to have a typical primitive aquasperm type, with an acrosome, nucleus, midpiece and flagellum (Publication I Fig. 4A). The pearshaped acrosome settles in a nuclear fossa (Publication I Fig. 4A, B). The transverse section of the acrosome shown in Publication I, Figure 4C illustrates that the structure is differentiated into two regions with different electron densities. The outer crescent-shaped basal ring is

electron-dense and represents approximately 50% of the total area of the acrosome. It is enclosed by an outer acrosomal membrane. The electron-lucent anterior, central and posterior region contains the axial rod at its centre. The electron-lucent part of the acrosome apex is arrow-shaped (Publication I Fig. 4A). The sperm nucleus is associated with the acrosome and accounts for approximately one-third of the total size of the sperm (Publication I Fig. 4D). The flagellum shows a structure typical of molluscs: the axoneme consisting of a central pair of microtubules encircled by four mitochondria (Publication I Fig. 4E). Mean values for length and diameter of the acrosome, nucleus and midpiece are given in Publication I, Table 5.

Key Points

● The separation of *D. marincovichi* and *D. obesulus* species according to the morphological characters of Coan (1983) is subject to a high degree of uncertainty.
● The shell W:H and H:L ratios of specimens revealed significant differences between stations (Fig. 1).
● Comparison of CO1 gene sequences revealed no genetic differentiation between species or stations (Fig. 1).
● The primitive aquasperm type revealed to be indistinguishable between both morphotypes.

3.2 *D. obesulus* and *M. donacium*: *in vitro*-experiments on larvae

DM

3.2.1 Early larval development under normal and El Niño temperatures

Immature specimens collected for brood stock were successfully conditioned within four weeks under *ad libitum* feeding conditions. Spawning was induced by temperature shock of 5°C over NTC. Larval development was observed and measured by light microscopy. Head length of spermatozoids was ~5 µm, tail

length was ~50 µm. Unfertilized oocytes had a diameter of 59.34 µm (n = 25, ±0.63 µm). Larval development followed successive stages typical of bivalve species and of Donacidae in particular. During the first 24 hours cell division was observed, resulting in a ciliated blastula, gastrula, a trochophore stage, and after 24 hours, a D-Veliger larvae is formed (Publication II, Fig. 1). After the D-Veliger stage the larva develops first a rudimentary foot, then a probing foot which is characteristic of larvae ready to settle before metamorphosis (Publication II, Fig. 1).

Larval length (LL, maximum anterior-posterior) increases during the third day after fertilization from 88.46 µm (n = 90, ±0.27 µm) to 160.85 µm (n = 90, ±1.95 µm) under NTC and from 88.65 µm (n = 90, ±0.32 µm) to 176.91 µm (n = 90, ±1.90 µm) at ENTC (Publication II, Fig. 2). The increase in larval height (dorsal-ventral) was less than the increase in length at both temperatures. Growth under NTC and ENTC was significantly different (F_1 = 358.0; p = 0.00). At 18 days post-fertilization, larvae at NTC attained a maximum length 213.5 µm, whereas larvae under ENTC reached a maximum length of 240.75 µm. Overall mortality during the experiment was very low: 0.79 ±0.11% at NTC, and 1.25 ±0.37% at ENTC (Publication II, Fig. 3). There was no significant difference in the mortality recorded for either treatment (F_1 = 2.82; p = 0.36).

D

3.2.2 Early larvae exposed to El Niño temperature and reduced salinity

No mortalities were recorded during a 48-hour exposure to salinities of 35, 25 and 15 ±1 at NTC and ENTC. Larvae exposed to the lowest salinity treatment (5 ±1) however exhibited 100% mortality after 16 hours in the NTC group and 32 hours at ENTC (Publication II, Fig. 4), a significant temperature dependent difference. (F_1 = 4.87; p = 0.03). At NCT LT_{50} was reached after 4.3 hours of the experiment while at ENCT LT_{50} was reached after 6.5 hours (Publication II, Fig. 4).

Activity of larvae, assessed as counts of actively swimming individuals, recorded every eight hours between 24 and 48 hours after the experiment started 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 lowest activity (Publication II, Fig. 5). Statistic analysis revealed no significant differences in activity of larvae between test temperatures (F_1 = 0.308; p = 0.59). However, significant differences were recorded between different salinity levels (15, 25 and 35 ±1) (F_2 = 18.29; p < 0.00). Tukey-post-hoc comparison revealed significant differences between salinities of 25 and 35 ±1 at NTC and ENTC (p = 0.01 and p = 0.01).

Key Points

● Spawning was successful induced by temperature shock treatment (NTC +5°C).

● Larvae reared under NTC and ENTC revealed significant differences in growth.

● Mortality of larvae was very low at NTC and ENTC (>2%).

● Larvae showed no mortality at normal and slightly reduced salinity (35-15) but suffered 100% mortality at very low salinity (5) at both NTC and ENTC.

3.3 *D. obesulus* and *M. donacium*: *in vitro*-experiments on juveniles and adults

DM

3.3.1 Adults exposed to normal, El Niño and La Niña conditions

Growth rate of *D. obesulus* was significantly dependent on shell size, with larger clams showing lower growth rates. Significant differences were also revealed between temperature treatments (Publication III, Tab. 1, Fig. 2). Growth was fastest under NTC, followed by ENTC and lowest at LNTC. Post-hoc comparisons showed significant differences between NTC and LNTC and between ENTC and LNTC (Tukey test; p < 0.01; p = 0.012 respectively), but no significant difference between NTC and ENTC (Tukey test: p = 0.497).

Mortality of *D. obesulus* revealed significant differences between experimental temperatures (Publication III, Tab. 2), being higher and earlier at LNTC (Publication

III, Fig. 4). Post-hoc comparisons showed significant differences in mortality between NTC and LNTC and between ENTC and LNTC (Tukey test; $p < 0.01$, $p = 0.02$, respectively) but no significant differences between NTC and ENTC (Tukey test; $p = 0.87$). This species proved highly sensitive to LNTC, with high mortality after 48 hours of exposure and total mortality after three weeks. In contrast, under NTC and ENTC a few dead specimens were observed after four days, rising slightly until day 18. Mortality did not surpass 15% by the end of the experiment. Growth rate of *M. donacium* was also inversely correlated to shell size and showed significant differences between temperature regimes (Publication III, Tab. 1, Fig. 3). A significant interaction between temperature and shell size was detected (Publication III, Tab. 1), indicating that differences between temperatures were less clear in larger clams. Multiple *a posteriori* comparisons showed that growth rate of *M. donacium* differed significantly between NTC and LNTC, and NTC and ENTC (Tukey test; $p = 0.021$; $p < 0.01$, respectively), but there was no significant difference between LNTC and ENTC (Tukey's HSD test: $p > 0.864$).

Of the two species tested, *M. donacium* was comparatively more resilient under changing temperatures (Publication III, Fig. 4). Our analysis revealed significant differences in mortality rates between experimental temperatures (Publication III, Tab. 2). Tukey tests showed significant differences between LNTC and ENTC ($p < 0.01$) and between NTC and ENTC ($p < 0.01$). However no significant differences were found between LNTC and NTC (Tukey test; $p = 0.91$) as all clams survived until the end of the experiment (Publication III, Fig. 4). Under ENTC, *M. donacium* showed a slight increase in mortality after the tenth day then held steady until day 17, after which the rate increased, reaching 67% at the end of the experiment (Publication III, Fig. 4).

As indicated by the LT_{50} values, *D. obesulus* responded much faster to temperature changes than *M. donacium*. The former species reached LT_{50} at LNTC after eight days, whereas LT_{50} of *M. donacium* at ENTC was reached after 27 days of the experiment (Publication III, Fig. 4). No other level of the treatment resulted in 50% mortality during the experimental period.

Key Points
● *D. obesulus* showed fastest growth under NTC and lowest growth and highest mortality under LNTC.
● *M. donacium* showed significant differences between temperature treatments and revealed highest mortality under ENTC.
● LT$_{50}$ indicated that *D. obesulus* responded faster to temperature changes that to *M. donacium*.

M

3.3.2 Adults and juveniles exposed to normal and El Niño conditions and reduced salinity

The growth rate of juvenile *M. donacium* varied between temperatures, but not between salinity levels (Publication IV, Tab. 3). Growth rate under ENTC decreased to approximately one-half of that at NTC (Publication IV, Fig. 2). The growth rate of adult clams varied between temperatures and between salinity levels (Publication IV, Tab. 3). Multiple comparisons showed that at ENTC, growth rate was significantly lower at a salinity of 10 than at 35 (Tukey's test, $p < 0.05$). In contrast, under NTC, no significant differences in growth rate were observed between salinity levels (Publication IV, Fig. 2). Overall, mortality was higher at ENTC at all salinity levels, reaching 50% after 23 days. In contrast, mortality was generally low under NTC. It was <20% at salinities of 35 and 20 and <50% at 10 (Publication IV, Fig. 3). The ANCOVA model revealed that juvenile clams died significantly faster at ENTC, but no significant differences were observed between salinity levels (Publication IV, Fig. 4, Tab. 4). In contrast, mortality of adults was significantly affected by both temperature and salinity, with a significant interaction between the main effects. Mortality was higher under ENTC, showing an additional increase at low salinities (10 and 20). Under NTC, adult clams died faster only at the lowest salinity, whereas no differences were observed between 20 and 35 (Publication IV, Fig. 4).

Key Points

● Juveniles exhibited lower growth at ENTC than at NTC.

● Under ENTC adults grow more slowly at salinity of 10 than at a salinity of 35; under NTC no growth difference was observed between salinities.

● Overall mortality is higher under ENTC than at NTC and increases further with decreasing salinity (35, 20, 10).

3.4 Publication I

GENETIC VARIABILITY, SHELL AND SPERM MORPHOLOGY SUGGEST THAT THE SURF CLAMS *DONAX MARINCOVICHI* AND *D. OBESULUS* ARE ONE SPECIES

DANIEL CARSTENSEN[1], JÜRGEN LAUDIEN[1], FLORIAN LEESE[1,2], WOLF ARNTZ[1] AND CHRISTOPH HELD[1]

[1]*Alfred Wegener Institute for Polar and Marine Research, PO Box 120161, D-27515 Bremerhaven, Germany; and*
[2]*Department of Animal Ecology, Evolution and Biodiversity, Ruhr University Bochum, Universitätsstrasse 150, D-44801 Bochum, Germany*

ABSTRACT

The taxonomy of two sympatric surf clams *Donax marincovichi* Coan, 1983 and *Donax obesulus* Reeve, 1854, inhabiting the coastal Humboldt Current Upwelling System is revisited. Because both species are exploited by artisanal fisheries, it is essential to verify that they are indeed distinct species that have to be managed separately. In this study, both taxa were sampled across their shared distributional area and specimens were indentified according to their respective morphological characteristics. Although width/height and height/length ratios revealed significant differences within sampling areas, the two morphotypes were frequently incongruent for taxonomically important morphometric parameters. In addition, they showed no significant mitochondrial genetic differentiation within or among populations and exhibited indistinguishable sperm ultrastructure. We conclude that the two morphotypes do not represent distinct species and should be included together under *D. obesulus*.

INTRODUCTION

The bivalve genus *Donax* is distributed almost worldwide, except for polar regions, and can be locally very abundant on exposed sandy beaches (Ansell, 1983). Donacidae occupy a major role in nearshore trophic webs, as they feed on phytoplankton, while in turn they are prey for gastropods, demersal fish, birds and mammals (Ansell, 1983). These bivalves represent an important resource for artisanal fisheries and local markets (Paredes & Cardoso, 2001; Rey, 2006, 2007, 2008; Riascos, 2006) and are exported, e.g. to Europe. Two species, *Donax marincovichi* Coan, 1983 and *Donax obesulus* Reeve, 1854, are distributed along the Humboldt Current Upwelling System from northern Chile to northern Ecuador (Fig. 1, Table 1). Within their shared distributional range both species reveal a broad range of shell shapes (Coan, 1983; Guzmán, Saá & Ortlieb, 1998).

The taxonomic status of several species of Donacidae has been much debated (Loesch, 1957; Wade, 1967a, b; Chanley, 1969; Morrison, 1971; Narchi, 1983; Bonsdorff & Nelson, 1992; Nelson, Bonsdorff & Adamkewicz, 1993; Guzmán *et al.*, 1998; Paredes & Cardoso, 2001; Laudien, Flint & van der Bank, 2003). The difficulty of species recognition is demonstrated by the reduction of 'valid' species from 64 (Ansell, 1983) to 45 in a recent taxonomic revision of the genus (Coan, Scott & Bernard, 2000). Clearly, confusion in taxonomical classification has led to numerous synonyms. The uncertain taxonomic status of the Chilean and Peruvian species *D. marincovichi* and *D. obesulus* is reflected in the current taxonomic literature (Olsson, 1961; Keen, 1971; Coan, 1983; Guzmán *et al.*, 1998; Paredes & Cardoso, 2001). In Chile these clams are informally known as 'machilla' and in Peru as 'mariposa', 'palabrita', 'concha blanca' or 'marucha' (Huaraz & Ishiyama, 1980; Soto, 1985; Paredes & Cardoso, 2001; Rey, 2006). Both species are exploited by the artisanal fisheries and are managed as one evolutionarily significant unit referred to as *Donax peruvianus* (Paredes & Cardoso, 2001; Rey, 2006, 2007, 2008). However, to verify

Correspondence: D. Carstensen; e-mail: daniel.carstensen@awi.de

management strategies and to optimize sustainable exploitation it is essential to know if *D. marincovichi* and *D. obesulus* can be treated as one species or if they have to be managed separately.

To clarify the taxonomic status of *D. marincovichi* and *D. obesulus* three different taxonomic methods were applied: (1) shells were classified after Coan (1983), followed by statistical evaluation of two morphometric parameters; (2) morphometric comparison of sperm ultrastructure of both species was performed using transmission electron microscopy (TEM); (3) sequence data of partial cytochrome *c* oxidase subunit 1 gene (COI) were used to estimate inter- and intraspecific similarities among geographically distant populations along the northern Chilean and Peruvian distributional area.

Sperm ultrastructural studies have provided a useful tool for taxonomic and phylogenetic investigations of bivalves for >45 years (Galtsoff & Gallardo, 1960).

In particular, genetic tools have been shown to be very suitable for resolving taxonomic and systematic problems, and techniques such as DNA barcoding have revealed a great capacity to define species boundaries (Kimura & Weiss, 1964; Levinton & Suchanek, 1978; Koehn *et al.*, 1984; Utter, 1991; Held, 2000; Hebert, Ratnasingham & DeWaard, 2003; Held & Wägele, 2005; Witt, Threlof & Hebert, 2006; Coghlan & Gosling, 2007). Genetic analyses are not only helpful in clarifying phylogenetic relationships, but also provide possibilities to analyse intraspecific relatedness between single populations and hence larval dispersal between regions and the dependence of recruitment on local stocks (Adamkewicz & Harasewych, 1994; Soares, 1999; Laudien *et al.*, 2003; Coghlan & Gosling, 2007).

For the clarification of the taxonomic status a combination of both morphological and genetic analyses is particularly effective for species identification (Jaernegren *et al.*, 2007).

MATERIAL AND METHODS

Material

Specimens ($n = 109$: *Donax marincovichi* $n = 56$ and *Donax obesulus* $n = 53$) were sampled at 10 locations along their
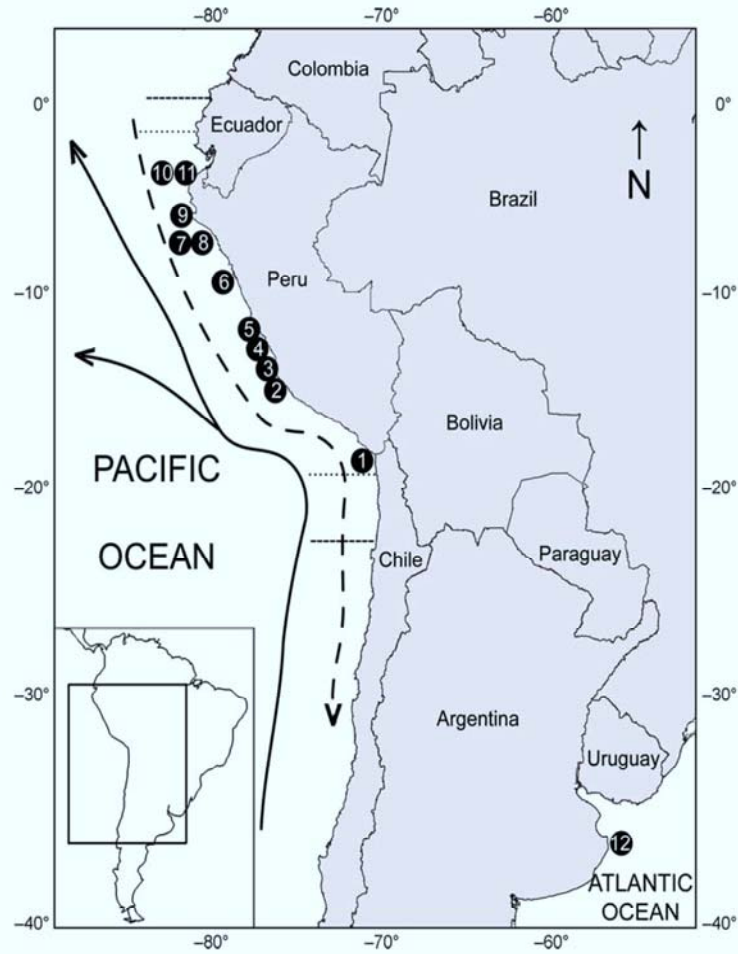
Figure 1. Distribution of *Donax marincovichi* (dotted line) (18°28′S; 70°20′W; 2°12′S; 80°58′W) and *Donax obesulus* (dashed line) (23°28′S; 70°31′W; 0°27′S; 80°7′W) and sampling stations (1–11) along the Chilean and Peruvian coast. Outgroups *Donax asper* and *Donax hanleyanus* were sampled at Stations 11 and 12. For further details see Table 1. Peru Coastal Current (solid arrows) and Peru Chile Undercurrent (dashed arrow), simplified after Tarazona & Arntz (2000).

Table 1. Sampling sites of *Donax marincovichi* (*n* = 56), *Donax obesulus* (*n* = 53) and *Donax* outgroups (*Donax asper* and *Donax hanleyanus*).

Station	Country	City nearby	Beach	Longitude	Latitude	D. marincovichi	D. obesulus
1	Chile	Arica	Chinchorro	S18°27′53.8″	W70°18′24.3″		11
2	Peru	Chincha	Violetas	S13°29′05.6″	W76°11′25.4″	11	
3	Peru	San Bartolo	Silencio	S13°24′33.0″	W76°11′49.1″	11	
4	Peru	Bujama	Sarapampa	S12°43′20.4″	W76°37′42.4″	10	1
5	Peru	Asia	Asia	S12°48′04.0″	W76°33′56.4″	10	1
6	Peru	Chimbote	El Dorado	S09°10′24.5″	W78°32′12.6″	8	2
7	Peru	Chiclayo	La Laguna	S07°04′08.7″	W79°44′00.2″		11
8	Peru	Chiclayo	La Laguna	S07°04′23.5″	W79°43′47.1″	6	5
9	Peru	Sechura	San Pedro	S05°29′49.8″	W80°53′53.7″		11
10	Peru	Tumbes	Hueso de Ballena	S03°30′20.5″	W80°29′04.0″		11
11	Peru	Tumbes	Hueso de Ballena	S03°30′20.5″	W80°29′04.0″	Donax asper	
12	Argentina	Villa Gesell	Mar Azul	S37°20′44.8″	W57°01′41.3″	Donax hanleyanus	

The numbers of the respective morphotypes indentified for each station are listed in the last two columns.

distributional range (Fig. 1, Table 1, Stations 1–10). Specimens of *Donax asper* from northern Peru (Tumbes; Fig. 1, Table 1, Station 11) and *Donax hanleyanus* from the Atlantic coast of Argentina (Villa Gesell; Fig. 1, Table 1, Station 12) were sampled as outgroups within the Donacidae. To avoid ontogenetic differences in shell morphology adult specimens (min, 15.02 mm; max, 29.97 mm) were chosen for analysis.

Table 2. Differentiation of *Donax marincovichi* and *D. obesulus* after Coan (1983).

	Donax marincovichi	Donax obesulus
1. Fig. 2A–D	Never has punctations	Punctations present on most specimens
2. Fig. 2E/F	Less inflated, W/H 0.57–0.69	More inflated, W/H 0.71–0.77
3. Fig. 2E/F	Beaks low	Beaks inflated
4. Fig. 2G/H	Periostracum dark	Periostracum light
5. Fig. 2G/H	Periostracum adherent in a wide marginal band	Periostracum light, marginal traces only
6. Fig. 2A–D	Surface silky	Surface shiny
7. Fig. 2I/J	Posterior lateral more distant from cardinals	Posterior lateral close to cardinals
8. Fig. 2K/L	Dorsal margin not produced above posterior lateral	Dorsal margin produced above posterior lateral
9. Fig. 2I–L	Anterior cardinal of right valve small, thin	Anterior cardinal of right valve thickened
10. Not considered	Maximum size 32 mm	Maximum size 38 mm

Shell morphology

Based on shell characteristics (following Coan, 1983) all individuals were identified as *D. marincovichi* or *D. obesulus* (Table 2). Specimens were measured with a vernier calliper (± 0.01 mm) to record length (anterior–posterior, L), height (ventro-dorsal, H) and width (left–right, W). Width/height (W/H) and height/length (H/L) ratios were calculated and the nonparametric Kruskal–Wallis test was used to evaluate statistical differences of each ratio among all stations. When the Kruskal–Wallis test revealed significant differences, *post hoc* comparisons were performed using the Dunn test at a level of 5% significance to evaluate paired (i.e. between every pair) differences (Zar, 1999). The character of 'maximum anterior–posterior shell length' specified by Coan (1983) for *D. marincovichi* (32 mm) and *D. obesulus* (38 mm) was not applied in this study as all specimens lay below these values (*D. marincovichi*, 29.75 mm; *D. obesulus*, 27.83 mm maximum lengths).

Sperm morphology

In order to compare sperm morphology, gonads of *D. marincovichi* and *D. obesulus* were dissected, c. 1 mm^3 of tissue (always from the same area) was fixed in 4% glutaraldehyde buffered with 0.1 M disodium phosphate buffer (pH 7.4) for 4 h at 4°C and then thoroughly rinsed in phosphate buffer. Tissue samples were dehydrated using an ascending series of ethanol (from 20% to absolute ethanol) and embedded in Araldite resin. Ultra-thin sections were produced using an LKB 2128 ultramicrotome after mounting the tissue on 200-μm mesh uncoated copper grids, stained with uranyl acetate (10–20 min) and lead citrate (10–20 min). For further details of preparation see Giménez *et al.* (2008). Ultrastructural sperm morphology was photographed with a TEM Philips EM 301 at 60 kV and a Jeol 1200 EX II at 80 kV. All measurements on sperm structures are based on TEM observations. As there are several important changes during maturation of sperm, such as changes in the acrosome and the positioning of the mitochondria (Pal, 1996), only mature sperm were analysed. Characterization of sperm morphology was realized by a precise identification of the general components of sperm (acrosome, nucleus, midpiece and flagellum).

Genetic analyses

For a subset of 79 *Donax* specimens (*D. asper*, $n = 9$; *D. hanleyanus*, $n = 9$; *D. marincovichi*, $n = 25$ and *D. obesulus*, $n = 36$) we sequenced a fragment of the mitochondrial COI gene. Individuals with opened and closed shells were preserved from each beach in >80% ethanol. Muscle tissue was extracted from the middle and apex region (c. 1 mm^2) of the foot and cleaned with ethanol (75%) to remove sand, detritus or external organic matter. The muscle tissue was cut into small pieces to decrease tissue lysis time. To avoid contamination of DNA, extraction was carried out under sterile conditions. DNA extraction was performed with the Qiagen DNA Mini kit using the standard tissue protocol. However, in a modification of this protocol, only 150 μl of elution buffer was used to increase the concentration of DNA.

Initially, DNA amplification of two mitochondrial genes (COI, 16S) was attempted using universal primers (Palumbi, Martin & Romano, 1991; Folmer *et al.*, 1994). The ribosomal large subunit gene (16S) did not amplify in many specimens and, therefore, further analyses focused on the COI gene, which amplified successfully using the primers LCO-1490 and HCO-2198 (Folmer *et al.*, 1994).

Amplification of the COI fragment was carried out in 25 μl reactions containing $1\times$ HotMaster buffer, 0.2 mM dNTPs, 0.5 μM of each primer, 1–3 μl of DNA template, 0.02 U/μl HotMaster Taq polymerase (5 prime, Hamburg, Germany), filled up to 25 μl with sterile H$_2$O. The PCR temperature profile for the COI amplification was: 94°C for 2 min, 38 cycles of 20 s at 94°C, 15 s at 42°C, 80 s at 65°C, followed by a final extension step of 7 min at 65°C.

PCR products were checked on a 1.5% TAE agarose gel and purified using a Qiagen QIAquick PCR purification kit according to the manufacturers' recommendations. Cycle-sequencing of PCR products was carried out using terminal primers given above and the BigDye Terminator v3.1 kit of Applied Biosystems (ABI), purified using DyeEx-Kit (Qiagen) according to the modified protocol and sequenced on an ABI 3130xl automated sequencer.

Sequences were aligned using MUSCLE (Edgar, 2004). As outgroup for the alignment, a sequence of *Macoma balthica* was used (GenBank accession number EF044136). Phylogenetic analyses were performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The optimal model of sequence evolution was identified using hierarchical likelihood ratio tests and the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.2 (Nylander, 2004). The MCMC analyses was performed using two independent runs with 10 million generations each and sampling every 100th tree. Convergence was reached after 60.000 generations (discarded as burn-in). A statistical parsimony network was calculated from the *D. marincovichi* and *D. obesulus* data set using TCS 1.21 (Clement *et al.*, 2000) and a 95% connection limit.

RESULTS

Shell morphology

Based on Coan's (1983) morphological characters, specimens ($n = 109$) from 10 different locations from northern Chile to
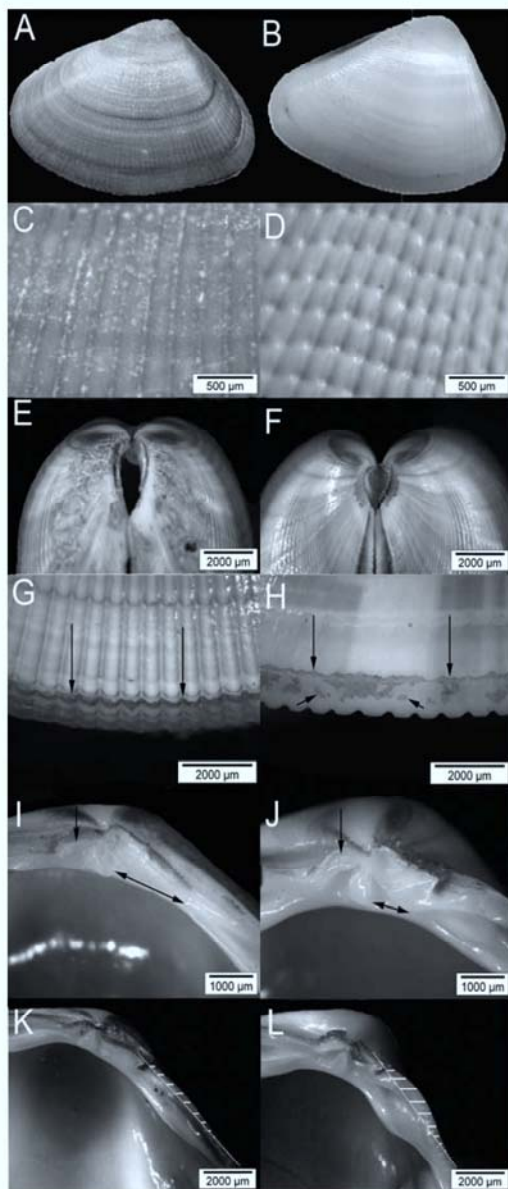
Figure 2. A. *Donax marincovichi* (left valve, 23 mm). Valve is less inflated, W/H between 0.57 and 0.69, maximum length 32 mm. **B.** *Donax obesulus* (left valve, 17 mm), valve is more inflated, W/H between 0.71 and 0.77. **C.** *Donax marincovichi* never has punctation, surface silky (32 ×). **D.** *Donax obesulus* shows punctation on most specimens, surface shiny (32 ×). **E.** *Donax marincovichi:* posterior dorsal half, beaks low (10 ×). **F.** *Donax obesulus:* posterior dorsal half, beaks inflated (10 ×). **G.** *Donax marincovichi:* left valve ventral margin: periostracum dark, adherent, in a wide marginal band (10 ×). **H.** *Donax obesulus:* left valve ventral margin: periostracum light, marginal traces only (10 ×). **I.** *Donax marincovichi:* right valve hinge region close to umbo: posterior lateral more distant (double-headed arrow) and anterior cardinal of right valve (single-headed arrow) small, thin (12.5 ×). **J.** *Donax obesulus:* right valve hinge region close to umbo: posterior lateral close to cardinals (double-headed arrow) and anterior cardinal (single-headed arrow) of right valve thickened (12.5 ×). **K.** *Donax marincovichi:* dorsal margin less (or not) produced above posterior lateral (hatched area) (7 ×). **L.** *Donax obesulus:* dorsal margin produced above posterior lateral (hatched area) (7 ×).

Table 3. Number of mismatches (inapplicable identification feature, as number and as percentage) for *Donax marincovichi* and *Donax obesulus* for each character (Fig. 2, Table 2).

	Donax marincovichi (n = 56)	%	*Donax obesulus* (n = 53)	%
1. Punctation	0	0	0	0
2. W/H	8	14	5	9
3. Beak	1	2	7	13
4. Periostracum colour	50	88	2	4
5. Periostracum marginal	20	35	13	25
6. Surface	9	16	1	2
7. Posterior lateral	37	65	5	9
8. Dorsal margin	0	0	13	12
9. Anterior cardinal	11	19	13	25
Total	133		58	

Maximal size (character 10, Table 2) was not considered as there were no specimens available reaching the maximum size given by Coan (1983).

northern Peru were identified to species level (Fig. 2, Table 1). However, only 20% of the specimens showed a perfect match with the morphotype expected from the species' description, while 80% of the specimens conflicted in at least one character by possessing a character state expected for the other species. Final assignment to species level was carried out by using the predominant number of morphological characteristics (Coan, 1983). The percentage of individuals exhibiting mismatches for each morphological character is presented in Table 3.

The northern Chilean population (Fig. 1, Table 1, Station 1) was composed only of clams identified as *Donax obesulus*. The central Peruvian beaches from Chincha to Chimbote (Fig. 1, Table 1, Stations 2–6) yielded predominantly *Donax marincovichi* morphotypes. In contrast, the population further north (Fig. 1, Table 1, Station 7) consisted exclusively of the *D. obesulus* morphotype, followed by Station 8 (Fig. 1, Table 1), which was inhabited by both morphotypes. The northernmost populations of Sechura and Tumbes (Fig. 1, Table 1, Stations 9 and 10) were composed of nominal *D. obesulus* morphotypes.

Among all the morphological characters used for identification in this study, only 'punctation' appeared to be reliable. As described by Coan (1983), it was predominant and/or prevalent on the *D. obesulus* morphotype, but never present on *D. marincovichi*. The most ambiguous identification features (Table 3) were 'colour' and 'distance from the posterior lateral to the cardinals' (respective mismatches of 46% and 38%), followed by 'location of the periostracum' (29%) and 'size of the anterior cardinal tooth' (18%). The characters 'dorsal margin produced or not produced above posterior lateral' and 'W/H ratio' conflicted in 12% of the cases. The characters 'surface' and 'shape of the beak' revealed 9% and 7% of mismatch, respectively (Table 3). Within the nine applied characters quantified by Coan (1983) the morphotype *D. marincovichi* (n = 56) possessed 133 mismatches, while morphotype *D. obesulus* (n = 53) revealed 58 mismatches (Table 3).

W/H, as well as the H/L ratio between all specimens, were tested and revealed significant differences (P < 0.001). Further comparisons between stations were made (Table 4), indicating that in terms of W/H and H/L ratios, stations from northern Chile and northern Peru (Fig. 1, Table 1, Stations 1, 7, 9 and 10), all dominated by *D. obesulus*, differed significantly from

Table 4. Pairwise comparison of different sampling sites (Table 1) by Dunn test applied to identify differences in W/H and H/L between single stations.

	W/H	H/L
1 vs 2	0.000*	0.003*
1 vs 3	0.000*	0.000*
1 vs 4	0.002*	0.018*
1 vs 6	0.003*	0.020*
2 vs 7	0.021*	0.037*
2 vs 8	0.151	0.025*
2 vs 9	0.000*	0.042*
2 vs 10	0.000*	0.000*
3 vs 7	1.000	0.008*
3 vs 8	1.000	0.005*
3 vs 9	0.000*	0.009*
3 vs 10	0.001*	0.000*
4 vs 9	0.001*	0.208
4 vs 10	0.013*	0.000*
5 vs 10	0.458	0.000*
6 vs 9	0.001*	0.211
6 vs 10	0.018*	0.000*
8 vs 9	0.044*	1.000

Asterisks indicate significant differences ($P < 0.05$).

central Peruvian stations (Fig. 1, Table 1, Stations 2–6 and 8), which are dominated by *D. marincovichi*. Specimens of the former stations are more wedge shaped (shorter and thicker) indicating a compacter shape than clams from the other sites (Fig. 3).

However, in addition to individuals displaying all morphological characters of one species and thus easily identified, individuals from all stations (except station 10) showed a number of transitional character states thus making unequivocal identification difficult or impossible (Fig. 3). Recent results increased the maximum value of the H/L ratio (Coan, 1983) (Table 2) for *D. marincovichi* to 0.62–0.73 and for *D. obesulus* to 0.66–0.85, respectively. Further sampling at Jahuay beach (Fig. 1, Table 1, Station 3) revealed that *D. marincovichi* reaches a maximum length up to 35 mm instead of 32 mm as reported by Coan (1983).

Sperm morphology

TEM observations of sperm structure of the two species *D. marincovichi* and *D. obesulus* (Coan, 1983) revealed that both demonstrate a typical primitive aquasperm type, containing an acrosome, nucleus, a short midpiece and a ring of mitochondria surrounding the flagellum (Fig. 4A). The pear-shaped acrosome settles in a nuclear fossa (Fig. 4A, B). A transverse section of the acrosome is provided in Figure 4C. It illustrates that the acrosome is differentiated into two regions with different electron densities. The surrounding outer crescent-shaped basal ring is electron dense and represents *c.* 50% of the total area of the acrosome. It is covered by an outer acrosomal membrane. The anterior, central and posterior region is electron lucent containing the axial rod in the centre. The electron-lucent part of the acrosome apex is arrow-shaped (Fig. 4B). The sperm nucleus capturing approximately one-third of the total size of the sperm is associated with the acrosome (Fig. 4D). The flagellum shows the structure typical of molluscs: the axoneme within the flagellum consists of a central pair of microtubules encircled by nine doublets (9 + 2 type). Its termination is surrounded by

four mitochondria (Fig. 4E). Mean values of length and diameter of the acrosome, nucleus and midpiece are given in Table 5.

Genetic analyses

DNA of good quality was extracted from specimens whose shells were cracked open immediately before preservation, presumably because diffusion of ethanol into tissue depends mainly on the permeability of the surface texture of the organism (Held, 2000). In contrast, DNA of individuals preserved intact showed greater degradation and lower yields. DNA extracted from the middle piece of the foot was on average less in quantity and lower in quality, i.e. more fragmented. Consequently, all tissues used for DNA extraction were obtained from the anterior apex of the foot of previously sectioned individuals.

The alignment of the COI gene sequences yielded a 567-bp alignment. Analyses of uncorrected *P*-distances and haplotype sharing in the statistical parsimony network revealed no consistent differences between *D. marincovichi* and *D. obesulus* (Figs 5, 6). Strong differences were recorded for *D. marincovichi* and *D. obesulus* and the two Donax outgroup species (>15% *P*-distance, Fig 5). The pairwise genetic difference in *D. obesulus*–*D. obesulus* or *D. marincovichi*–*D. marincovichi* pairs was equal to within-species pairwise comparisons, i.e. *D. marincovichi*–*D. obesulus* pairs and *vice versa* (0–1.2%). In addition, no genetic differentiation was observed between geographically separated populations.

For details of analysed specimens ($n = 61$) and number of matches and mismatches of identification after Coan (1983) see Supplementary material.

DISCUSSION

The W/H and H/L ratios obtained during this study revealed significant differences between *Donax marincovichi* and *D. obesulus* among sampling areas (Table 4). However, following the key of Coan (1983) (Fig. 2 and Table 2) these as well as other distinguishing characteristics frequently revealed incongruence concerning identification of the two species. Of the identified specimens 80% displayed characters of both morphotypes (Table 3). In order to clarify the taxonomic status we applied genetic tools and observed the sperm morphology of both morphotypes.

The genetic analysis indicated that COI gene sequences are useful for species discrimination within the genus *Donax*. The observed divergence levels (0–1.2%) of both morphotypes pooled (Fig. 5) are typical of intraspecific levels (Held, 2000; Hebert *et al.*, 2003; Cardenas, Castilla & Viard, 2009). No consistent spatial structure was found in the distribution of genetic variance among geographically separated populations, suggesting extensive and ongoing gene flow between geographically separated populations and also between nominal *D. obesulus* and *D. marincovichi*. The same molecular marker system was highly informative when distinguishing the two *Donax* species included in the outgroup (*D. asper* and *D. hanleyanus*).

In addition, the examination of sperm morphology showed that sperm of both morphotypes were of a similar aqua sperm type (Fig. 4), which is typical of most members of the family Veneroidea (Healy, 1995). This sperm type is characterized by a conical acrosome, a short nucleus, a midpiece consisting of a ring of round mitochondria surrounding the centrioles and a single simple flagellum with a 9+2 axoneme (Healy, 1995, 2000). Comparison of the main components of the respective sperm revealed no differences in morphology. The electron
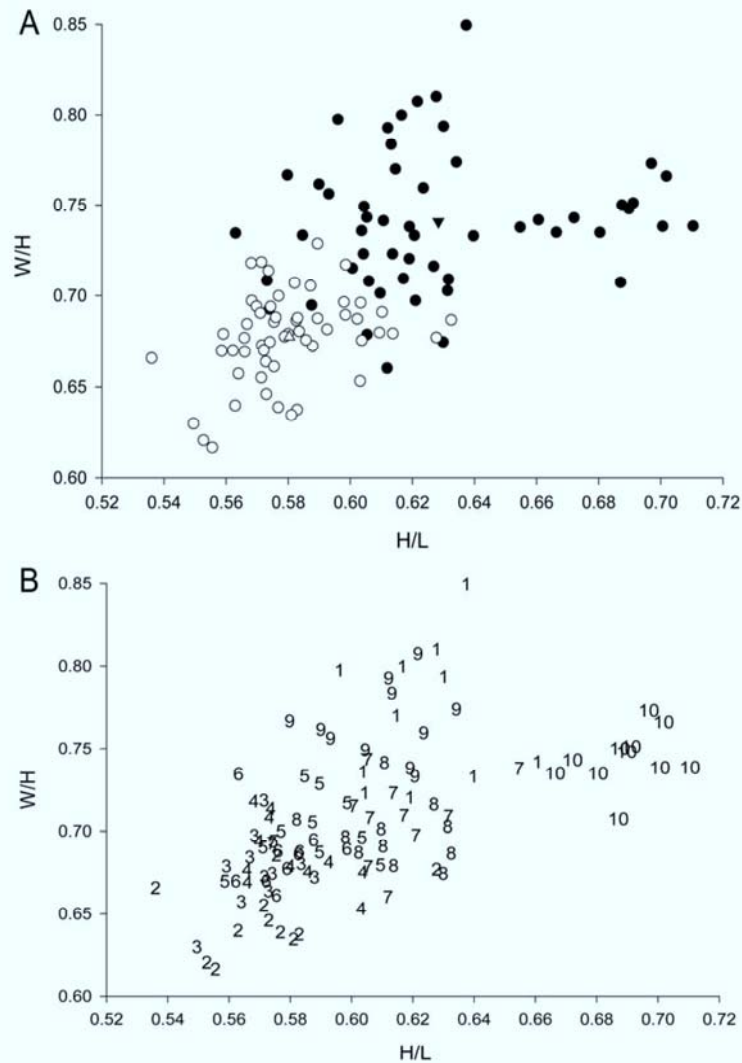
Figure 3. A. Shell shape variability of *Donax marincovichi* (open circles; mean: open triangle) and *Donax obesulus* (solid circles; mean: solid triangle) from stations along the Pacific coast of northern Chile and Peru. Width/height (W/H) ratios are plotted *vs* height/length (H/L). **B.** Shell shape variability of *Donax marincovichi* and *Donax obesulus* from Stations 1 to 10.

lucent part of the acrosome apex has an arrow-shaped point (Fig. 4B), which appears to be characteristic. A review of sperm of Donacidae (van der Horst *et al.*, 1986; Hodgson, Bernard & van der Horst, 1990; Sousa & Oliveira, 1994; Healy, 1995; Matos *et al.*, 1995; *D. hanleyanus*, M. Herrmann, unpublished) showed that the present sperm type differs in shape and size from those previously reported (Fig. 4, Table 5).

Neither significant mitochondrial genetic differentiation within or among populations, nor differences in sperm ultra-structure, separate the two morphotypes defined by Coan (1983) as distinct species. Therefore we propose that the differences between the morphotypes might be primarily the result of phenotypic plasticity caused by different local environments. We suggest that *D. marincovichi* and *D. obesulus* are better characterized as two different ecomorphs (sensu Nelson *et al.*, 1993) of the species *D. obesulus*.

Within the study area geographically separated populations spanned approximately 1900 km (linear distance) from the southernmost station (Chinchorro Beach in Tarapacá, Chile)

to the northernmost station (Sechura Bay in Tumbes, Peru). Meroplanktic larval development of *Donax* species takes about three to four weeks, depending on environmental conditions (food availability and temperature), and can be delayed under unfavourable conditions (Chanley, 1969; Helm, Bourne & Lovatelli, 2004). The Peruvian Coastal Current (Fig. 1) transports water masses at speeds of up to 15 cm s^{-1}, which is equivalent to approximately 400 km per month (Tarazona & Arntz, 2000), implying that the analysed sub-populations may be interconnected via stepping-stone larval exchange within a modest number of generations. Similar long-distance inter-populational genetic connectivity has been recorded for *D. serra* populations from the Benguela Current Upwelling System (Laudien *et al.*, 2003) and for *D. deltoides* populations connected by the East Australian Current (Murray-Jones & Ayre, 1997).

Our results concur with numerous publications demonstrating that shell shape of Donacidae may vary greatly and that it is unreliable as a taxonomic feature (Olsson, 1961; Keen, 1971; Coan, 1983; Guzmán *et al.*, 1998). This family is well known to
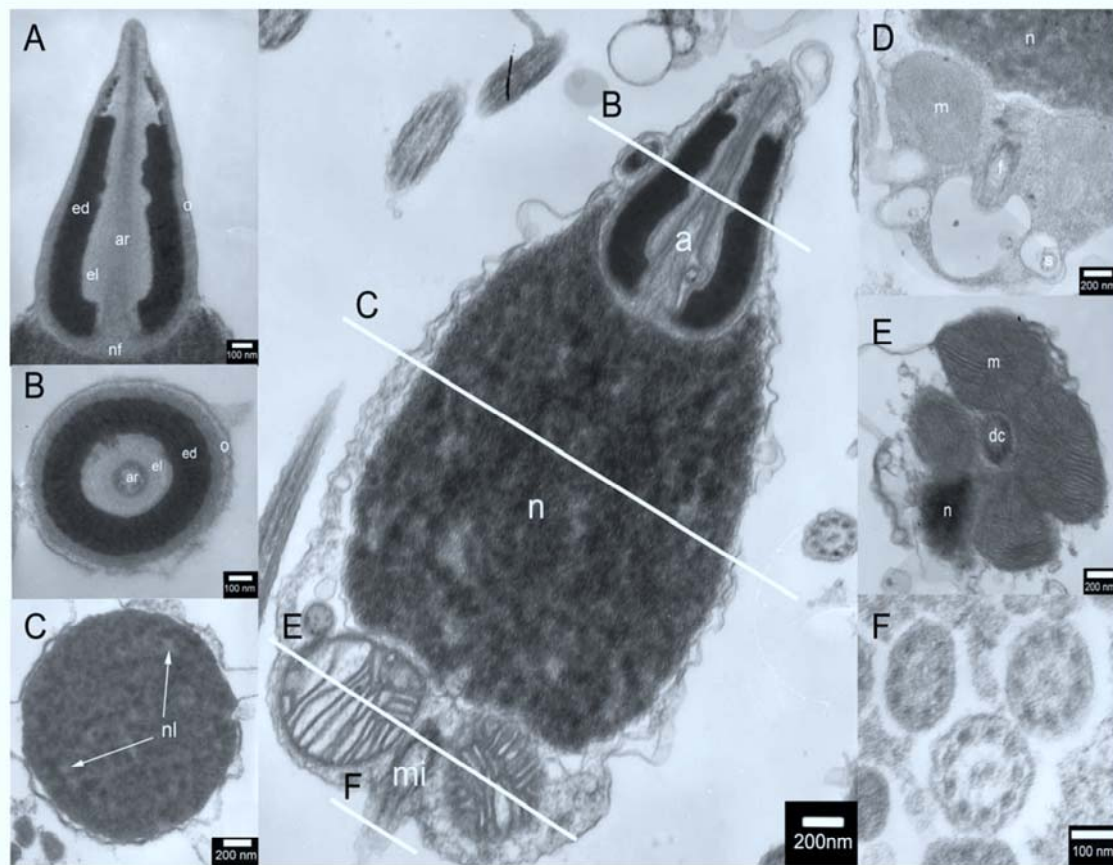
Figure 4. Overview in mid-longitudinal section through sperm type shared by *Donax obesulus* and *Donax marincovichi*, showing level of transverse sections (20,000 ×). **A.** Detail, mid-longitudinal section of acrosome (50,000 ×). **B.** Transverse section of acrosome (60,000 ×). **C.** Transverse section of nucleus with brighter spots of nuclear lacunae (20,000 ×). **D.** Detail, longitudinal section of satellite (30,000 ×). **E.** Transverse section of four mitochondria, part of the nucleus and distal centriole (30,000 ×). **F.** Transverse section of flagellum showing 9 + 2 axoneme (80,000 ×). Abbreviations: a, acrosome; ar, axial rod; dc, distal centriole; ed, electron-dense basal ring; el, electron-lucent region; f, flagellum; m, mitochondrion; mi, midpiece; n, nucleus; nf, nuclear fossa; nl, nuclear lacunae; o, outer plasma membrane; s, satellite.

Table 5. Comparison of sperm ultrastructure of *Donax* species: mean ($\pm$ SD) measurements (length and diameter) of acrosome, nucleus and midpiece.

Species (*n*)	Acrosome (μm)	Nucleus (μm)	Midpiece (μm)	Author
Donax sp. (39)	1.38 × 0.83 ($\pm$ 0.17/$\pm$ 0.06)	2.11 × 1.83 ($\pm$ 0.19/$\pm$ 0.20)	0.68 × 1.54 ($\pm$ 0.16/$\pm$ 0.29)	This study
Donax deltoides (5)	1.18 × 0.88	0.67 × 0.75	0.6 × 1.75	Healy (1995)
Donax serra (20)	0.9 × 1.0	1.33 × 1.5	No data	Hodgson (1990)
Donax sordidus (20)	1.0 × 0.8	1.5 × 1.4	No data	Hodgson (1990)
Donax madagascariensis (20)	1.07 × 0.98	1.56 × 1.4	No data	Hodgson (1990)

span a wide spectrum of shell colour, pattern and shape. High heterogeneity has been documented for *D. variabilis* populations over short linear distances; at six stations covering 114 km of total distance, each population presented a unique combination in shell length and morph frequencies (Adamkewicz, 1989). Extreme diversity of shell characters and the absence of clear diagnostic features for Donacidae have also been reported in other studies (Wade, 1967a, b; Adamkewicz, 1989; Donn, 1990; Adamkewicz & Harasewych, 1994; McLachlan *et al.*, 1995; Laudien *et al.*, 2003), suggesting that variation in size, colour, shape and sculpture of the shells may be due to phenotypic plasticity rather than an expression of genetic differentiation between the populations. McLachlan *et al.* (1995) studied the influence of beach morphodynamics on shell shape. Reflective beaches favour smaller, uniform and more wedge-shaped shells, while dissipative (Dean's parameter 5–10; for details see Defeo & McLachlan, 2005) beaches instead favour larger valves with a broader spectrum of shapes. This was also documented for *D. serra* inhabiting reflective (Dean's parameter 0–2; Defeo & McLachlan, 2005) *vs* intermediate (Dean's parameter 2–5) to dissipative beaches (Laudien *et al.*, 2003). Donn (1990) stressed the influence of locality and population density and documented that a high-intertidal population of *D. serra* at a higher density had thicker
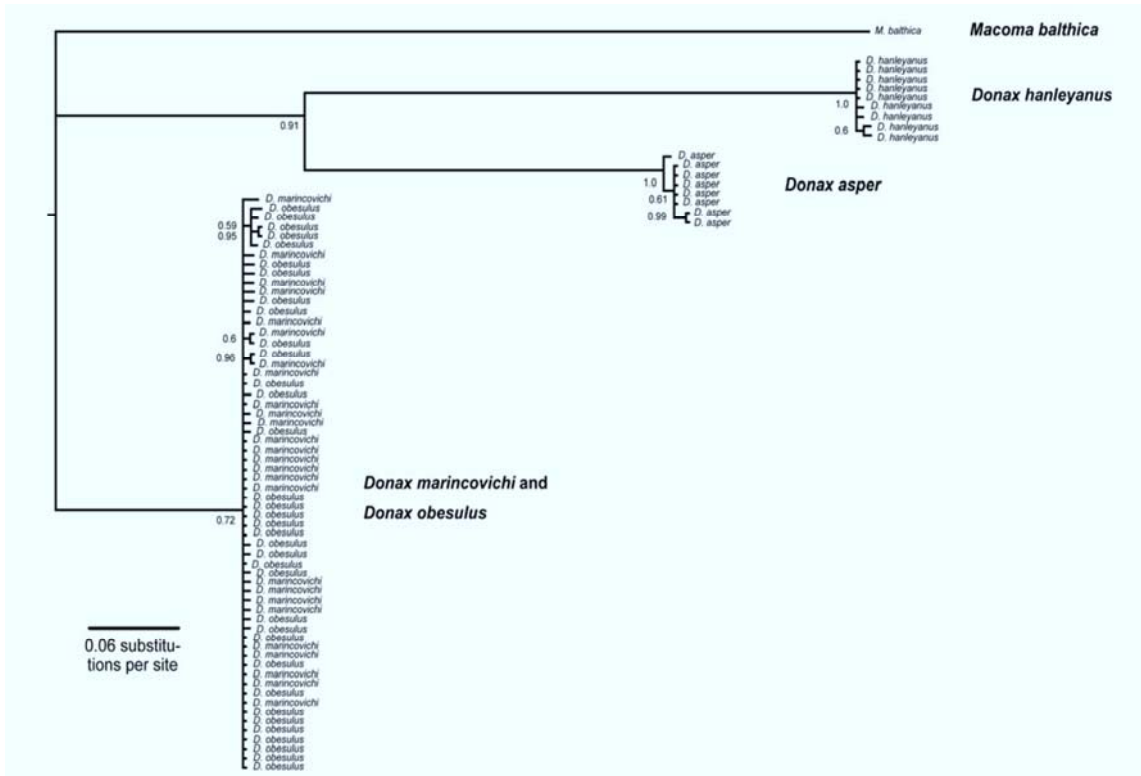
Figure 5. Majority rule (>50%) consensus tree obtained from Bayesian analysis of the mitochondrial COI gene sequence data for *Donax marincovichi*, *Donax obesulus* and the outgroups *Donax asper*, *Donax hanleyanus* and *Macoma balthica*. Numbers below branches are posterior probability values.
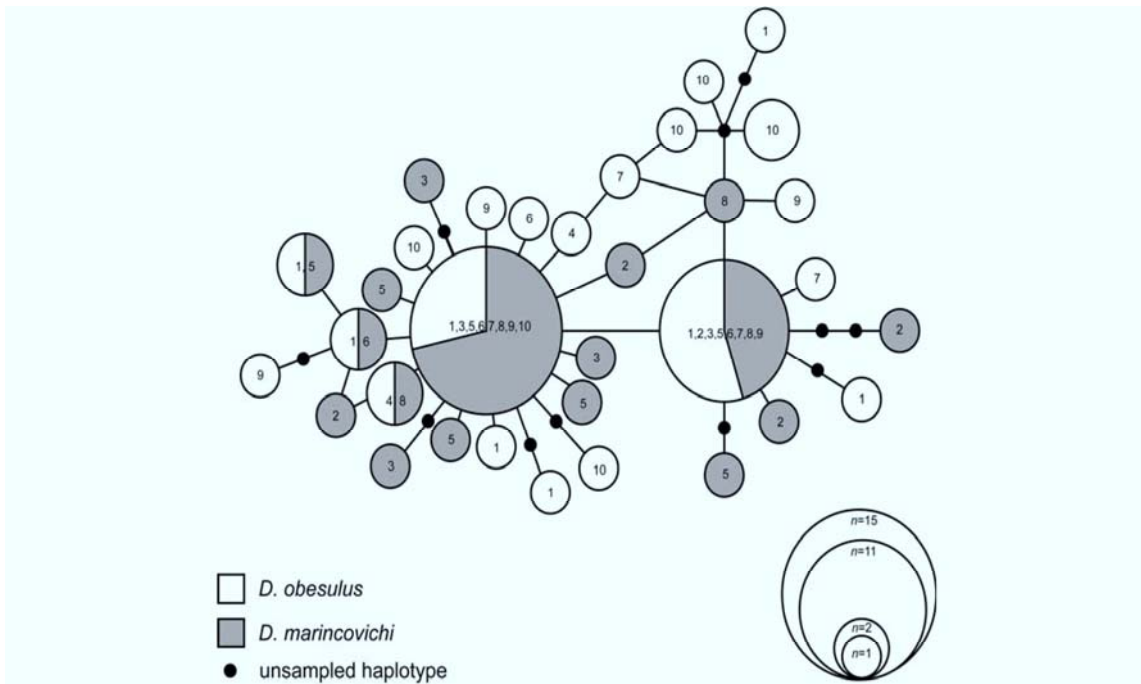

Figure 6. Statistical parsimony network (95% connection limit) for the putative *Donax marincovichi* and *Donax obesulus* specimens sampled at station 1–10. The diameter corresponds to the number of specimens found with a specific haplotype. The number(s) within each haplotype refer(s) to the station(s) at which the haplotype was found.

and heavier shells, whereas low-intertidal or subtidal populations possessed flatter, more rounded shells. Considering the observed specimens, it can be suggested that the *D. marincovichi* morphotype may be more adapted to intermediate beaches because it has a flatter and less wedge-shaped form (Fig. 3), whereas the *D. obesulus* morphotype from northern Chile and Peru may be more adapted to reflective beaches, because it is shorter and more wedge-shaped (Fig. 3) (cf. McLachlan *et al.*, 1995).

The diversity of synonyms of Donacidae reflects taxonomical confusion that is mainly due to the use of unreliable shell characteristics for species determination. Future research should include faster-evolving nuclear markers (e.g. AFLP, microsatellites) that could extend our findings based on mitochondrial sequences, and resolve more recent evolutionary events and at a finer geographic resolution than the present study (Held & Leese, 2007).

In conclusion, as a consequence of environmentally driven phenotypic plasticity, analysis of shell morphology may be unsuitable for the delimitation of *Donax* species. Phenotypic plasticity in shape, dimensions, sculpture and colour can be considered adaptive for species living in environments that are physically and biologically dynamic, where it can give rise to distinctive ecomorphs.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

REFERENCES

ADAMKEWICZ, S.L. 1989. Differences in the frequencies of several characters in the clam *Donax variabilis* around Cape Hatteras, North Carolina. *Veliger*, **32**: 21–28.

ADAMKEWICZ, S.L. & HARASEWYCH, M.G. 1994. Use of random amplified polymorphic DNA (RAPD) markers to asses relationships among beach clams of the genus *Donax. The Nautilus*, **2**: 51–60.

ANSELL, A.D. 1983. The biology of the genus *Donax.* In: *Developments in hydrobiology.* Vol. 19: *Sandy Beaches as Ecosystems* (A. McLachlan, T. Erasmus & W.E. Junk eds), pp. 607–635. W. Junk, The Hague, The Netherlands.

BONSDORFF, E. & NELSON, W.G. 1992. The ecology of coquina clams *Donax variabilis* Say, 1822, and *Donax parvula* Philippi, 1849, on the east coast of Florida. *Veliger*, **35**: 358–365.

CARDENAS, L., CASTILLA, J.C. & VIARD, F. 2009. A phylogeographical analysis across three biogeographical provinces of the south-eastern Pacific: the case of the marine gastropod *Concholepas concholepas. Journal of Biogeography* **36**: 969–981.

CHANLEY, P. 1969. *Donax fossor*: a summer range extension of *Donax variabilis. Nautilus*, **83**: 1–14.

CLEMENT, M., POSADA, D. & CRANDALL, K.A. 2000. TCS: a computer program to estimate gene genealogy. *Molecular Ecology* **9**: 1657–1659.

COAN, E. 1983. The Eastern Pacific Donacidae. *Veliger*, **25**: 273–298.

COAN, E.V., SCOTT, P. & BERNARD, F.R. 2000. *Bivalve seashells of western North America: marine bivalve mollusks from Arctic Alaska to Baja California.* Museum of Natural History Monographs, Santa Barbara: 764 p.

COGHLAN, B. & GOSLING, E. 2007. Genetic structure of hybrid mussel populations in the west of Ireland: two hypotheses revisited. *Marine Biology*, **150**: 841–852.

DEFEO, O. & MCLACHLAN, A. 2005. Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. *Marine Ecology Progress Series*, **295**: 1–20.

DONN, T.E.J. 1990. Morphometrics of *Donax serra* Röding (Bivalvia: Donacidae) populations with contrasting zonation patterns. *Journal of Coastal Research*, **6**: 893–901.

EDGAR, R. 2004. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**: 1792–1797.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnologie*, **3**: 294–299.

GALTSOFF, P.S. & GALLARDO, C.S. 1960. Ultrastructure of the spermatozoon of the oyster *Crassotrea virginica. Journal of Ultrastructural Research*, **3**: 241–253.

GIMÉNEZ, J., HEALY, J.M., HERMIDA, G.N., NOSTRO, F.L. & PENCHASZÁDEH, P.E. 2008. Ultrastructure and potential taxonomic importance of euspermatozoa and paraspermatozoa in the volutid gastropods *Zidona dufresnei* and *Provocator mirabilis* (Caenogastropoda, Mollusca). *Zoomorphology*, **127**: 161–173.

GUZMÁN, N., SAÁ, S. & ORTLIEB, L. 1998. Catálogo descriptivo de los moluscos litorales (Gastropoda y Pelecypoda) de la zona de Antofagasta 23°S (Chile). *Estudios Oceanológicos, Universidad de Antofagasta*, **17**: 17–86.

HEALY, J.M. 1995. Comparative spermatozoal ultrastructure and its taxonomic and phylogenetic significance in the bivalve order Veneroida. In: *Advances in spermatozoal phylogeny and taxonomy* (B.G.M. Jamieson, J. Ausio & J.-L. Justine eds), pp. 155–166. Mémoires du Muséum National D' Histoire Naturelle, Paris.

HEALY, J.M., KEYS, J.L. & DADDOW, L.Y.M. 2000. Comparative sperm ultrastructure in pteriomorphian bivalves with special reference to phylogenetic and taxonomic implications. In: *The evolutionary biology of the Bivalvia* (E.M. Harper, J.D. Taylor & J.A. Crame eds), pp. 169–190. Geological Society Special Publication.

HEBERT, P.D.N., RATNASINGHAM, S. & DEWAARD, J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **270**: 96–99.

HELD, C. 2000. Phylogeny and biogeography of serolid isopods (Crustacea, Isopoda, Serolidae) and the use of ribosomal expansion segments in molecular systematics. *Molecular Phylogenetics and Evolution*, **15**: 165–178.

HELD, C. & LEESE, F. 2007. The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. *Polar Biology*, **30**: 513–521.

HELD, C. & WÄGELE, J.W. 2005. Cryptic speciation in the giant Antarctic Isopod *Glyptonotus antarcticus* (Isopoda: Valvifera: Chaetiliidae). *Scientia Marina*, **69**: 175–181.

HELM, M.M., BOURNE, N. & LOVATELLI, A. 2004. Hatchery culture of bivalves. A practical manual. *FAO Fisheries Technical Paper*, **471**: 177.

HODGSON, A.N., BERNARD, R.T.F. & VAN DER HORST, G. 1990. Comparative spermatology of three species of *Donax* (Bivalvia) from South Africa. *Journal of Molluscan Studies*, **56**: 257–265.

HUARAZ, F. & ISHIYAMA, V. 1980. Madurez sexual de la 'concha mariposa' (*Donax peruvianus*) de la playa de Jahuay, Ica, Perú. *Revista de Ciencias U.N.M.S.M.*, **72**: 47–56.

HUELSENBECK, J. & RONQUIST, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755

JAERNEGREN, J., SCHANDER, C., SNELI, J.-A., ROENNINGEN, V. & YOUNG, C.M. 2007. Four genes, morphology and ecology: distinguishing a new species of *Acesta* (Mollusca; Bivalvia) from the Gulf of Mexico. *Marine Biology*, **152**: 43–55.

KEEN, A.M. 1971. *Sea shells of tropical West America*. Edn 2. Stanford University Press, Standford, California.

KIMURA, M. & WEISS, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**: 561–576.

KOEHN, R.K., HALL, J.G., INNES, D.J. & ZERA, A.J. 1984. Genetic differentiation of *Mytilus edulis* in eastern North America. *Marine Biology*, **79**: 117–126.

LAUDIEN, J., FLINT, N.S. & BANK, F.H.V.D. 2003. Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches. *Biochemical Systematics and Ecology*, **31**: 751–772.

LEVINTON, J.S. & SUCHANEK, T.H. 1978. Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. *Marine Biology*, **49**: 363–375.

LOESCH, H.C. 1957. Studies of the ecology of two species of *Donax* on Mustang Island, Texas. *Publications of the Institute of Marine Science*, **4**: 201–227.

MATOS, E., MATOS, P., CORRAL, L. & AZEVEDO, C. 1995. Estudo ultraestrutural da espermatogenese de *Donax striatus* Linnaeus (Mollusca, Bivalvia) do litoral norte do Brasil. *Revista Brasileira de Zoologia*, **12**: 221–227.

MCLACHLAN, A., JARAMILLO, E., DEFEO, O., DUGAN, J.E., RUYCK, A.D. & COETZEE, P. 1995. Adaptions of bivalves to different beach types. *Journal of Experimental Marine Biology and Ecology*, **187**: 147–160.

MORRISON, J.P.E. 1971. Western Atlantic *Donax*. *Proceedings of the Biological Society of Washington*, **83**: 545–568.

MURRAY-JONES, S.E. & AYRE, D.J. 1997. High levels of gene flow in the surf bivalve *Donax deltoides* (Bivalvia: Donacidae) on the east coast of Australia. *Marine Biology*, **128**: 83–89.

NARCHI, W. 1983. *Donax hanleyanus* Philippi, 1847, proposed conservation and proposed suppression of *Donax hilairea* Guerin, 1832 (Mollusca, Bivalvia). Z.N. (S.) 2152. *Bulletin of Zoological Nomenclature*, **40**: 188–189.

NELSON, W.G., BONSDORFF, E. & ADAMKEWICZ, L. 1993. Ecological, morphological, and genetic differences between the sympatric bivalves *Donax variabilis* Say, 1822, and *Donax parvula* Philippi, 1849. *Veliger*, **36**: 317–322.

NYLANDER, J. 2004. MrModeltest2.2. Computer software distributed by the University of Uppsala.

OLSSON, A.A. 1961. *Mollusks of the tropical eastern Pacific, Panamic Pacific Pelecypoda*. Paleontological Research Institute, Ithaca.

PAL, S.G. 1996. Some observations on the testes of *Donax lubrica* (Bivalvia: Donacidae). *Malacological Review*, **6**: 55–62.

PALUMBI, S.R., MARTIN, A. & ROMANO, S. 1991. *The simple fool's guide to PCR, Version 2.0*. University of Hawaii, Honolulu.

PAREDES, C. & CARDOSO, F. 2001. El género *Donax* en la costa peruana (Bivalvia: Tellinoidea). *Revista Peruana de Biología*, **8**: 1–13.

REEVE, L.A. 1854. Monograph of the genus *Donax*. In: *Conchologia iconica* (L.A. Reeve ed.). London.

REY, R. 2006. Prohíben la extracción, recepción, transporte, procesamiento y comercialización del recurso marucha en tallas inferiores a 22 milímetros de longitud valvar: *Resolución Ministerial Nº 298-2006-Produce*: 2. Perú: Imarpe, Informe N° 300-2006-PRODUCE/DGEPP-Dch.

REY, R. 2007. Prohíben la extracción, recepción, transporte, procesamiento y comercialización del recurso marucha en tallas inferiores a 22 milímetros de longitud valvar: *Resolución Ministerial N° 445-2007-Produce*: 2. Perú: Imarpe, Informe N° 544-2007-PRODUCE/DGEPP-Dch.

REY, R. 2008. Prohíben actividades de extracción, procesamiento, transporte y comercialización del recurso marucha o palabritas en el litoral y ámbito del departamento de Lambayeque: *Resolución Ministerial N° 607-2008-Produce*: 2. Perú: Imarpe, Informe N° 452-2008-PRODUCE/DGEPP-Dch.

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. *Marine Biology*, **148**: 1283–1293.

SOARES, A.G., SCAPINI, F., BROWN, A.C. & MCLACHLAN, A. 1999. Phenotypic plasticity, genetic similarity and evolutionary inertia in changing environments. *Journal of Molluscan Studies*, **65**: 136–139.

SOTO, R. 1985. Efectos del fenómeno El Niño 1982-83 en ecosistemas de la 1 Región. *Investigación Pesquera (Chile)*, **32**: 199–206.

SOUSA, M. & OLIVEIRA, E. 1994. Ultrastructural and cytochemical study of spermatogenesis in *Donax trunculus* (Mollusca, Bivalvia). *Journal of Submicroscopial Cytology and Pathology*, **26**: 305–311.

TARAZONA, J. & ARNTZ, W. 2000. The Peruvian coastal upwelling system. *Ecological Studies*, **144**: 229–244.

UTTER, F.M. 1991. Biochemical genetics and fisheries managment: a historical perspective. *Journal of Fish Biology*, **39**: 1–20.

VAN DER HORST, G., HODGSON, A.N., BERNARD, R., SALIE, W. & MAASDORP, L.J.W. 1986. Sperm ultrastructure in two *Donax* (Bivalvia) species. *Proceedings of the Electron Microscopial Society of South Africa*, **16**: 69–70.

WADE, B.A. 1967a. On the taxonomy, morphology, and ecology of the beach clam, *Donax striatus*. *Bulletin of Marine Science*, **17**: 723–740.

WADE, B.A. 1967b. Studies on the biology of the West Indian beach clam, *Donax denticulatus* L. I. Ecology. *Bulletin of Marine Science*, **17**: 149–174.

WITT, J.D.S., THRELOFF, D.L. & HEBERT, P.D.N. 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology*, **15**: 3073–3082.

ZAR, J.H. 1999. *Biostatistical analysis*. Edn 4. Prentice-Hall Inc., Upper Saddle River.

3.5 Publication II

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

DANIEL CARSTENSEN,[1]* JÜRGEN LAUDIEN,[1,2] WALTER SIELFELD,[3] MARCELO E. OLIVA[4] AND WOLF E. ARNTZ[1]

[1]*Alfred Wegener Institute for Polar and Marine Research, PO Box 120161, D-27515 Bremerhaven, Germany;* [2]*Institute for Applied Ecology Ltd., Alte Dorfstrasse 11, 18184 Neu Broderstorf, Germany;* [3]*Departamento de Ciencias del Mar, Universidad Arturo Prat, Av. Arturo Prat 2120, Iquique, Chile;* [4]*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 ± 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 *D. 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 *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 & Fahrbach 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 & Fahrbach 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°27′S, 70°18′W) to the north of Peru (3°30′S, 80°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. 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 annual mean sea surface temperature (17.8 ± 0.2°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 ± 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 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 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-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 ± 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 trochophore 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 ($F_1 = 358.0$, $P = 0.00$). 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: $0.79 \pm 0.11\%$ at NTC, and $1.25 \pm 0.37\%$ at ENTC (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 ± 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 ($F_1 = 4.87$, $P = 0.03$). Under NTC, LT_{50} was reached after 4.3 h; under ENTC, 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 ± 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 ($F_1 = 0.308$, $P = 0.59$). However, within different salinity levels (15, 25, and 35 ± 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 ± 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
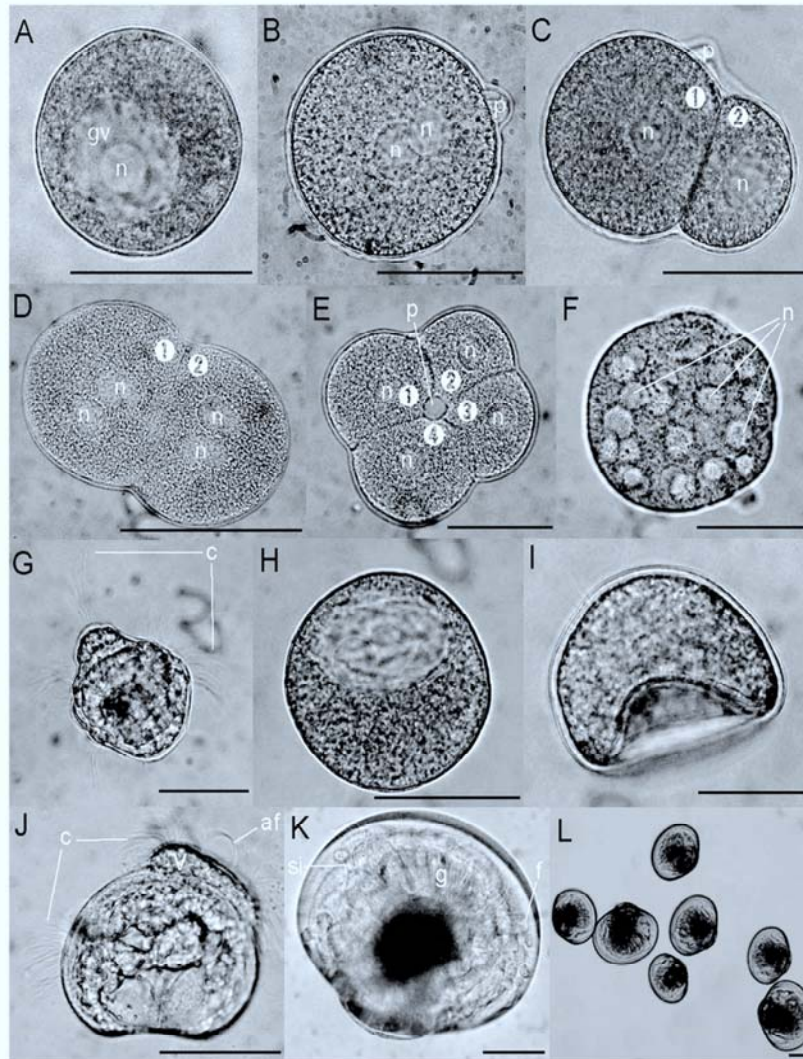
Figure 1. Light microphotograph (Leica DM LS2, Solms, Germany) taken at a magnification of 400 × (except view L, 100 ×) with a Canon Powershot S50 (Tokyo, Japan) digital camera. All scale bars are equivalent to 50 µm. (A) Spawned oocyte with large germinal vesicle (gv) and nucleolus (n), which indicates that the egg is still not fertilized (62.5 µm, <1 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 µm; nucleolus, 16 µm; 4.5 h). (C) Two-cell embryo with unequal blastomeres (➊ and ➋). The polar body (p) is located in the cleavage plane (87.5 × 62.5 µm; nucleolus, 12.5 µm; 5 h). (D) Two-cell embryo with equal blastomeres (➊ and ➋) in transition to a 4-cell embryo (nucleolus divided; 100 × 87.5 µm, 6 h). (E) Four-cell embryo with 4 equal blastomeres (➊, ➋, ➌, and ➍; 100 × 95 µm, 6 h). (F) Multicellular stage, nucleolus (n; 82.5 µm, 9.5 h). (G) Early trochophore, ciliate (c) indicated by lines (75 µm, <24 h). (H) Early gastrula stage (75 µm, 9.5 h). (I) Late gastrula stage (80 µm, <24 h). (J) D-Veliger with velum (v), cilia (c), and apical flagellum (af; 80 × 67.5 µm, 2.5 days). (K) Foot-stage larvae, siphon (si), gills (g), and foot (f; 200 × 170 µm, 18 days). (L) Foot-stage larvae overview (~187.5–250 µm, 18 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. obesulus* 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).
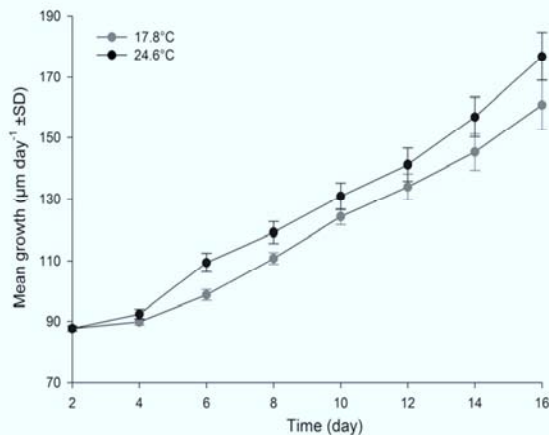
Figure 2. Mean growth rate of *Donax obesulus* larvae cultured at NTC (17.8 ± 0.2°C) and ENTC (24.6 ± 0.2°C) for 16 days.

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 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
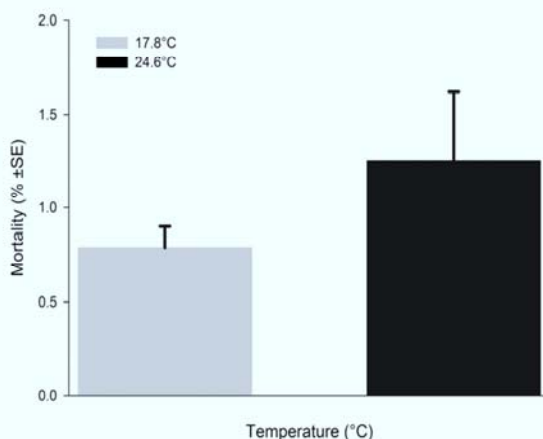

Figure 3. Total mortality of *Donax obesulus* larvae during a 16-day growth experiment carried out under NTC (17.8 ± 0.2°C) and ENTC (24.6 ± 0.2°C).

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 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 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 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
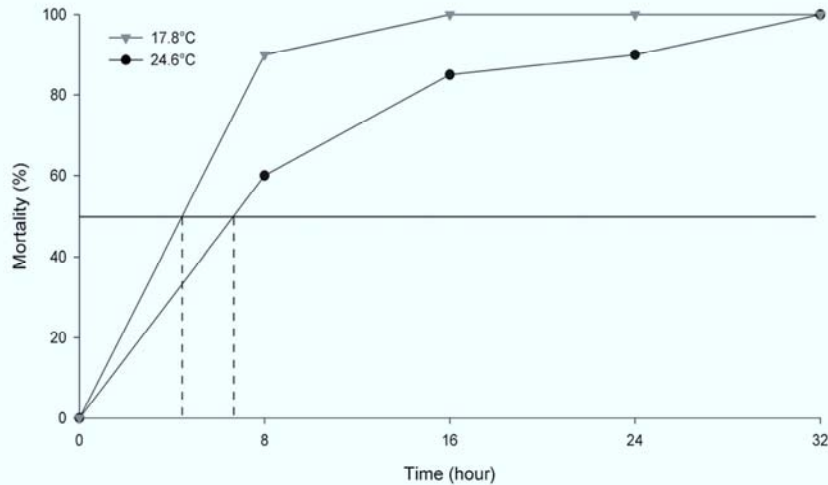
Figure 4. Mortality of *Donax obesulus* larvae (*n* = 20) exposed for 48 h to a salinity of 5 ± 1 under NTC (17.8 ± 0.2°C) and ENTC (24.6 ± 0.2°C). Vertical dashed lines indicate the time at which 50% of the larvae had died (LT$_{50}$) under NTC (left) and ENTC (right).

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 (≥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.

Figure 5. Activity of early larvae (24–48 h) recorded as counts of swimming larvae exposed to salinities of 15, 25, and 35 ± 1 (each treatment, *n* = 20) at NTC (17.8 ± 0.2°C) and ENTC (24.6 ± 0.2°C).

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.
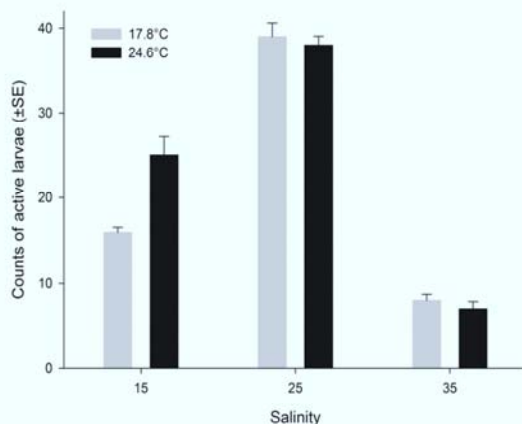
Vol. 19. Sandy beaches as ecosystems. The Hague, Netherlands: Dr. W. Junk. pp. 607–635

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 marincovichi* 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 Jahuay, 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 palabritas en el litoral y ámbito del departamento de Lambayeque. Resolución ministerial no. 607-2008-Produce: 2. Informe no. 452-2008-PRODUCE/DGEPP-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.

3.6 Publication III

Recurrent, thermally-induced shifts in species distribution range in the Humboldt current upwelling system

D. Carstensen [a,*], J.M. Riascos [b], O. Heilmayer [c], W.E. Arntz [a], J. Laudien [a]

[a] Alfred Wegener Institute for Polar and Marine Research, Section Bentho-Pelagic Processes, P.O. Box 120161, D-27515 Bremerhaven, Germany
[b] Universidad de Antofagasta, Instituto de Investigaciones Oceanológicas, Avenida Universidad de Antofagasta 02800, Antofagasta, Chile
[c] German Aerospace Center, Heinrich-Konen-Str. 1, D-53225 Bonn, Germany

ARTICLE INFO

ABSTRACT

El Niño-Southern Oscillation (ENSO) is a global climate variablility, which fundamentally influences environmental patterns of the Humboldt Current System (HCS) off Chile and Peru. The surf clams *Donax obesulus* and *Mesodesma donacium* are dominant and highly productive bivalves of exposed sandy beaches of the HCS. Existing knowledge indicates that El Niño (EN, warm phase of ENSO) and La Niña (LN, cold phase of ENSO) affect populations of both species in a different way, although understanding of the mechanisms underlying these effects is still lacking. The aim of this study was to test hypotheses attempting to explain field observations on the effect of strong EN or LN events by using controlled experimental conditions. Growth and mortality rates of both species were registered during a four-week experiment under EN temperature conditions, normal temperature conditions and LN temperature conditions. While *D. obesulus* exhibited reduced growth and higher mortality under LN conditions, *M. donacium* showed reduced growth and higher mortality under EN conditions. The results clearly indicate different temperature tolerance windows for each species, possibly reflecting the evolutionary origins of the Donacidae and Mesodesmatidae in regions with contrasting temperature regimes. These results provide experimental support for previous hypotheses suggesting that thermal tolerance is the driving factor behind observed changes in the species distributions of *D. obesulus* and *M. donacium* during the extreme phases of ENSO.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The Humboldt Current System (HCS) is among the most productive marine ecosystems in the world. Strong, coastal upwelling driven by trade winds brings deep, nutrient-rich water into the euphotic layer, fueling vast primary production. Unlike other coastal ecosystems at similar latitudes, the HCS exhibits cool, stable temperature conditions throughout the year. According to Thiel et al. (2007, Fig. 1), three biogeographical units can be distinguished within the HCS: (i) the first northern unit dominated by subtropical and temperate biota and extending from northern Peru to northern Chile (4–6°S to 30–36°S); (ii) the second southern unit dominated by a subantarctic and temperate biota extending from the Chilean archipelago to the Magellan Province (41–43°S to 54°S); and (iii) in between, a transitional unit characterized by strong numerical reductions in subantarctic and subtropical species at its northern and southern borders

respectively (30–36°S to 41–43°S). These biogeographical units are subject to continual fundamental changes caused by the El Niño-Southern Oscillation (ENSO), the strongest signal in the interannual variation of the ocean–atmosphere system (Wang et al., 1999). During El Niño (EN, the warm phase of ENSO) and La Niña (LN, the cold phase of ENSO) events, multiple and contrasting abiotic changes trigger strong biotic changes, including alternations in species composition, abundance and biomass (e.g. Tarazona et al., 1985; Arntz and Fahrbach 1991; Castilla and Camus, 1992; Riascos, 2006; Thatje et al., 2008). Taking into account the normally stable thermal regime of the HCS and the strong thermal anomalies associated to the extremes of ENSO, temperature has been proposed as a key factor driving changes at the population and community level, and thereby influencing community structure and the latitudinal distributions of marine benthic organisms within this system (e.g. Soto, 1985; Tomicic, 1985; Arntz and Fahrbach 1991; Díaz and Ortlieb, 1993; Urban, 1994; Laudien et al., 2007). Unfortunately, cause-and-effect explanations of the described changes during extremes of ENSO are hampered by the lack of experimental and physiological evidence (Arntz et al., 2006; Thatje et al., 2008).

* Corresponding author. Tel.: +49 (0) 47148311315; fax: +49 (0) 47148311918.
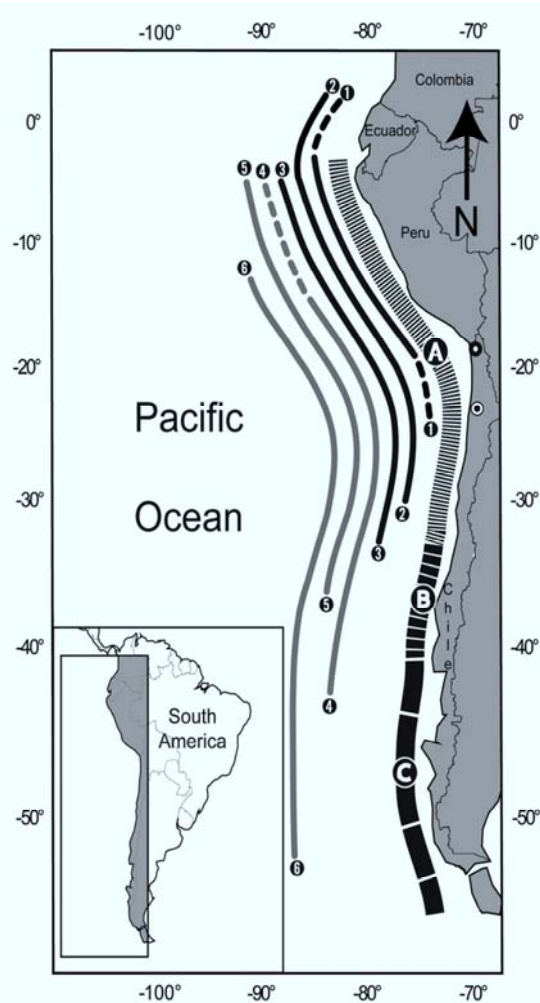E-mail address: Daniel.Carstensen@awi.de (D. Carstensen).

Fig. 1. Biogeographical units of the Humboldt Current System after Thiel et al. (2007): A: The Peruvian province, B: The transition zone and C: The Magellanic province. Sample location of *Donax obesulus* ⊙ in Arica; Chinchorro beach and *Mesodesma donacium* ⊙ in Antofagasta; Hornitos beach. Lines in black indicate species with tropical origin: ❶ Black and black dashed lines indicate historic distribution of *D. obesulus* after Coan (1983). Black line indicates current distribution after an ample sampling. ❷ Distribution of *Argopecten purpuratus* and ❸ *Thais chocolata* after Guzmán and Viana (1998a). Lines in grey indicate species with tropical origin: ❹ Grey and grey dashed lines indicate historic distribution of *M. donacium* after Tarifeño (1980). Grey line indicates current distribution after an ample sampling. ❺ Distribution of *Semimytilus algosus* after Olsson (1961). ❻ Distribution of *Aulacomya ater* after Guzmán and Viana (1998a). For further details see Table 3.

Donax obesulus Reeve, 1854 and *Mesodesma donacium* Lamarck, 1818 are two common surf clams of reflective and dissipative sandy beaches. Whereas *D. obesulus* is restricted to the intertidal zone, *M. donacium* inhabits both, the intertidal (juveniles) and the shallow subtidal (adults) (Riascos et al., 2008). Both species show high abundance along their distribution range in the HCS (Carstensen, 2010; Arntz et al., 1987). These species occupy the same niche and have similar ecological roles (Tarazona et al., 1986; Beu, 2006). Therefore, strong competitive interactions may be expected, such as reported between *Donax hanleyanus* and *Mesodesma mactroides* at the Atlantic coast of South America (Defeo and de Alava 1995; Herrmann et al., 2009). As poikilothermic organisms, their temperature is directly dictated by the temperature of the

surroundings. A small increase of the 'normal operating temperature range' of an organism can reduce scope for growth and reproduction (Pörtner et al., 2005, Pörtner and Knust, 2007; Wang and Overgaard, 2007).

Within their distributional range both species are affected differently by extreme phases of ENSO (Soto, 1985; Arntz et al., 1987, 1988, Arntz and Fahrbach, 1991; Díaz and Ortlieb, 1993; Thiel et al., 2007). Historically, *D. obesulus* (synonyms: *D. marincovichi* and *D. peruvianus*; Carstensen et al., 2009) was distributed from the Ecuadorian coast (0°26′S) to the North of Chile (23°05′S) (Coan, 1983; Fig. 1) and temporarily expands its distribution range southward during strong EN episodes (Tomicic, 1985). In contrast, the historic distribution of *M. donacium* spans from Chiloé, southern Chile (16°36′S) to Sechura, northern Peru (42°23′S) (Tarifeño, 1980; Fig. 1). During EN, the species suffers mass mortalities in the northern part of its geographical range (Tomicic, 1985; Arntz et al., 1987; Guzmán et al., 1998b).

Generally, the distributional ranges of species are determined by different and interacting contributory factors, including environmental conditions, species interactions and population demographics (Sagarin et al., 2006). Apart from purely descriptive accounts, some evidences suggest that distribution shifts may not be entirely explained by corresponding shifts in temperature. First, it is not clear whether sympatric *D. obesulus* and *M. donacium* develop competitive interactions (Arntz et al., 1987). Second, *M. donacium* reveals a remarkably poor ability to recover its former distribution following EN events in spite of several cycles of warm and cold (Arntz et al., 2006). Third, parasitic interactions and, interspecific interactions generally have been shown to play a role in mortality events and species distribution (Dugan et al., 2004; Riascos et al., 2008). In this context, determining the physiological responses of particular species to temperature would provide a mechanistic understanding of the observed changes and discriminate this factors from others contributing to changes in distribution.

Therefore, this study aimed to (i) evaluate experimentally the effect of temperatures typically observed during EN and LN events on growth and mortality of the two surf clams, and (ii) compare these results with the observed changes in distribution. Therefore, bivalves were collected from the current southernmost extent of the *D. obesulus* disributional range of, and from the northernmost point in the *M. donacium* disributional range, both within the area impacted by the cold and warm episodes of ENSO, and used in a series of in vitro-experiments.

2. Material and methods

2.1. Distribution and sampling site

Given that EN and LN have caused changes in the historical distributional range of both study species, an ample sampling and a compilation of historical data were performed to determine the current southern distribution limit of *D. obesulus* (Carstensen, 2010), and the current northern distribution limit of *M. donacium* along the Peruvian and Chilean coast (Riascos, 2008). The southernmost population of *D. obesulus* was located at Chinchorro beach (Northern Chile, Arica, 18°27′53.8′ S; 70°18′24.3′W; Fig. 1). The northernmost population comprising the full range of size classes of *M. donacium* was found at Hornitos beach (Antofagasta, 22°54.99′S; 70°17.42′W; Fig. 1). Specimens of both species were collected in February 2007 (*D. obesulus*, n = 104; *M. donacium*, n = 122) and transported to the laboratory. To minimize stress, clams were transported within 12 h of collection and transferred immediately to laboratory conditions at the Marine Laboratory of the University of Antofagasta.

2.2. In vitro-experiment

During the first two weeks after collecting, the bivalves were acclimatized in the laboratory at the temperatures corresponding to local conditions at the collection sites (*D. obesulus*: 17.8 °C; *M. donacium*: 17.4 °C). Low mortality (<5%) was observed during the acclimatization period. The sampling sites for each species lies approximately 800 km apart, hence the slight difference in temperature. Considering this, experimental temperatures were set to mimic normal temperature conditions (NTC), El Niño typical temperature conditions (ENTC) and La Niña typical temperature conditions (LNTC) at each sampling site (Fig. 1). Temperatures were defined according to the analysis of a long-term (1980–2006) sea surface temperature (SST) data archived in the database of the Servicio Hidrográfico y Oceanográfico de la Armada de Chile (available from http://www.shoa.cl). For *D. obesulus*, NTC was defined as the long-term annual mean SST (17.8 °C), ENTC reflected the highest monthly mean SST registered during EN 1982–1983 (24.6 °C), and LNTC mimicked the lowest monthly mean SST during LN 1990 (14.9 °C) at the Arica station (18°28'S, 70°19'). Similarly, for *M. donacium* NTC was set to 17.4 °C, ENTC was 24.2 °C, and LNTC was 14.5 °C.

After acclimatization, the shell length (maximal anterior–posterior shell length; SL) of each specimen was measured to the nearest 0.01 mm with a vernier caliper and the specimen was tagged with a consecutive code to ensure individual identification. Thereafter, clams were incubated for 3 h in moderately aerated, filtered seawater containing 50 mg l^{-1} Calcein (Sigma, CAS 1461-15-0) to incorporate a non-deleterious fluorescent mark in the shell, that would allow the measurement of short-term (i.e. daily) growth increments (Riascos et al., 2007). Thereafter, clams were randomly assigned to a single factor (temperature), three-level (NTC, ENTC, LNTC) experimental design, with three replicate aquaria per level and 12 individuals of *D. obesulus* and 16 individuals of *M. donacium* per replicate.

Specimens were maintained under experimental conditions in closed circulation systems for 31 days (March 2007) in a 12:12 h day–night cycle. Aquaria were filled with filtered (1 μm) seawater and sufficient sand for clams to be able to retract completely (~13 cm depth). Experimental temperature was maintained within a 0.3 °C fluctuation range, using a computer-controlled system. Sand was aerated and ~15% of the water was replaced daily to minimize water pollution by accumulation of nutrients (NH_4^+, NO_2^-, NO_3^-) from excreted waste. Animals were fed daily with a mixture (50:50) of *Isochrysis galbana* (T-ISO) and *Chaetoceros calcitrans* (~40.000 cells ml^{-1}) *ad libitum*. Dead animals (open shell, foot and siphons extended) were removed on a daily basis and shells were cleaned and stored for further analysis.

At the end of the experiment, all animals were sacrificed, soft parts were removed and shells oven-dried at 65 °C for 24 h. A section through the right valve of each clam was obtained by cutting across the longest growth axis and the section embedded in epoxy-resin. In order to clearly identify the Calcein mark included in the shell, the surface of the resulting section was polished using a speed grinder–polisher (Buehler, Alpha, 2 Speed Grinder–Polisher) with grinding paper (200 μm–5 μm) and a polycrystalline diamond suspension (1 μm–0.05 μm). Microgrowth was measured (to the nearest μm) from the Calcein mark to the shell edge, by analyzing digital fluorescence micrographs (blue light, 460–490 nm). The daily growth rate (μm day^{-1}) was calculated for each specimen. Mortality of each species was calculated from the daily percentage of surviving clams throughout the experimental period (31 days). A dosage–mortality approach (Urban, 1994) was used to determine the point at 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.

2.3. Statistical analysis

To evaluate the effect of temperature on growth rate (μm day^{-1}), the general linear model (GLM) procedure in JMP 7.0.1 (SAS Institute Inc.) was used to apply a one-way analysis of covariance (ANCOVA) model. The model treated temperature as a fixed effect, growth rate as the response variable and shell length as the covariate under the full interaction (separate slopes) model approach. Therefore, the interactions between the covariate and the main effects were included as additional effects. In order to deal with variation within replicates and between replicates, a nested design was used (aquaria within temperature), thus including an additional effect in the model. Tukey's honestly significant difference test was used for post-hoc comparisons of least squared means between effect levels.

To analyse mortality, the slope of a linear regression of the relationship between mortality (proportions of dead clams) and time (days) was calculated for each replicate, following the approach of Menge et al. (2007). Mortality data were square root-transformed, thus obtaining good fits ($r^2 \geq 0.762$) of all relationships to the linear model. The slope of the regression could thus be taken as an appropriate measure of mortality. An analysis of variance (ANOVA), using temperature as a three-level-treatment and the slope of the regression as the response variable was used to test for effects of temperature on mortality of each species. Significant differences for between-levels were tested by the Tukey post-hoc-Test. Previously, the Shapiro–Wilk test and Barlett's test were used to assess normality and homogeneity of variances to meet ANOVA and ANCOVA assumptions respectively.

3. Results

3.1. Growth rate

Growth rate of *D. obesulus* was significantly dependent on shell size, with larger clams showing lower growth rates, and revealing significant differences between temperatures (Table 1, Fig. 2). Growth was fastest under NTC, followed by ENTC and slowest at LNTC. There was no significant interaction between temperature and shell size, and within aquaria variability was not significant. Post-hoc comparisons showed significant differences between NTC and LNTC and between ENTC and LNTC (Tukey test; $p < 0.01$; $p = 0.012$ respectively), but no differences between NTC and ENTC (Tukey test: $p = 0.497$).

Table 1
Results of ANCOVA performed on *Donax obesulus* and *Mesodesma donacium* growth rate, with temperature as the main factor and shell size as the covariant. Significant probability values are highlighted in bold.

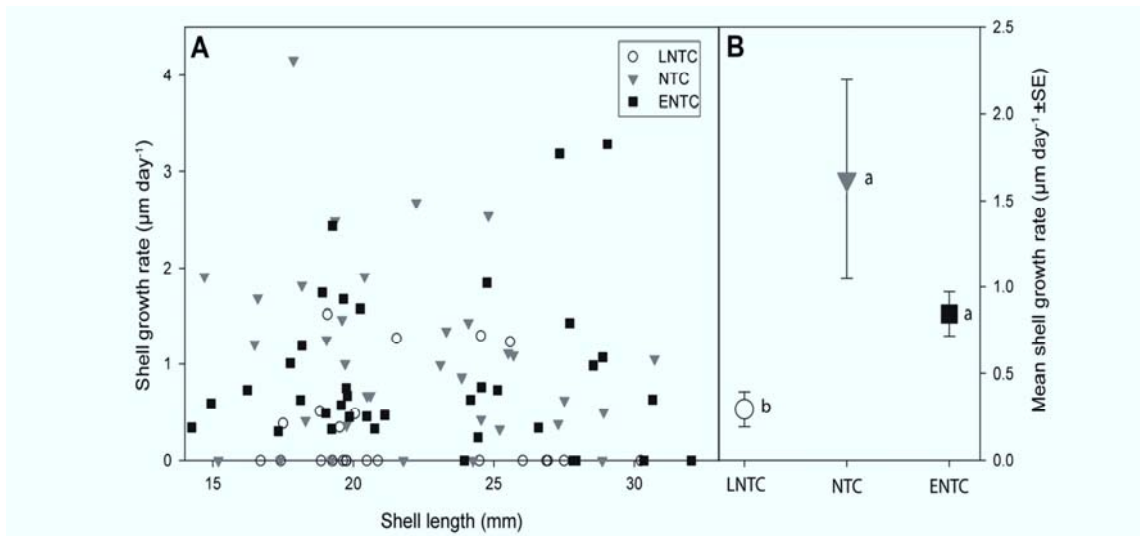| Source of variation	DF	Sum of Squares	F-ratio	Prob > F
Donax obesulus				
Temperature (T)	2	8.865	9.215	**<0.001**
Shell size (SL)	1	5.627	11.698	**0.001**
T × SL	2	2.234	2.993	0.104
Aquaria [T]	6	3.751	4.215	0.061
Error	84	40.405		
Mesodesma donacium				
Temperature (T)	2	16.591	7.936	**<0.001**
Shell size (SL)	1	11.317	10.827	**0.001**
T × SL	2	20.425	9.770	**<0.001**
Aquaria [T]	6	1.799	0.2869	0.941
Error	74	77.346		

Fig. 2. (A): Shell growth rate (μm day^{-1}) of *Donax obesulus* under three different experimental temperature conditions: normal (NTC), La Niña (LNTC) and El Niño (ENTC). (B): comparison of mean shell growth rates (±Standard Error) for each temperature condition. Levels not sharing the same letters are significantly different (Tukey's test, $p < 0.05$).

The growth rate of *M. donacium* was inversely correlated to shell size and showed significant differences between temperature regimes (Table 1, Fig. 3). A significant interaction between temperature and shell size was detected (Table 1), indicating that differences between temperatures were less clear among larger clams. Multiple *a posteriori* comparisons showed that growth rate of *M. donacium* differed significantly between NTC and LNTC and between NTC and ENTC (Tukey test; $p = 0.021$; $p < 0.01$, respectively), but did not differ between LNTC and ENTC (Tukey's HSD test: $p > 0.864$).

3.2. Mortality

Mortality of *D. obesulus* revealed significant differences between experimental temperatures (Table 2), being higher and earlier at LNTC (Fig. 4). Post-hoc comparisons showed significant differences

between NTC and LNTC and between ENTC and LNTC (Tukey test; $p < 0.01$, $p = 0.02$, respectively) but no significant differences between NTC and ENTC (Tukey test; $p = 0.87$). This species showed high sensitivity to LNTC, with high mortality after 48 h of exposure and 100% mortality after three weeks. In contrast, under NTC and ENTC, a few dead specimens were observed after four days, rising slightly until day 18. Mortality did not exceed 15% at the end of the experiment.

M. donacium was comparatively more resistant to changing temperatures than *D. obesulus* (Fig. 4). Our analysis revealed significant differences in mortality rates of *M. donacium* between experimental temperatures (Table 2). Tukey tests showed significant differences between LNTC and ENTC ($p < 0.01$) and between NTC and ENTC ($p < 0.01$). In contrast, no differences were found between LNTC and NTC (Tukey test; $p = 0.91$) as all clams survived until the end of the experiment (Fig. 4). Under ENTC *M. donacium*
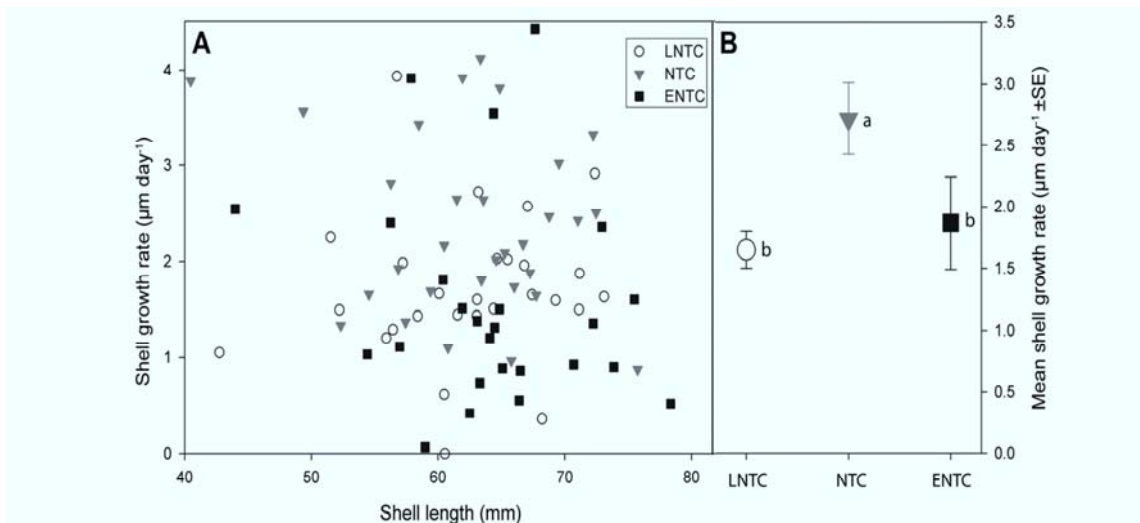

Fig. 3. (A): Shell growth rate (μm day^{-1}) of *Mesodesma donacium* under three different experimental temperature conditions: normal (NTC), La Niña (LNTC) and El Niño (ENTC). (B): comparison of mean shell growth rates (±Standard Error) for each temperature condition. Levels not sharing the same letters are significantly different (Tukey's test, $p < 0.05$).

Table 2

Results of ANOVA performed to test differences of mortality of *Donax obesulus* and *Mesodesma donacium* among three temperatures. Significant probability values are highlighted in bold.

Source of variation	DF	Sum of squares	F-ratio	Prob > F
Donax obesulus				
Temperature	2	0.0002	7.3095	**0.0246**
Error	6	0.0001		
Mesodesma donacium				
Temperature	2	0.0014	42.0577	**<0.001**
Error	6	0.0001		

showed a slight increase in mortality after the tenth day with a constant trend until day 17, thereafter increasing to 67% until the end of the experiment (Fig. 4).

As indicated by the LT_{50} values, *D. obesulus* responded much faster to temperature changes compared to *M. donacium*. The former species reached LT_{50} under LNTC after eight days, whereas LT_{50} of *M. donacium* under ENTC was reached at day 27 of the experiment (Fig. 4). No other level of the treatment resulted in 50% of mortality during the experimental period.

4. Discussion

Temperature has been considered the most significant abiotic factor controlling growth, reproduction, nutrition and other physiological processes in marine bivalves, (Laudien et al., 2001; Heilmayer et al., 2004; Miyaji et al., 2007; Riascos et al., 2009). SST in the costal HCS shows little seasonal variability and similar conditions over a broad range of latitudes. Therefore marine species are sensitive to anomalous thermal changes (Urban, 1994; Camus, 2001; Riascos et al., 2009). This study showed a high, contrasting sensitivity to short-term anomalous temperatures, with sublethal and lethal effects in both species. While *D. obesulus* was sensitive to LNTC, and tolerant to ENTC, *M. donacium* was sensitive to both ENTC and LNTC.

In general, the present results support the hypothesis that temperature is an important factor causing regime shifts during EN. In the period before and during EN 1982–1983 Arntz et al. (1987) followed recently-recruited cohorts of *D. obesulus* off southern Lima (~12°S). This species showed significantly reduced growth when SST peaked in February and March 1983, but was able to survive this strong warming event (Arntz and Fahrbach, 1991). In contrast, *M. donacium* populations exhibited mass mortality when
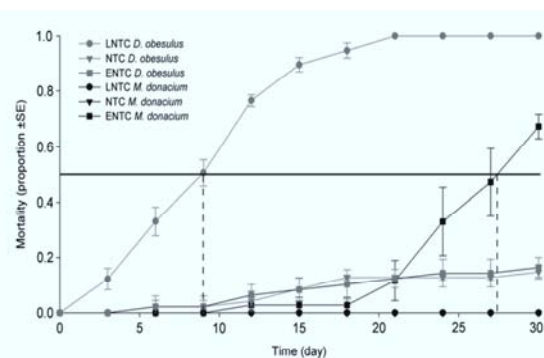

Fig. 4. Mortality of *Donax obesulus* and *Mesodesma donacium* under three different experimental temperature conditions: normal (NTC), La Niña (LNTC) and El Niño (ENTC). Vertical dashed lines indicate LT_{50} values for *M. donacium* (right) and *D. obesulus* (left).

SST peaked. Interestingly, in that studies, specimens of *M. donacium* were seen to open their shells a few days before they died, coinciding with observations made under laboratory conditions in the current study. Accordingly, the local extinction of *M. donacium* described above and the survival of *D. obesulus* after strong EN was observed repeatedly elsewhere off Peru and northern Chile (Tomicic, 1985; Arntz et al., 1987; Carbajal et al., 1995; Aburto and Stotz, 2003; Quiroz et al., 2006; Thiel et al., 2007; Riascos et al., 2009). A southward extension of the geographic distribution of *D. obesulus* has been reported following EN warming, enabling this species to temporarily reach as far south as ~23°S (Tomicic, 1985; Carbajal et al., 1995), about 800 km south of its current southern distribution limit.

Despite the fact that the present results reflect responses to extreme values of a single factor and that they are spatially limited to one end of the current species distribution boundaries they may provide insights into the mechanism underlying observed changes related to EN events. However, for most EN/LN episodes, SST anomalies may be far less extreme, and species may also be affected by other factors which may either reduce or increase any potential negative temperature effects, depending on the physical characteristics of the locality. On the other hand, this study provides insights into the responses of *D. obesulus* and *M. donacium* to LN, which have been only marginally addressed in previous studies. One striking aspect of the results is that although the two clams showed opposite responses to ENTC, they both seem to be sensitive to LNTC, showing slower growth in comparison with NTC. This is probably the reason why (i) *D. obesulus* has been unable to establish viable populations within southern areas colonized during EN (Tomicic, 1985), and (ii) remnants of *M. donacium* populations are not able to rapidly recolonize northern beaches during LN (Arntz et al., 1987, 2006), as would be expected under a contrasting response pattern. Despite the fact that *D. obesulus* and *M. donacium* are sympatrically distributed along large parts of the coastal HCS, their biogeography reflects different environmental affinities, which may explain their contrasting responses to the extremes of ENSO cycle. The family Donacidae primarily inhabits warm areas, with most of the living species (>75%) restricted to the tropics (Ansell, 1983). *D. obesulus* most likely represents a species of tropical affinity with adaptations to cooler waters. It is thus able to survive during strong EN and to expand temporarily southwards, but hampered in its ability to establish viable populations in those areas under LN conditions. In contrast, recent species of the family Mesodesmatidae generally inhabit cold and temperate waters (Marins and Levy, 1999; Beu, 2006). Therefore, the distribution and population stability of *M. donacium* seem closely linked to the interannual thermal variability associated to ENSO in the HCS (Riascos et al., 2009).

Shallow-water molluscs display significant changes in abundance associated to ENSO (e.g. Arntz and Fahrbach, 1991; Arntz et al., 2006; Thiel et al., 2007). Table 3 shows changes in abundance reported for several shallow-water molluscs distributed along the HCS (Fig. 1). Clearly, all these species show a strong nexus between their tropical/temperate origin and their changing abundance during EN (Fig. 1). Although studies exhibiting changes in abundance associated with LN appear to be almost non-existent, the present results suggest that such changes may also be dependent on biogeographical affinity. On the other hand, distributional ranges are similar for all species of tropical origin. These molluscs (Table 1) inhabit exclusively the first biogeographical unit (Thiel et al., 2007), reflecting the distributional range of *D. obesulus* (Thiel et al., 2007; Fig. 1 and Table 1). Conversely, all temperate species inhabit mainly the area of the second and third biogeographical unit (Thiel et al., 2007; Fig. 1 and Table 1), as in the case of *M. donacium*.

Table 3

Effects of El Niño events on population abundance (decrease (↓); increase (↑) of shallow-water molluscs of the Peruvian and Chilean coast (Arntz and Tarazona, 1990).

Species (affect EN)	Origin	Distribution	References
❶ *Donax obesulus* (↑)	tropical	0°26′S to 23°05′S	Olsson (1961); Coan (1983)
❷ *Argopecten purpuratus* (↑)	tropical	0°55′S to 29°57′S	Wolff (1987); Guzmán et al. (1998b)
❸ *Thais chocolata* (↑)	tropical	05°04′S to 33°02′S	Keen (1971); Guzmán et al. (1998b)
❹ *Mesodesma donacium* (↓)	subantarctic	16°36′S to 42°23′S	von Ihering (1907); Tarifeño (1980)
❺ *Semimytilus algosus* (↓)	temperate	03°34′S to 36°54′S	Olsson (1961); Caro and Castilla (2004)
❻ *Aulacomya ater* (↓)	temperate	12°03′S to 52°46′S	Jaramillo and Navarro (1995); Guzmán et al. (1998b)

The responses to changing temperature regimes depicted here may also operate at larger temporal scales. Inter-decadal shifts in the biological regimes of pelagic ecosystems corresponding to shifts in fundamental physical properties of the HCS are now being recognized (e.g. Bakun and Broad, 2003; Chavez et al., 2003; Montecinos et al., 2003; Ayón and Swartzman, 2008). Although no attempt has been made to evaluate potential effects in coastal populations of the HCS, a strong effect seems feasible (Southward et al., 1995). Moreover, the hypothesized increase in the frequency of ENSO under a scenario of ongoing global warming (Timmermann et al., 1999) may significantly affect current distribution and abundance patterns of coastal species in the HCS.

Even though our results support the hypothesis of a strong link between temperature tolerance, anomalous temperature and distribution shifts of *D. obesulus* and *M. donacium*, we cannot expect those shifts to be explained solely in terms of this relationship. There are several further biotic and abiotic processes that have to be taken into account for a proper understanding of the biological responses of these species to ENSO. A whole array of abiotic factors is modified during ENSO, notably salinity, wave action, sediment load, upwelling strength and hence nutrient and food availability, which act simultaneously to produce an integrated effect on these species (e.g. Arntz et al., 2006) that may be difficult to address in experimental studies.

Interspecific interactions play an important role in defining population processes and community structure in sandy beach ecosystems (see review by Defeo and McLachlan, 2005). These interactions may be significantly modified under strong environmental stress and may modulate the responses of species to the extremes of ENSO. These interactions may be implicated in the poor ability of *M. donacium* to recover its former abundance and distribution and in the inability of *D. obesulus* to thrive at southern beaches in the wake of EN events. However, it is not clear whether competition occurs between these clams: After the mass mortality of *M. donacium* in Peru during EN 1982–1983, *D. obesulus* and the mole crab *Emerita analoga* never reached the density formerly attained by *M. donacium*; instead, an impoverished community consisting mainly of small opportunistic polychaetes became established, leading Arntz et al. (1987) to suggest that no competition exists between these commonly dominant species. On the other hand, the parasitic association between *M. donacium* and the spionid *Polydora bioccipitalis* may also play a role in the process of recovery of abundance and distribution range after strong warming events (Riascos et al., 2008).

Acknowledgements

References

Aburto, J., Stotz, W., 2003. Una experiencia de co-manejo de bivalvos en el marco de una nueva herramienta de administración pesquera en Chile: las áreas de manejo. Policy Matters 12, 200–204.

Ansell, A.D., 1983. The biology of the genus Donax. In: McLachlan, A., Erasmus, T., Junk, W.E. (Eds.), Developments in Hydrobiology Vol. 19: Sandy Beaches as Ecosystems, vol. 19. Dr. W. Junk, The Hague, Netherlands, pp. 607–635.

Arntz, W., Tarazona, J., 1990. Effects of El Niño 1982–1983 on Benthos, Fish and Fisheries off the South American Pacific Coast. In: Glynn, P.W. (Ed.), Global Ecological Consequences of the 1982–1983 El Niño-Southern Oscillation. Elsevier Oceanography Series, pp. 323–360.

Arntz, W.E., Brey, T., Tarazona, J., Robles, A., 1987. Changes in the structure of a shallow sandy-beach community in Peru during an El Niño event. South African Journal of Marine Science 5, 645–658.

Arntz, W.E., Fahrbach, E., 1991. El Niño-Klimaexperiment der Natur: Physikalische Ursachen und biologische Folgen. Birkhäuser, Basel, 264 pp.

Arntz, W.E., Gallardo, V.A., Gutiérrez, D., Isla, E., Levin, L.A., Mendo, J., Neira, C., Rowe, G.T., Tarazona, J., Wolff, M., 2006. El Niño and similar perturbation effects on the benthos of the Humboldt, California, and Benguela Current upwelling ecosystems. Advances in Geosciences 6, 243–265.

Arntz, W.E., Valdivia, E., Zeballos, J., 1988. Impact of El Niño 1982–83 on the commercially exploited invertebrates (mariscos) of the Peruvian shore. Meeresforschung 32, 3–22.

Ayón, P., Swartzman, G., 2008. Changes in the long-term distribution of zooplankton in the Humboldt Current Ecosystem off Peru, 1961–2005, and its relationship to regime shifts and environmental factors. Fisheries Oceanography 17, 421–431.

Bakun, A., Broad, K., 2003. Environmental 'loopholes' and fish population dynamics: comparative pattern recognition with focus on El Niño effects in the Pacific. Fisheries Oceanography 12, 458–473.

Beu, A.G., 2006. Marine Mollusca of oxygen isotope stages of the last 2 million years in New Zealand. Part 2. Biostratigraphically useful and new Pliocene to recent bivalves. Journal of the Royal Society of New Zealand 36, 151–338.

Camus, P.A., 2001. Biogeografía marina de Chile continental. Revista Chilena de Historia Natural 74, 587–617.

Carbajal, W., Carrasco, F.D., Curo, P., Zamora, J., 1995. Cambios en la estructura comunitaria de la macroinfauna de playas arenosas, después del fenómeno El Niño 1986–1987, en Lambayeque, Perú. Gayana Oceanologia 3, 29–40.

Caro, A.U., Castilla, J.C., 2004. Predator-inducible defences and local intrapopulation variability of the intertidal mussel *Semimytilus algosus* in Central Chile. Marine Ecology Progress Series 276, 115–123.

Carstensen, D., 2010. Environmentally induced responses of Donax obesulus and Mesodesma donacium (Bivalvia) inhabiting the Humboldt Current System, Ph. D. thesis. University Bremen. 160 p.

Carstensen, D., Laudien, J., Leese, F., Arntz, W.E., Held, C., 2009. Genetic variability, shell and sperm morphology suggest that the surf clams *Donax marincovichi* and *D. obesulus* are one species. Journal of Molluscan Studies 75, 381–390.

Castilla, J.C., Camus, P.A., 1992. The Humboldt-El Niño scenario: coastal benthic resources and anthropogenic influences, with particular references to the 1982/83 ENSO. South African Journal of Marine Science 12, 703–712.

Chavez, F.P., Ryan, J., Lluch-Cota, S.E., Ñiquen, C.M., 2003. From anchovies to sardines and back: multidecadal change in the Pacific Ocean. Science 299, 217–221.

Coan, E., 1983. The Eastern Pacific Donacidae. The Veliger 25, 273–298.

Defeo, O., de Alava, A., 1995. Effects of human activities on long-term trends in sandy beach populations: the wedge clam *Donax hanleyanus* in Uruguay. Marine Ecology Progress Series 123, 73–82.

Defeo, O., McLachlan, A., 2005. Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. Marine Ecology Progress Series 295, 1–20.

Díaz, A., Ortlieb, L., 1993. El fenómeno 'El Niño' y los moluscos de la costa peruana. Bulletin de l'Institut Francais d'Études Andines 22, 159–177.

Dugan, J.E., Jaramillo, E., Hubbard, D.M., Contreras, H., Duarte, C., 2004. Competitive interactions in macroinfaunal animals of exposed sandy beaches. Oecologia 139, 630–640.

Guzmán, J.M., Viana, M.T., 1998a. Growth of abalone *Haliotis fulgens* fed diets with and without fish meal, compared to a commercial diet. Aquaculture 165, 321–331.

Guzmán, N., Saá, S., Ortlieb, L., 1998b. Catálogo descriptivo de los moluscos litorales (Gastropoda y Pelecypoda) de la zona de Antofagasta 23°S (Chile). Estudios Oceanológicos 17, 17–86.

Heilmayer, O., Brey, T., Pörtner, H.O., 2004. Growth efficiency and temperature in scallops: a comparative analysis of species adapted to different temperatures. Functional Ecology 18, 641–647.

Herrmann, M., Carstensen, D., Fischer, S., Laudien, J., Penchaszádeh, P.E., Arntz, W.E., 2009. Population structure, growth and production of the wedge clam *Donax hanleyanus* (Bivalvia: Donacidae) from northern Argentinean beaches. Journal of Shellfish Research 28, 1–16.

Jaramillo, R., Navarro, J., 1995. Reproductive cycle of the Chilean ribbed mussel, *Aulacomya ater* (Molina, 1782). Journal of Shellfish Research 14, 165–171.

Keen, A.M., 1971. Sea Shells of Tropical West America, 2 ed. Standford University Press, Standford, California, 1064 pp.

Laudien, J., Brey, T., Arntz, W.E., 2001. Reproduction and recruitment patterns of the surf clam *Donax serra* (Bivalvia, Donacidae) on two Namibian sandy beaches. South African Journal of Marine Science 23, 53–60.

Laudien, J., Rojo, M.E., Oliva, M.E., Arntz, W.E., Thatje, S., 2007. Sublittoral soft bottom communities and diversity of Mejillones bay in northern Chile (Humboldt current upwelling system). Helgoland Marine Research 61, 103–116.

Marins, L.F., Levy, J.A., 1999. High genetic distance between marine bivalves of the genus *Mesodesma* inhabiting the Atlantic and Pacific coasts of South America. Comparative Biochemistry and Physiology 124, 313–319.

Menge, B.A., Daley, B.A., Sanford, E., Dahlhoff, E.P., Lubchenco, J., 2007. Mussel zonation in New Zealand: an integrative eco-physiological approach. Marine Ecology Progress Series 345, 129–140.

Miyaji, T., Tanabe, K., Schöne, B.R., 2007. Environmental controls on daily shell growth of *Phacosoma japonicum* (Bivalvia: Veneridae) from Japan. Marine Ecology Progress Series 336, 141–150.

Montecinos, A., Purca, S., Pizarro, O., 2003. Interannual-to-interdecadal sea surface temperature variability along the western coast of South America. Geophysical Research Letters 30, 1570.

Olsson, A.A., 1961. Mollusks of the Tropical Eastern Pacific, Panamic Pacific Pelecypoda. Paleontological Research Institute, Ithaca, 574 pp.

Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315, 95–97.

Pörtner, H.O., Storch, D., Heilmayer, O., 2005. Constraints and trade-offs in climate-dependent adaptation: energy budgets and growth in a latitudinal cline. Sciencias Marinas 69, 271–285.

Quiroz., M., Tejada, A., Gonzales, A., Castillo, R., 2006.esEstrategia para reforzar el repoblamiento natural del recurso macha (Mesodesma donacium, Lamarck 1818) en el litoral de la provincia de Islay, región Arequipa-Perú. Informe Instituto del Mar del Perú CCMC13, p. 33–4.

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. Marine Biology 148, 1283–1293.

Riascos, J.M., 2008. Thriving and declining: population dynamics of the macha (Mesodesma donacium, Bivalvia, Mesodesmatidae) along a latitudinal gradient of the Humboldt Current Upwelling System, Ph.D. thesis. University Bremen. 124 p.

Riascos, J.M., Carstensen, D., Laudien, J., Arntz, W., Oliva, M.E., Güntner, A., Heilmayer, O., 2009. Thriving and declining: temperature and salinity shaping life-history and population stability of *Mesodesma donacium* in the Humboldt Upwelling System. Marine Ecology Progress Series 385, 151–163.

Riascos, J.M., Guzmán, N., Laudien, J., Heilmayer, O., Oliva, M.E., 2007. Suitability of three stains to mark shells of *Concholepas concholepas* (Gastropoda) and *Mesodesma donacium* (Bivalvia). Journal of Shellfish Research 26, 1–7.

Riascos, J.M., Heilmayer, O., Oliva, M.E., Laudien, J., Arntz, W.E., 2008. Infestation of the surf clam *Mesodesma donacium* by the spinoid polychaete *Polydora bio-cipitalis*. Journal of Sea Research 59, 217–227.

Sagarin, R.D., Gaines, S.D., Gaylord, B., 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. Trends in Ecology and Evolution 21, 524–530.

Soto, R., 1985. Efectos del fenómeno El Niño 1982–83 en ecosistemas de la I Región. Investigación Pesquera 32, 199–206.

Southward, A.J., Hawkins, S.J., Burrows, M.T., 1995. Seventy years observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. Journal of Thermal Biology 20, 127–155.

Tarazona, J., Arntz, W.E., Canahuire, E., Ayala, Z., Robles, A., 1985. Modificaciones producidas durante 'El Niño' en la infauna bentónica de áreas someras del ecosistema de afloramiento peruano. In: Arntz, W.E., Landa, A., Tarazona, J. (Eds.), 'El Niño', su impacto en la fauna marina. Boln. Inst. Mar. Perú, Callao, pp. 55–63. Spec. Issue.

Tarazona, J., Paredes, C., Igreda, M., 1986. Estructura del macrobentos en las playas arenosas de la zona de Lima, Perú, 74. Revista de Ciencias U.N.M.S.M. 103–116.

Tarifeño, E., 1980. Studies on the biology of the surf-clam *Mesodesma donacium* (Lamarck, 1818) (Bivalvia: Mesodesmatidae) from Chilean sandy beaches, Ph.D. thesis. University of California, Los Ángeles, 203 p.

Thatje, S., Heilmayer, O., Laudien, J., 2008. Climate variability and El Niño Southern Oscillation: implications for natural coastal resources and management. Helgoland Marine Research 62, 5–14.

Thiel, M., Macaya, E.C., Acuña, E., Arntz, W., Bastias, H., Brokordt, K., Camus, P.A., Castilla, J.C., Castro, L.R., Cortés, M., Dumont, C.P., Escribano, R., Fernández, M., Gajardo, J.A., Gaymer, C.F., Gómez, I., González, A.E., González, H.E., Haye, P.A., Illanes, J.E., Iriarte, J.L., Lancellotti, D.A., Luna-Jorquera, G., Luxordo, C., Manríquez, P.H., Marin, V., Muñoz, P., Navarrete, S.A., Pérez, E., Poulin, E., Sellanes, J., Sepúlveda, H.H., Stotz, W., Tala, F., Thomas, A., Vargas, C.A., Vásquez, J.A., Vega, J.M.A., 2007. The Humboldt current system of northern and central Chile, oceanographic processes, ecological interactions and socioeconomic feedback. Oceanography and Marine Biology 45, 195–345.

Timmermann, A., Oberhuber, J., Bacher, A., Esch, M., Latif, M., Roeckner, E., 1999. Increased El Niño frequency in a climate model forced by future greenhouse warming. Nature 398, 694–697.

Tomicic, J.J., 1985. El Niño 1982–83 effects on the littoral communities of the Peninsula de Mejillones. Investigacion Pesquera 32, 209–213.

Urban, H.J., 1994. Upper temperature tolerance of ten bivalve species off Peru and Chile related to El Niño. Marine Ecology Progress Series 107, 139–145.

von Ihering, H., 1907. Les mollusques fossiles du tertiaire et du cretacé supérieur de l'Argentine. Anales del Museo Nacional de Buenos Aires ser. II, 611 pp.

Wang, H.J., Zhang, R.H., Cole, J., Chavez, F., 1999. El Niño and the related phenomenon Southern Oscillation (ENSO): the largest signal in interannual climate variation. Proceedings of the Academy of Natural Sciences 96, 11071–11072.

Wang, T., Overgaard, J., 2007. The heartbreak of adapting to global warming. Science 315, 49–50.

Wolff, M., 1987. Population dynamics of the Peruvian scallop *Argopecten purpuratus* during the El Niño phenomenon of 1983. Canadian Journal of Fisheries and Aquatic Sciences 44, 1684–1691.

3.7 Publication IV

Thriving and declining: climate variability shaping life-history and population persistence of *Mesodesma donacium* in the Humboldt Upwelling System

José M. Riascos[1,2,*], Daniel Carstensen[2], Jürgen Laudien[2], Wolf E. Arntz[2], Marcelo E. Oliva[1], Andreas Güntner[3], Olaf Heilmayer[2,4]

[1]Universidad de Antofagasta, Instituto de Investigaciones Oceanológicas, Avenida Angamos 601 Antofagasta, Chile
[2]Alfred Wegener Institute for Polar and Marine Research, Am Alten Hafen 26, 27568 Bremerhaven, Germany
[3]Helmholtz Centre Postdam, GFZ German Research Centre for Geosciences, Telegrafenberg, 14473 Potsdam, Germany
[4]German Aerospace Center (DLR), Heinrich Konen-Str. 1, 53227 Bonn, Germany

ABSTRACT: Large-scale environmental patterns in the Humboldt Current System (HCS) show major changes during strong El Niño episodes, leading to the mass mortality of dominant species in coastal ecosystems. Here we explore how these changes affect the life-history traits of the surf clam *Mesodesma donacium*. Growth and mortality rates under normal temperature and salinity were compared to those under anomalous (El Niño) higher temperature and reduced salinity. Moreover, the reproductive spatial–temporal patterns along the distribution range were studied, and their relationship to large-scale environmental variability was assessed. *M. donacium* is highly sensitive to temperature changes, supporting the hypothesis of temperature as the key factor leading to mass mortality events of this clam in northern populations. In contrast, this species, particularly juveniles, was remarkably tolerant to low salinity, which may be related to submarine groundwater discharge in Hornitos, northern Chile. The enhanced osmotic tolerance by juveniles may represent an adaptation of early life stages allowing settlement in vacant areas at outlets of estuarine areas. The strong seasonality in freshwater input and in upwelling strength seems to be linked to the spatial and temporal patterns in the reproductive cycle. Owing to its origin and thermal sensitivity, the expansion and dominance of *M. donacium* from the Pliocene/Pleistocene transition until the present seem closely linked to the establishment and development of the cold HCS. Therefore, the recurrence of warming events (particularly El Niño since at least the Holocene) has submitted this cold-water species to a continuous local extinction–recolonization process.

KEY WORDS: El Niño · Fresh water input · Geographic distribution · Reproductive cycle · Sandy beach ecology · Submarine groundwater discharge · Macroecology

INTRODUCTION

The surf clam *Mesodesma donacium* (Lamarck, 1818) is an important species from both an economical and ecological perspective. It is one of the most important species for Chilean and Peruvian benthic fisheries in the Humboldt Current System (HCS), an ecosystem ranking among the most productive marine systems worldwide (Tarifeño 1980, Defeo et al. 1993, McLach-lan et al. 1996, Thiel et al. 2007). *M. donacium* often exhibits high densities and extremely high annual production (up to 2900 g shell-free dry mass m^{-2}), thus representing >95% of the shallow soft-bottom community (Arntz et al. 1987). This filter-feeding clam inhabits the swash zone of exposed, high-energy intermediate and dissipative sandy beaches, often located near river mouths, where it typically burrows down to a depth around 10 cm, though it can sometimes reach

25 cm when disturbed (Tarifeño 1980, Jaramillo et al. 1994). In Chile, adult clams are primarily confined to the subtidal, at water depths ranging between 3 and 15 m, while the vast majority of juveniles occur mainly in the intertidal zone, often in the outlets of estuarine areas (Tarifeño 1980, Jaramillo et al. 1994, Riascos et al. 2008a), although an inverse depth pattern between juvenile and adult clams has been described for Peruvian populations (Arntz et al. 1987). The reproductive cycle of *M. donacium* has been found to be influenced by local variability in water temperature and food availability (Tarifeño 1980, McLachlan et al. 1996). The long meroplanktonic larval stage (30 to 45 d) allows plenty of time for the phytoplanktophagous larvae to find suitable unoccupied sediments in which to settle (Tarifeño 1980).

Environmental conditions in the HCS are broadly characterized by nutrient-rich, cool waters, showing limited seasonal temperature variability compared to that in other coastal ecosystems at similar latitudes (e.g. Arntz et al. 1987, Camus 2001, Thiel et al. 2007). The influence of the year-round upwelling of cold subsurface water causes an atypical weak north–south temperature gradient and extends the influence of cold environmental conditions northward (Menzies 1962, Camus 2001). As a consequence, many species in the HCS exhibit broad distributional ranges and are adapted to fairly constant low water temperatures in this area (Santelices 1980, Brattström & Johanssen 1983, Urban 1994). However, during strong El Niño events (the warm phase of the El Niño–Southern Oscillation) warmer, nutrient-poor, less-saline waters are transported into the nearshore zone by coastal Kelvin waves, triggering several physical changes in the water column (Arntz et al. 2006). These changes result in mass mortalities of *Mesodesma donacium* and leave an impoverished sandy beach community mainly consisting of small opportunistic organisms (Arntz et al. 1987). Originally distributed from Peru (Sechura, 5° 10′ S) to southern Chile (Chiloe, 43° 20′ S) (Tarifeño 1980), *M. donacium* was set back south to 14° S (southern Peru) after the severe El Niño in 1982-83 (Arntz et al. 1987) and further south after the El Niño in 1997-98, which wiped out large populations in Arica (18° 20′ S) and Coquimbo (29° 55′ S) (Thiel et al. 2007), leaving only some minor, scattered populations in between.

The shallow-water fauna in the HCS is closely adapted to sea surface temperature. This distinctive feature defines large-scale biogeographical patterns in this region (Castilla 1979, Camus 2001). Therefore, thermal anomalies are expected to explain changes in performance and distribution of marine bivalves during El Niño (Urban 1994). On the other hand, reduced salinity seems particularly important to understand *Mesodesma donacium* responses to El Niño, given the

strong increases in freshwater input to the coastal zone during these events (e.g. Waylen & Caviedes 1990). However, the lack of experimental work addressing factor-specific effects precludes convincing explanations for the numerous changes that have been described (Arntz et al. 2006, Thatje et al. 2008). Establishing links between the life-history traits of *M. donacium* and large-scale environmental settings during non-El Niño conditions is a crucial step in assessing the responses to El Niño and subsequent recovery. Reproduction and recruitment are key processes controlling long-term variability and persistence of marine bivalve populations (Bricelj et al. 1987, Lima et al. 2000, Beukema & Dekker 2007). Based on both, observational and experimental data sources, this paper aims to: (1) determine the effects of higher temperature and reduced salinity, expected under El Niño conditions, on growth and mortality of *M. donacium*; (2) describe the latitudinal patterns of reproductive activity and assess the link with large-scale environmental variability; and (3) analyse consequences for the long-term development and persistence of this species in Chile and Peru.

MATERIALS AND METHODS

Effects of temperature and salinity on growth and mortality. Specimens of *Mesodesma donacium* (n = 135) were collected in February 2006 from Hornitos, northern Chile (22° 54′ S; Fig. 1), which is currently the northernmost population of this species. Juvenile clams were collected in the intertidal, whereas adults were collected in the shallow subtidal (i.e. up to 5 m). After sampling, clams were immediately transported to the laboratory and acclimated for 25 d at ambient temperature (17.4°C) and salinity (~35 psu) in a 12 h day: 12 h night cycle before being transferred to experimental conditions. Clams were fed ad libitum with a mixed diet (1:1) of micro-algae (*Chaetoceros calcitrans* and *Isochrysis galbana*). Low mortality (9 clams) was observed during this period.

After acclimation, clams were incubated for 3 h in slightly aerated filtered seawater containing 50 mg l^{-1} calcein (Sigma, CAS 1461-15-0). This procedure was used to incorporate a fluorescent band in shells of *Mesodesma donacium* in order to determine short-term growth increments after the treatment (for details see Riascos et al. 2007). After calcein marking, individuals were divided into 2 groups based on anterior–posterior shell length (mm): juveniles: (6.0 to 29.9 mm; average = 13.5 mm) and adults (52.7 to 72.4 mm; average = 62.6 mm). Clams from each group were randomly assigned to a 3 × 2 factor experimental design: 3 salinities (10 ± 1, 20 ± 1 and 35 ± 1 psu) and 2 temperatures
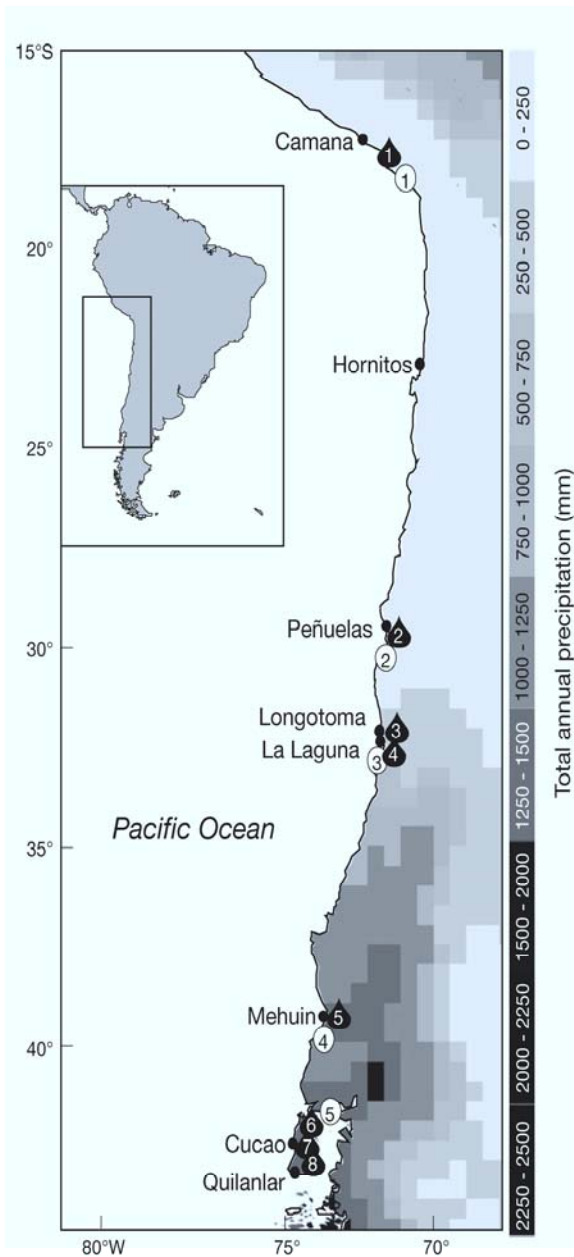
Fig. 1. Total annual precipitation (modified from Legates & Willmott 1990) in the study area, geographic location of sandy beaches (black dots) considered in the analysis of the reproductive cycle of *Mesodesma donacium* and location of stations for sea surface temperature (circles — 1: Ilo; 2: Coquimbo; 3: Valparaíso; 4: Corral; 5: Puerto Montt) and precipitation (drop symbols — 1: Mollendo; 2: La Serena; 3: Petorca; 4: La Ligua; 5: Toltén; 6: Tres Cruces; 7: Piruquina; 8: Castro). Data sources as indicated in Table 2

(17.4 ± 0.5°C, the historical annual average, hereafter called 'the normal temperature' and 24.2 ± 0.5°C, the maximum temperature registered during El Niño 1982-83, hereafter called 'the El Niño temperature'; data from CENDHOC 2008). Each combination was run with

3 replicates and 7 clams replicate^{-1}. Clams were conditioned over 2 wk to high temperature and low salinities by gradually increasing temperature and/or reducing salinity. They were fed as stated before and held in experimental conditions for 1 mo in 35 l tanks (1/3 sterilised sand) in temperature-controlled rooms and a 12 h day:12 h night cycle. Seawater was filtered (0.1 µm), continuously aerated and exchanged daily. Twice a day, sand was aerated, dead clams were counted and removed, and shells were kept for further analysis. After 43 d, the remaining individuals were sacrificed and shell length was measured to the nearest 0.1 mm. All shells were cleaned, oven-dried at 65°C for 24 h and processed according to Riascos et al. (2007), to determine the micro-growth increment (calcein mark to shell edge) to the nearest 1 µm under a fluorescence microscope using blue light (460 to 490 nm) and the individual growth rate (µm d^{-1}).

To evaluate the effect of temperature and salinity on growth rate, the general linear model (GLM) procedure in JMP 7.0.1 (SAS Institute Inc.) was used to apply a 2-way analysis of covariance (ANCOVA) model for juveniles and adults, separately. The model treated salinity and temperature as fixed effects and shell length as the covariate and used the full interaction (separate slopes) model approach. Therefore, the interactions between the covariate and the main effects were included as additional effects. In order to assess the effect of temperature and salinity on mortality of juvenile and adult clams, the time (in days) elapsed between the beginning of the experiment and the death of each clam was estimated and modelled using a 2-way ANCOVA model under the GLM procedure. Salinity and temperature were treated as fixed effects, and shell length was used as covariate.

In a preliminary stage, the 'aquarium' effect was included in the models to assess the significance of the between-aquaria variability using the difference in the residual sum of squares of the fitted model with and without this effect, resulting in non-significance in all cases. Prior to analysis, mortality data were square-root transformed in order to meet assumptions of the ANCOVA model. Tukey's honestly significant difference test was used for post hoc comparisons of least-squared means between effect levels.

Gametogenic cycle and its relationship with environmental variability. We integrated information on the gametogenic cycle of *Mesodesma donacium* scattered in local sources (Table 1) and published them in the open-access library PANGAEA (Publishing Network for Geoscientific & Environmental Data). These data sets refer to 8 sandy beaches and include our own data from a 2 yr study in Hornitos (northern Chile). Thus, our study covers the entire current distribution range of *M. donacium* (Camaná, 16° 36′ S, to Quilanlar,

42° 23' S; Fig. 1). With the exception of the 11 mo study by Rubilar et al. (2008) (Table 1), only data sets based on standard histological gonad examinations, taken at monthly intervals for at least 1 yr were included. Two studies performed during strong El Niño/La Niña years were excluded, because they included short-term and scattered samplings that did not provide sufficient and reliable evidence on deviations in reproductive patterns under normal conditions. In each study, tissue samples were previously fixed either in aqueous Bouin's fixative or in formaldehyde-acetic-alcohol and stained thereafter with haematoxylin-eosin or Arteta trichromic stain. Individuals were classified into 4 or 5 gametogenic stages (Table 1). However, 2 consecutive, distinct stages were consistently distinguished in each study for both males and females. In the 'active' or ripe stage, which represents the period of gamete maturation, the acini are large and crowded, with complete walls and are full of free germ cells. Abundant mature sperm form dense masses in the follicles, and oocytes are large and numerous, with ovoid to spherical shape, typically showing a prominent nucleolus. The early germ line in this stage is occasionally represented by a few oogones and previtellogenic oocytes of small size. In contrast, in the 'spawning' or spent stage, representing the period of gamete release, the acini are small, with a rough basal membrane and only a few residual free germ cells, often in a stage of degeneration. In order to determine the transition from the final stages of gametogenesis to the spawned state, significant reductions in the proportions of active stages coinciding with a significant increase in the proportion of spawning states between months were evaluated using the McNemar test for dependent samples (Zar 1999) and interpreted as indicative of spawning events.

Overall, original studies did not include environmental data or they were based on snapshot measurements. Long-term monthly mean data on sea surface temperature (SST; °C), river inflow ($m^3 s^{-1}$) and coastal precipitation (mm) were obtained from the nearest hydrological or meteorological governmental stations in Chile and Peru (Table 2, Fig. 1). Moreover, the long-term (1981 to 2008) monthly mean upwelling index ($m^3 s^{-1}$ per 100 m of coastline) was computed for each location (1° of nominal resolution) using the Live

Table 1. *Mesodesma donacium*. Details of data sets included in the analysis of the gametogenic cycle, including geographical location, corresponding sampling periods, monthly sample size (N) and number of gonad stages (GS) used to characterize the cycle. All studies were based on standard histological techniques

Location	Latitude	Sampling period	N	GS	Source
Camaná	16° 36' S	Mar 1973 to Feb 1974	65	4	Salgado & Ishiyama (2008)
Hornitos	22° 54' S	May 2005 to Apr 2007	18–30	4	Riascos et al. (2008c)
Peñuelas	29° 50' S	Jun 1994 to Jul 1995	20–30	5	Jerez et al. (2008)
Longotoma	32° 22' S	Jun 1994 to Jul 1995	20–30	5	Jerez et al. (2008)
La Laguna	32° 37' S	Aug 1969 to Nov 1970	50	5	Tarifeño (2008)
Mehuin	39° 26' S	Jul 1989 to Jun 1991	30	4	Filun (2008)
Cucao	42° 35' S	Sep 2000 to Aug 2001	16–89	5	Rubilar et al. (2008)
Quilanlar	43° 23' S	Oct 2000 to Aug 2001	27–64	5	Rubilar et al. (2008)

Table 2. Station names and sources of environmental data used to assess the environmental influence on the reproductive cycle of *Mesodesma donacium* at several locations along the Peruvian–Chilean coast. The base period (in parentheses) used to calculate long-term monthly means is given if available. SST: sea surface temperature; n.a.: not available

Study site	SST station (yr)	River inflow station (yr)	Precipitation station (yr)
Camaná	Ilo[a] (1995–2007)	Camaná[c] (1973–1974)	Mollendo[e] (1888–1900)
Hornitos	Hornitos[f] (2005–2007)	–	–
Peñuelas	Coquimbo[b] (1982–2006)	Elqui[d] (2000–2005)	La Serena[e] (1869–1982)
Longotoma	Valparaíso[b] (1982–2006)	Petorca[d] (2001–2007)	Petorca[e] (1931–1960)
La Ligua[d] (1979–2002)	La Ligua[e] (1931–1960)		
La Laguna	Valparaíso[b] (1982–2006)	Petorca[d] (2001–2007)	Petorca[e] (1931–1960)
La Ligua[d] (1979–2002)	La Ligua[e] (1931–1960)		
Mehuin	Corral[b] (1982–2006)	Cruces[d] (1969–2001)	Toltén[e] (1931–1965)
Cucao	Puerto Montt[b] (1982–2006)	Deñal (n.a.)	Tres Cruces[e] (1931–1960)
Quilanlar	Puerto Montt[b] (1982–2006)	Quilanlar (n.a.)	Castro[e] (1930–1962)

[a]IMARPE (2008) (Peru), [b]CENDHOC (2008) (Chile), [c]INRENA (1994), [d]DGA (2008) (Chile), [e]Vörösmarty et al. (1998), [f]Present study

Access Server (Pacific Fisheries Environmental Laboratories, NOAA; available from http://las.pfeg.noaa.gov). The index was calculated at 1° offshore to avoid potential effects of extreme coastal topography on the calculations. For Hornitos, our own data on SST and salinity were registered. An Onset StowAway logger was installed on a floating structure located in front of the beach to register SST at hourly intervals and salinity every 15 min during monthly samplings using a multiparameter datasonde (Yellow Springs Instrument Company, Model 6600).

A multivariate approach of linking environmental variability patterns to biotic patterns developed by Clarke & Ainsworth (1993) was adapted to assess the temporal influence of SST and precipitation, river flow, or salinity on gametogenic stage composition. A Bray-Curtis similarity matrix was calculated for each locality using untransformed gonad stage percentages between months. The original classification of gonad stages of the corresponding study (Table 1) was used to calculate Bray-Curtis matrices. Environmental variables were normalised ($X_n = X - X/S$) to account for scale differences and enable comparisons, and between-month Euclidean distance matrices were calculated for each locality.

To determine whether the monthly pattern of gonad stage composition may be explained by monthly changes in environmental factors, the BIO-ENV analysis of the PRIMER V6.1.6 software package (Clarke & Gorley 2006) was used. This is a non-parametric permutation procedure calculating the Spearman rank correlation (ρ) between Bray-Curtis similarity matrices and Euclidean distance matrices. A global permutation test of the significance of ρ was calculated under the null hypothesis that there is no relationship between the 2 matrices. The BIO-ENV procedure defined single or suites of environmental variables that best 'explain' the variability of gonad stage composition.

RESULTS

Effects of temperature and salinity on growth and mortality

Growth rate of juvenile *Mesodesma donacium* varied between temperatures, but not between salinity levels (Table 3). Growth rate at El Niño temperature decreased to approximately one-half that at normal temperature (Fig. 2). Only the temperature term showed a significant interaction with the covariant, reflecting stronger effects on smaller clams. The growth rate of adult clams varied between temperatures and between salinity levels (Table 3). Multiple comparisons showed that, at El Niño temperature, growth rate was significantly lower at 10 psu than at 35 psu (Tukey's test, p < 0.05). In contrast, at normal temperature, no significant differences in growth rate were observed between salinity levels (Fig. 2).

Table 3. *Mesodesma donacium*. Results of the analyses of covariance testing the effects of temperature and salinity on growth rate of juvenile and adult clams. SL: shell length; boldface print: significant at p < 0.05

| Source	df	SS	F ratio	Prob > F
Juveniles				
Temperature (A)	1	44.204	279.418	**<0.001**
Salinity (B)	2	1.079	3.0132	0.061
SL (covariant)	1	23.014	145.477	**<0.001**
SL × A	1	5.915	37.389	**<0.001**
SL × B	2	0.197	0.623	0.541
A × B	2	0.175	0.554	0.578
Error	25	6.960		
Adults				
Temperature (A)	1	9.817	136.132	**<0.001**
Salinity (B)	2	0.848	5.885	**0.004**
SL (covariant)	1	2.099	29.107	**<0.001**
SL × A	1	0.287	3.985	0.051
SL × B	2	0.092	0.641	0.530
A × B	2	0.390	2.704	0.075
Error	64	18.076		
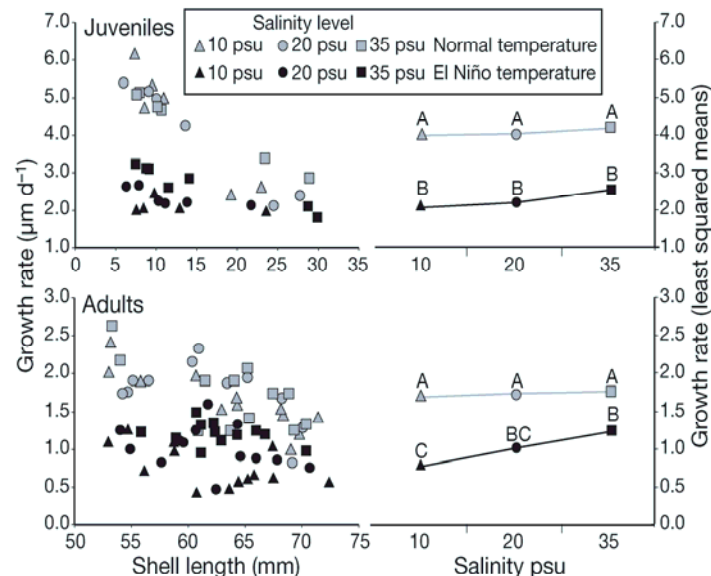
Fig. 2. *Mesodesma donacium*. Growth rate against initial shell length of juvenile and adult clams submitted to different experimental temperatures and salinities and comparisons of least-squared means of each treatment. Levels not sharing the same letter are significantly different (Tukey's test, p < 0.05)

Overall, mortality was higher at El Niño temperature at all salinity levels, reaching 50% after 23 d. In contrast, mortality was generally low at normal temperature. It was <20% at 35 and 20 psu and <50% at 10 psu (Fig. 3). The ANCOVA model revealed that juvenile clams died significantly faster at El Niño temperature, but no differences were observed between salinity levels (Fig. 4, Table 4). Non-significant interaction was observed between the main effects, and shell length was not significant as a covariant (Table 4). Therefore, the interaction between the main effects and the covariant was excluded from the model. In contrast, mortality of adults was significantly affected by both temperature and salinity, with
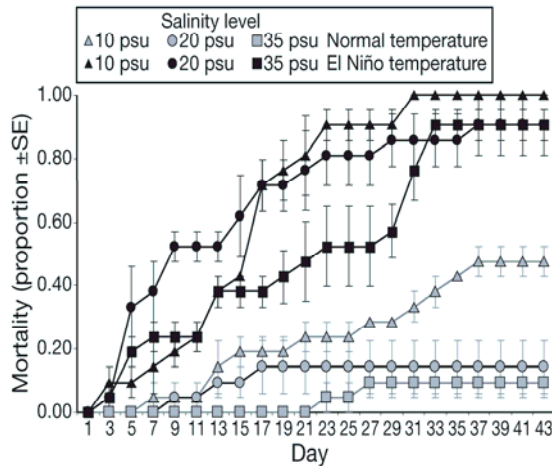
Fig. 3. *Mesodesma donacium*. Mortality of clams submitted to different experimental temperatures (normal and El Niño) and salinities (10, 20 and 35 psu) throughout a 45 d experiment

Table 4. *Mesodesma donacium*. Results of the analyses of covariance testing the effects of temperature and salinity on mortality (days elapsed until dead) of juvenile and adult clams. SL: shell length; boldface print: significant at p < 0.05

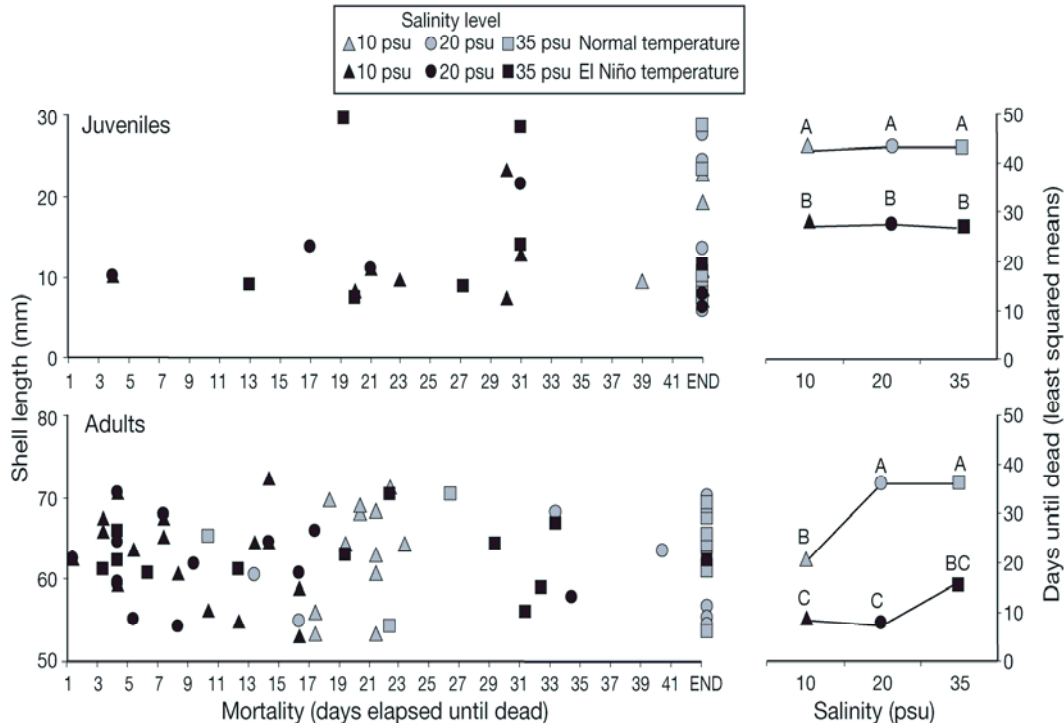| Source	df	SS	F ratio	Prob > F
Juveniles				
Temperature (A)	1	20.058	26.648	**<0.001**
Salinity (B)	2	0.067	0.0446	0.956
SL (covariant)	1	0.008	0.011	0.915
A × B	2	0.133	0.088	0.915
Error	29	6.960		
Adults				
Temperature (A)	1	83.935	64.854	**<0.001**
Salinity (B)	2	20.792	8.033	**<0.001**
SL (covariant)	1	0.051	0.0395	0.843
A × B	2	9.922	3.833	**0.026**
Error	64	18.076		

Fig. 4. *Mesodesma donacium*. Mortality against initial shell length of juvenile and adult clams submitted to different experimental temperatures and salinities and comparisons of least-squared means of each treatment. END: individuals surviving until the 43rd day, i.e. the end of the experiment. Levels not sharing the same letter are significantly different (Tukey's test, p < 0.05)

Fig. 5. *Mesodesma donacium*. Spatial and temporal patterns of reproductive activity, based on histological examination of gonad tissue. Transition between active and spawned gonad state (stars) based on significant drops in the active stage frequency coinciding with a significant increase in the spawning state frequency between months (see 'Materials and methods')

a significant interaction between the main effects. Mortality was higher under El Niño conditions, showing an additional increase at low salinities (10 and 20 psu). Under normal temperature conditions, adult clams died faster only at the lowest salinity, whereas no differences were observed between 20 and 35 psu (Fig. 4).

Influence of environmental variability on the gametogenic cycle

Reproductive activity of *Mesodesma donacium* along the distribution range showed a consistent temporal pattern with few departures (Fig. 5). The breeding season generally started in winter (seasons hereafter referring to austral) and extended into spring. Overall, a single spawning event was observed throughout the year, starting in late winter or spring and extending into summer. In contrast, gonad activity was continuous, and spawning events were biannual (spring and autumn) at Mehuin (39°S), thereby showing a departure from the above-mentioned pattern.

BIO-ENV analyses (Table 5) showed a significant correlation between the gonad stage composition and environmental factors analysed: the Spearman rank correlation ρ was significant for 6 out of 7 study sites, the exception being Peñuelas. Environmental variables showing significant correlations with the gametogenic cycle were plotted in Fig. 6. Stronger correlations were

obtained for northern beaches. Generally, the upwelling index was significantly correlated with gonad development only at northern beaches (Camaná and Hornitos). The transition between the breeding period and the spawning season coincided with the season of stronger upwelling. In contrast, precipitation and river inflow were mainly correlated with reproductive stages at central and southern places; in this case the breeding–spawning transition occurred shortly after the seasonal precipitation/river inflow maximum. Temperature was involved in significant global correlations only at Mehuin.
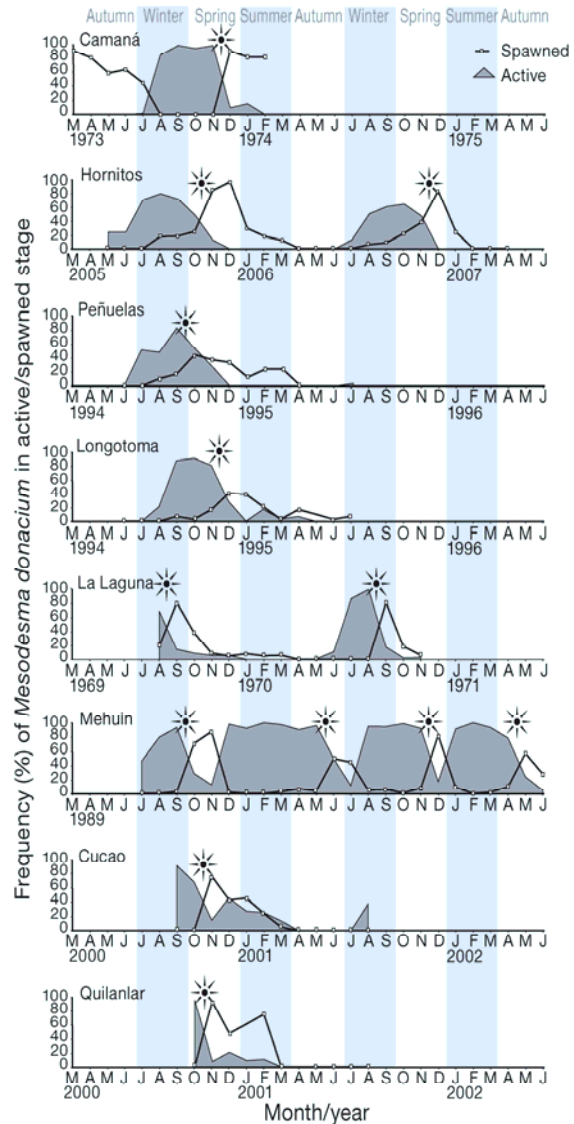
Table 5. *Mesodesma donacium*. Results of BIO-ENV analyses testing the relationship between gonad stage composition and environmental changes. Significant ($p < 0.05$) Spearman rank correlation coefficients (ρ) are shown in boldface print and the corresponding selection of the best 'explanatory' variable(s) is given

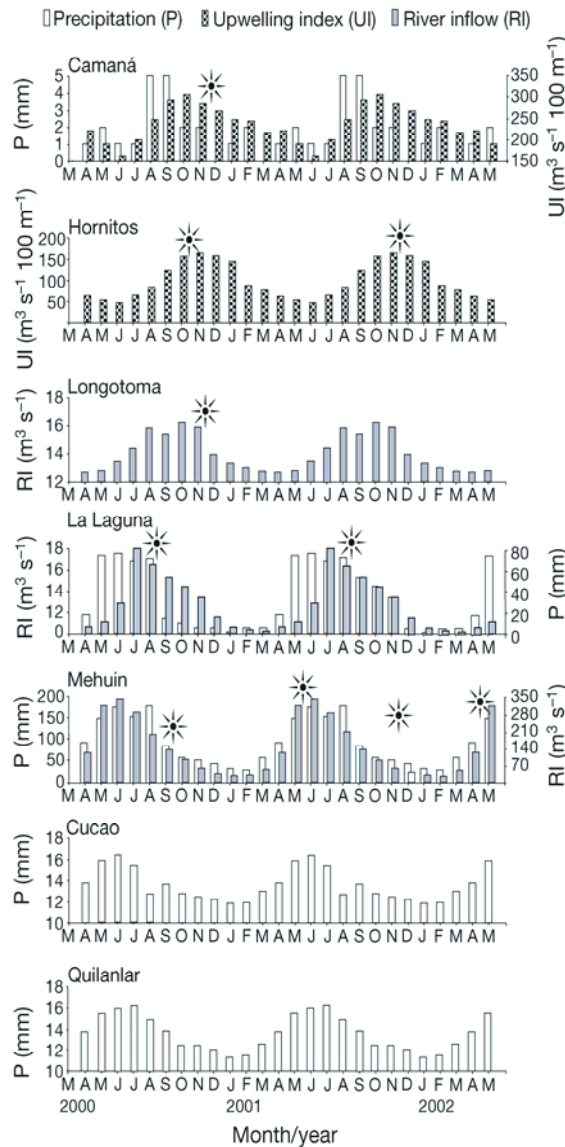| Location	ρ	p	Selection of variable(s)
Camaná	**0.727**	<0.01	Upwelling index, precipitation (Mollendo)
Hornitos	**0.525**	0.01	Upwelling index
Peñuelas	0.245	0.10	–
Longotoma	**0.585**	0.01	River inflow (Petorca)
La Laguna	**0.649**	<0.01	River inflow (Ligua), precipitation (Ligua)
Mehuin	**0.387**	0.01	River inflow (Cruces), precipitation (Toltén)
Cucao	**0.340**	0.04	Precipitation (Tres Cruces)
Quilanlar	**0.341**	0.03	Precipitation (Castro)

Fig. 6. Long-term variability of environmental factors showing significant correlations with the reproductive cycle of *Mesodesma donacium* in the study area. Transition between active and spawned gonad state of *M. donacium* (star symbols) at the corresponding location are indicated. Environmental data sources as indicated in Table 2

DISCUSSION

Influence of temperature and salinity on growth and mortality

The observed short-term effects of increased temperature on the growth and mortality of juvenile and adult *Mesodesma donacium* from Hornitos give strong experimental support to the hypothesis that the anomalous higher temperature during El Niño is the main factor explaining the mortality of this clam. However, the actual effects of anomalous temperature on *M. donacium* along its distribution range depend on a number of interacting factors including the strength, extent and spatial features of El Niño, the effects of associated physical changes, the dynamics of parasitic interactions under environmental stress (Riascos et al. 2008a) and the potential for behavioural thermoregulation (e.g. migration to deeper waters; Arntz et al. 1987) and increased parasitic interactions. Fig. 7a shows that the magnitude of thermal changes during El Niño depends on the geographic location, being weaker toward higher latitudes. As El Niño thermal anomalies are propagated southward by coastal Kelvin waves, their magnitude commonly declines towards higher latitudes, producing a latitudinal impact gradient (Camus 2008). It is not possible to define a threshold for the thermal tolerance of *M. donacium* across its geographic range based solely on our results. However, it is reasonable to expect that during strong El Niños the thermal tolerance and the potential of behavioural thermoregulation of populations north of Hornitos would be surpassed, given the stronger thermal changes in northern beaches, which may last for several months. A local adaptation of clams from northern beaches to higher temperature does not seem feasible, because there is not a strong latitudinal gradient of temperature along the HCS under normal conditions (Fig. 7a). Moreover, northern beaches have to be recolonized after strong El Niño episodes from southern populations, likely adapted to slightly cooler waters. Considering the populations south of Hornitos, even assuming that the weaker thermal changes would not produce mortality, they may still produce sublethal stress for several months on the northern and central Chilean coast. This stress, along with the stress produced by associated changes in salinity (i.e. lower salinity further reduces growth and increases mortality at El Niño temperatures; Figs. 3 & 4) and other abiotic factors (e.g. food availability, sediment load and wave action among others) during strong El Niño events may ultimately lead to mass mortality events. In fact, these events have only been reported between ~12 and ~30° S, coinciding with the latitudinal range where thermal anomalies during El Niño are more clear (Fig. 7a).

In contrast to its sensitivity to high temperature, *Mesodesma donacium* was less vulnerable to reduced salinity, with a higher tolerance for juvenile clams. The ability of *M. donacium* to cope with such a wide salinity range resembles that of euryhaline species; an intriguing result in view of the lack of coastal precipitation and river discharge in Hornitos, located off the Atacama Desert of northern Chile, one of the most arid zones in the world. Nevertheless, salinity in Hornitos showed relatively high variability (Fig. 8). Mean salin-
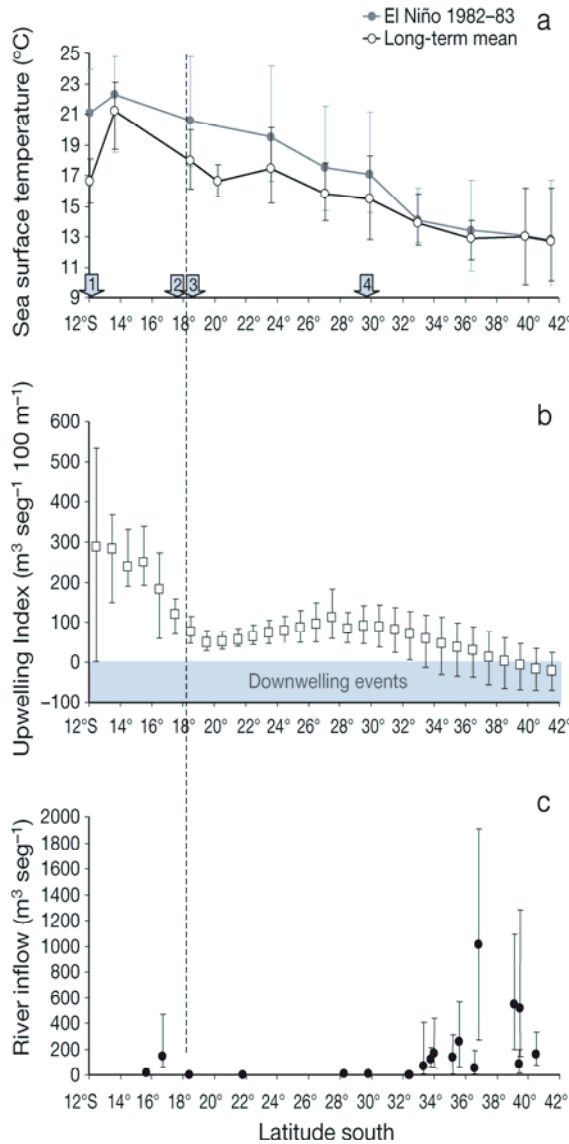
Fig. 7. Long-term annual mean and mean seasonal variability ('error bars': upper/lower bar represents the highest/lowest long-term monthly mean) of environmentals factors among latitudes (centesimal units) in the Humboldt Current System of Chile and Peru. (a) Long-term (Peru: 1956 to 1982; Chile: 1982 to 2006) annual mean sea surface temperature (SST) and mean seasonal oscillations compared with SST during El Niño 1982-83. Numbered arrows show locations of mass mortality events of *Mesodesma donacium* reported in literature in connection with El Niño (1: Arntz et al. 1987; 2: Quiroz & Barriga 1998; 3: Jerez et al. 1999; 4: Aburto & Stotz 2003). (b) Long-term (1981 to 2008) annual mean upwelling index and mean seasonal oscillations. (c) Long-term (variable number of years in record) annual mean river inflow and mean seasonal oscillations. Vertical dotted line: Peruvian–Chilean political border. Data sources: SST: IMARPE (2008) (Peru) and CEND-HOC (2008) (Chile); upwelling index: Pacific Fisheries Environmental Laboratories, NOAA; river inflow: INRENA (1994) (Peru), DGA (2008) (Chile) and Vörösmarty et al. (1998)
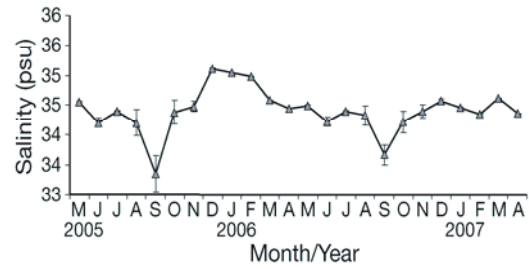

Fig. 8. Temporal variability of salinity at Hornitos, northern Chile, between May 2005 and April 2007

ity in September significantly differed from that during the rest of the year (Student's *t*-test = −3.628; p < 0.01). These drops in salinity coincided with the occurrence of submarine seepage through holes (~25 cm diameter) distributed along a narrow belt of about 500 m parallel to the coast line in the shallow subtidal (1.5 to 2.5 m depth). These holes were observed interspersed among the adult clam belt, but never in the intertidal. Water collected from several holes revealed low salinity (average = 19.5 psu; SD = 11.3; n = 12). The features of the groundwater flow at Hornitos most likely correspond to submarine groundwater discharge (SGWD; sensu Church 1996), which occurs where an unconfined aquifer is hydraulically connected with the sea through permeable bottom sediments and where its (head) water level is above sea level (Johannes 1980). Although unconfined aquifers are common along the Chilean–Peruvian desert coast (e.g. Rojas et al. 1995, Squeo et al. 2006), this is the first observation of recent SGWD on the western coast of South America. On sandy shores, SGWD may affect both the intertidal and subtidal, leading to strong salinity changes, particularly at the upper tidal levels during low tides (Johannes 1980, McLachlan & McGwynne 1986). It is difficult to confirm whether the osmotic tolerance of *M. donacium* is related to the SGWD observed in Hornitos. Our salinity measurements may not accurately reflect the magnitude and temporal variability of this flux of freshwater, the measurement of which is inherently difficult (Burnett et al. 2001, Gallardo & Marui 2006). The high osmotic tolerance may, instead, represent a more general adaptation of this species to a significant influence of freshwater discharge along its geographic distribution.

Reproductive cycle

Given the frequent correlation between seasonal temperature change and seasonal reproductive activity of marine animals, temperature was long assumed to be a causative factor for reproductive rhythms in the

marine realm (Giese & Pearse 1974). However, the existence of seasonal reproductive rhythms in places with slight seasonal temperature fluctuations led to the recognition that other seasonal factors may fluctuate more dramatically and may be more important for synchronizing reproduction (Pearse & Lockhart 2004). The reproductive cycle of *Mesodesma donacium* along its geographic range showed clear seasonality, with a low correlation with seasonal changes in temperature. The persistent northward flow of the cold Humboldt Current causes slight seasonality in the temperature of the HCS, typically a few degrees (Fig. 7a); this change is smaller than that of other marine systems at comparable latitudes (Arntz et al. 1987), and it appears unlikely that this slight fluctuation may act to synchronize reproduction. In contrast, strong effects of temperature on the intraannual variation of reproduction of several shallow-water bivalves, including the surf clam *M. mactroides*, have been reported for the mid-temperate Atlantic beaches of South America, where the temperature seasonality is high (e.g. Defeo et al. 1992, Morsan & Kroeck 2005, Fiori & Defeo 2006, Hermann et al. 2009). In fact, between-ocean differences in thermal patterns have been suggested to explain differences in the biogeographic patterns of populations of the Pan-American species *Excirolana brasiliensis* (Cardoso & Defeo 2004).

According to our analyses, the reproductive cycle is mostly correlated to the upwelling index, river inflow and precipitation, which all display strong seasonal and geographical patterns. Upwelling strength and river inflow vary by several orders of magnitude between seasons (Fig. 7b,c). This enormous variability, particularly of river inflow, can be expected to have a strong local influence on the nutrient input and phytoplankton production and, thus, to influence the reproductive cycles of bivalves (e.g. Riascos 2006, Riascos et al. 2008b). Our results show a link between increased upwelling strength and river inflow (i.e. food availability) and the season of gamete maturation of *Mesodesma donacium*. However, 2 factors may obscure the results of our analyses: (1) the potentially confounding effect of interannual differences on reproductive activities among the populations analysed here and (2) the offshore calculation of the upwelling index may not accurately reflect the nearshore nutrient availability, where upwelling is often influenced by beach geomorphology (Thiel et al. 2007).

Macroscale variations in upwelling strength, river inflow (Fig. 7b,c) and precipitation (Fig. 1) along the HCS seem to better explain the observed shift between the upwelling-influenced reproductive cycle of northern beaches and the river-influenced reproductive cycle of central and southern beaches. Upwelling strength decreases with latitude, while river inflow

and precipitation increase with latitude. The lack of a significant correlation between environmental factors and the reproductive cycle at Peñuelas (29° 50′ S) may be related to its location near a 'transitional zone' between 30 and 33° S, where upwelling is weaker and the frequency of both downwelling events and river inflow increases. On the other hand, the observed continuous gonad activity and biannual pattern of spawning events at Mehuin (39° 26′ S) could be explained by the stream flow regime of large rivers influencing the coastal zone up to 75 km offshore (Dávila et al. 2002). River runoff is an integral response to precipitation patterns, snow melt, delayed groundwater discharge and human water use throughout the river basins. Rivers in this region have a mixed pluvial–nival regime, with one peak flow due to winter precipitation and a second peak flow in summer due to snowmelt at higher altitudes (Dávila et al. 2002, Houston 2006).

Latitudinal variations in river inflow, upwelling strength and the higher osmotic tolerance of juvenile clams observed in our experiments may explain conflicting evidence regarding the spatial segregation between juvenile and adult *Mesodesma donacium*. According to Tarifeño (1980) and Jaramillo et al. (1994), juvenile clams settle near river mouths at both La Ligua (~32° S) and Mehuin (~39° S), showing an alongshore spatial segregation from adults, which seems a common pattern among sandy beach clams (see Donn 1987). The enhanced ability of juveniles to cope with low salinities may represent an early physiological trait allowing larvae to settle in vacant areas by active or passive processes (e.g. Mann et al. 1991, Shanks & Brink 2005) and thereby to reduce strong intraspecific and interspecific competition for food and habitat quality (Tarifeño 1980, Dugan et al. 2004) and increasing recruitment success. However, contradictory evidence regarding spatial segregation related to salinity has been reported by Ortiz & Stotz (1996). They pointed out that abundances of juveniles were lower near small river inlets in Coquimbo (~29° S). This might be related to the fact that river runoff is minor and intermittent throughout the year in Coquimbo Bay, whereas that at La Ligua and Mehuin is perennial and more abundant. Therefore, if some relationship does exist between a salinity gradient and alongshore distribution of different sizes, it would be hard to detect in Coquimbo. Moreover, as larvae are commonly transported onshore during downwelling and offshore during upwelling (Shanks & Brink 2005), the prevalence of downwelling currents throughout the year in southern Chile would favour larval retention and, thereby, increase interspecific interactions.

However, establishing a link between differential osmotic tolerance and spatial size segregation in the field related to salinity remains speculative because:

(1) the results of our experiment are limited to a single population involving a few animals, (2) observations regarding the spatial distribution of *Mesodesma donacium* are mostly circumstantial and do not take into account its inherently dynamic nature, and (3) appropriate quantitative models are needed to study spatial patterns on sandy beaches (see Defeo & Rueda 2002, Schoeman & Richardson 2002). Clearly, more experimental and field work on the environmental factors affecting early life stages is needed to conciliate this evidence, as it may shed light on the recruitment process, one of the key factors regulating the population dynamics of sandy beach clams (Lima et al. 2000).

Implications for population persistence

Our findings depict how macroscale spatial patterns and seasonal variability in temperature, river inflow and upwelling strength in the HCS may affect growth, mortality and the reproductive cycle of *Mesodesma donacium*. However, these findings may be useful in interpreting how long-term environmental variability in the HCS has affected population persistence.

Earlier fossil records of *Mesodesma donacium* have been found in late Pliocene deposits at Horcón and Quebrada Blanca, central Chile (Herm 1969), and at Tablazos, northern Peru (DeVries 1986). A well-recognized mass extinction, presumably caused by the development of the oxygen minimum zone after the establishment of the HCS during the mid-to late Miocene (McRoberts & Newton 1995, Ibaraki 1997, Tsuchi 1997, Rivadeneira & Marquet 2007), took probably place during the Pliocene/Pleistocene transition (Herm 1969, DeVries 2001, Rivadeneira & Marquet 2007). Coinciding with this mass extinction, several species with sub-Antarctic affinity, which were formerly scarce during the Pliocene, became dominant in their corresponding biotope in the Pleistocene (Herm 1969, DeVries 1986). In general, the development of the HCS involved both the northward advance of sub-Antarctic biota and the northward retraction of a former tropical/subtropical biota, with consequences that still persist (Brattström & Johanssen 1983, Camus 2001). Therefore, the expansion and dominance of *M. donacium*, a species with a presumably Antarctic origin (von Ihering 1907, Marins & Levy 1999), seem closely linked to the expansion of cold-water conditions through the Humboldt Current. Given its sensitivity to high temperature, the recurrent warming effect of El Niño, at least since the Holocene (Ortlieb et al. 2000), has caused a recurrent southward retraction of *M. donacium* populations as demonstrated by changes in diversity and abundance of species in kitchen middens found along the Peruvian coast (Quilter & Stocker 1983, Sandweiss 1996). A progressive southern retraction of *M. donacium*, along with several sub-Antarctic species, may also be foreseeable under the scenario of an increasing frequency of El Niño events, which has been discussed in connection with ongoing global warming (Timmermann et al. 1999).

Overall, the ecological, biogeographical and evolutionary consequences derived from the recurrent local extinction–recolonization dynamics undergone by species that are heavily affected by El Niño are not understood (Thiel et al. 2007). While we focus here on the ecological implications of these dynamics, further studies on the latitudinal patterns of life-history traits of *Mesodesma donacium* and their environmental drivers may be crucial for a broader understanding of the biogeographical and evolutionary consequences.

LITERATURE CITED

Aburto J, Stotz W (2003) Una experiencia de co-manejo de bivalvos en el marco de una nueva herramienta de administración pesquera en Chile: las areas de manejo. Policy matters 12:200–204. www.iucn.org/about/union/commissions/ceesp/ceesp_publications/pm/

Arntz WE, Brey T, Tarazona J, Robles J (1987) Changes in the structure of a shallow sandy-beach community in Peru during an El Niño event. In: Payne AIL, Gulland JA, Brink HK (eds) The Benguela and comparable ecosystems. S Afr J Mar Sci 5:645–658

Arntz WE, Gallardo VW, Gutiérrez D, Isla E and others (2006) El Niño and similar perturbation effects on the benthos of the Humboldt, California, and Benguela Current upwelling ecosystems. Adv Geosci 6:243–265

▶ Beukema JJ, Dekker R (2007) Variability in annual recruitment success as a determinant of long-term and large-scale variation in annual production of intertidal Wadden Sea mussels (*Mytilus edulis*). Helgol Mar Res 61:71–86

Brattström H, Johanssen A (1983) Ecological and regional zoogeography of the marine benthic fauna of Chile. Sarsia 68:289–339

▶ Bricelj VM, Epp J, Maloui RE (1987) Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. Mar Ecol Prog Ser 36:123–137

▶ Burnett WC, Makoto T, Oberdorfer J (2001) Measurement and significance of the direct discharge of groundwater into the coastal zone. J Sea Res 46:109–116

Camus PA (2001) Biogeografía marina de Chile continental. Rev Chil Hist Nat 74:587–617

▶ Camus PA (2008) Understanding biological impacts of ENSO on the eastern Pacific: an evolving scenario. Int J Environ Health 2:5–19

Cardoso R, Defeo O (2004) Biogeographic patterns in life history traits of the Pan-American sandy beach isopod *Excirolana braziliensis*. Estuar Coast Shelf Sci 61:559–568

Castilla JC (1979) Características bióticas del Pacífico Sur-Oriental, con especial referencia al sector chileno. Rev Com Perm Pac Sur 10:167–182

CENDHOC (Centro Nacional de Datos Hidrográficos y Oceanográficos de Chile) (2008) Gráficos y estadísticas de temperaturas superficiales y nivel del mar de estaciones ambientales costeras. Centro Hidrográfico y Oceanográfico de la Armada, Valparaiso. Available at: www.shoa.cl/cendoc-jsp/index.jsp (accessed on 8 January 2008)

▶ Church TM (1996) An underground route for the water cycle. Nature 380:579–580

▶ Clarke KR, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. Mar Ecol Prog Ser 92:205–219

Clarke KR, Gorley RN (2006) Primer v6: user manual/tutorial. PRIMER-E, Plymouth

▶ Dávila PM, Figueroa D, Müller E (2002) Freshwater input into the coastal ocean and its relation with the salinity distribution off austral Chile (35–55°S). Cont Shelf Res 22:521–534

▶ Defeo O, Rueda M (2002) Spatial structure, sampling design and abundance estimates in sandy beach macroinfauna: some warnings and new perspectives. Mar Biol 140:1215–1225

▶ Defeo O, Ortiz E, Castilla JC (1992) Growth, mortality and recruitment of the yellow clam *Mesodesma mactroides* on Uruguayan beaches. Mar Biol 114:429–437

Defeo O, de Alava A, Valdivieso V, Castilla JC (1993) Historical landings and management options for the genus *Mesodesma* in coasts of South America. Biol Pesq (Chile) 22:41–54

DeVries TJ (1986) The geology and paleontology of the Tablazos in northwest Peru. PhD thesis, Ohio State University, Columbus, OH

DeVries TJ (2001) Contrasting patterns of Pliocene and Pleistocene extinctions of marine mollusks in western North and South America. In: Proceedings of the 97th annual meeting of the Geological Society of America, Boston, MA, 33:A35

DGA (Dirección General de Aguas) (2008) Servicios satelitales en tiempo real. Dirección General de Aguas, Santiago. Available at: http://dgasatel.moptt.cl/index.asp (accessed on 5 January 2008)

▶ Donn TE (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 JE, Jaramillo E, Hubbard DM, Contreras H, Duarte C (2004) Competitive interactions in macrofaunal animals of exposed sandy beaches. Oecologia 139:630–640

Filun LO (2008) Gametogenic cycle of *Mesodesma donacium* in Mehuin Beach, Valdivia, Chile—July 1989 to June 1991. Pangaea doi:10.1594/PANGAEA.695910

▶ Fiori S, Defeo O (2006) Biogeographic patterns in life-history traits of the yellow clam, *Mesodesma mactroides*, in sandy beaches of South America. J Coast Res 22:872–880

▶ Gallardo AH, Marui A (2006) Submarine groundwater discharge: an outlook of recent advances and current knowledge. Geo-Mar Lett 26:102–113

Giese AC, Pearse JS (1974) Introduction. In: Giese AC, Pearse JS (eds) Reproduction of marine invertebrates. Academic Press, New York, p 1–40

Herm D (1969) Marines Pliozän und Pleistozän in Nord- und Mittel-Chile unter besonderer Berücksichtigung der Entwicklung der Mollusken-Faunen. Zitteliana 2:1–159

Hermann M, Alfaya JEF, Lepore M, Penchaszadeh PE, Laudien J (2009) Reproductive cycle and gonad development of the northern Argentinean *Mesodesma mactroides* (Bivalvia: Mesodesmatidae). Helgol Mar Res doi:10.1007/s10152-009-0150-2

▶ Houston J (2006) Variability of precipitation in the Atacama desert: its causes and hydrological impact. Int J Climatol 26:2181–2198

▶ Ibaraki M (1997) Closing of the Central American seaway and Neogene coastal upwelling along the Pacific coast of America. Tectonophysics 281:99–104

IMARPE (Instituto del Mar del Perú) (2008) Informes semanales de temperatura. IMARPE, Lima Available at: www.imarpe.gob.pe/ambiente.php (accessed on 3 April 2008)

INRENA (Instituto Nacional de Recursos Naturales) (1994) Descargas de los ríos y almacenamiento de reservorios y represas de la costa peruana. Technical report, INRENA, Peru

Jaramillo E, Pino M, Filun L, Gonzalez M (1994) Longshore distribution of *Mesodesma donacium* (Bivalvia: Mesodesmatidae) on a sandy beach of the south of Chile. Veliger 37:192–200

Jerez G, Ariz L, Baros V, Olguin A and others (1999) Estudio biológico pesquero del recurso macha en la I y III regions. Technical Report FIP 97-93, Instituto de Fomento Pesquero Valparioso

Jerez G, Aris L, Brown D, Roa R, Miranda H (2008) Gametogenic cycle of *Mesodesma donacium* in Longotoma and Peñuelas, Chile, June 1994 to July 1995. Pangaea

▶ Johannes RE (1980) The ecological significance of the submarine discharge of groundwater. Mar Ecol Prog Ser 3:365–373

▶ Legates DR, Willmott CJ (1990) Mean seasonal and spatial variability in gauge-corrected, global precipitation. Int J Climatol 10:111–127

▶ Lima M, Brazeiro A, Defeo O (2000) Population dynamics of the yellow clam *Mesodesma mactroides*: recruitment variability, density-dependence and stochastic processes. Mar Ecol Prog Ser 207:97–108

▶ Mann R, Campos BM, Luckenback MW (1991) Swimming rate and responses of larvae of three mactrid bivalves to salinity discontinuities. Mar Ecol Prog Ser 68:257–269

▶ Marins LF, Levy JA (1999) High genetic distance between marine bivalves of the genus *Mesodesma* inhabiting the Atlantic and Pacific coasts of South America. Comp Biochem Physiol A 124:313–319

▶ McLachlan A, McGwynne L (1986) Do sandy beaches accumulate nitrogen? Mar Ecol Prog Ser 34:191–195

McLachlan A, Dugan J, Defeo O, Ansell AD, Hubbard DM, Jaramillo E, Penchaszadeh PE (1996) Beach clam fisheries. Oceanogr Mar Biol 34:163–232

▶ McRoberts CA, Newton CR (1995) Selective extinction among end-Triassic European bivalves. Geology 23:102–104

Menzies RJ (1962) The zoogeography, ecology and systematics of the Chilean marine isopods. Lunds Universitets Arsskrift NF 57:1–162

▶ Morsan EM, Kroeck MA (2005) Reproductive cycle of purple clam, *Amiantis purpurata* (Bivalvia: Veneridae) in northern Patagonia (Argentina). J Mar Biol Assoc UK 85:367–373

Ortiz MA, Stotz WB (1996) Distribución de juveniles recientemente asentados de *Mesodesma donacium* (Lamarck, 1818) (Mollusca: Bivalvia: Mesodesmatidae) en tres bahías de la Cuarta Región: variables físicas y químicas que le carcterizan. Biol Pesq (Chile) 25:27–40

Ortlieb L, Escribano R, Follegati R, Zuniga O and others (2000) Recording of ocean-climate changes during the last 2000 years in a hypoxic marine environment off northern Chile (23° S). Rev Chil Hist Nat 73:221–242

Pearse JS, Lockhart S (2004) Reproduction in cold water: paradigm changes in the 20th century and a role for cidaroid sea urchins. Deep-Sea Res II 51:1533–1549

Quilter J, Stocker T (1983) Subsistence economies and the origins of Andean complex societies. Am Anthropol 85: 545–562

Quiroz M, Barriga E (1998) Evaluación del recurso 'macha' (*Mesodesma donacium*) en el litoral de Moquegua y Tacna, Marzo de 1998. Inf Prog Inst Mar Peru 86:1–11

Riascos JM (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 JM, Guzmán N, Laudien J, Heilmayer O, Oliva M (2007) Suitability of three stains to mark shells of *Concholepas concholepas* (Gastropoda) and *Mesodesma donacium* (Bivalvia). J Shellfish Res 26:43–49

Riascos JM, Heilmayer O, Oliva M, Laudien J, Arntz WE (2008a) Infestation of the surf clam *Mesodesma donacium* by the spionid polychaete *Polydora bioccipitalis*. J Sea Res 59:217–227

Riascos JM, Heilmayer O, Laudien J (2008b) Population dynamics of the tropical bivalve *Cardita affinis* from Málaga Bay, Colombian Pacific related to La Niña 1999–2000. Helgol Mar Res 62:63–71

Riascos JM, Laudien J, Heilmayer O (2008c) Gametogenic cycle of *Mesodesma donacium* in Hornitos (II Región) Chile between May 2005 and April 2007. Pangaea doi:10.1594/PANGAEA.695933

Rivadeneira MM, Marquet PA (2007) Selective extinction of late Neogene bivalves on the temperate Pacific coast of South America. Paleobiology 33:455–468

Rojas R, Howard G, Bartram J (1995) Groundwater quality and water supply in Lima, Peru. In: Nash H, McCall GJH (eds) Groundwater quality. Chapman & Hall, London, p 159–167

Rubilar P, Ariz L, Ojeda V, Lozada E and others (2008) Gametogenic cycle of *Mesodesma donacium* in Cucao and Quilanlar, Chile, September 2000 to August 2001. Pangaea doi:10.1594/PANGAEA.695934

Salgado I, Ishiyama V (2008) Gametogenic cycle of *Mesodesma donacium* in Camaná, Arequipa, Perú, March 1973 to February 1974. Pangaea doi:10.1594/PANGAEA.695907

Sandweiss DH (1996) Environmental change and its consequences for human society on the Central Andean coast: a malacological perspective. In: Reitz EJ, Newsom LA, Scudder SJ (eds) Case studies in environmental archaeology. Plenum Press, New York, p 127–147

Santelices B (1980) Phytogeographic characterization of the

temperate coast of Pacific South America. Phycologia 19: 1–12

Schoeman DS, Richardson AJ (2002) Investigating biotic and abiotic factors affecting recruitment of an intertidal clam on an exposed sandy beach using a generalized additive model. J Exp Mar Biol Ecol 276:67–81

Shanks A, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. Mar Ecol Prog Ser 302:1–12

Squeo FA, Aravena R, Aguirre E, Pollastri A, Jorquera CB, Ehleringer JR (2006) Groundwater dynamics in a coastal aquifer in north-central Chile: implications for groundwater recharge in an arid ecosystem. J Arid Environ 67: 240–254

Tarifeño E (1980) Studies on the biology of the surf-clam *Mesodesma donacium* (Lamarck, 1818) (Bivalvia: Mesodesmatidae) from Chilean sandy beaches. PhD thesis, University of California, Los Angeles, CA

Tarifeño E (2008) Gametogenic cycle of *Mesodesma donacium* in La Laguna beach—V Región, Chile, August 1969 to November 1970. Pangaea doi:10.1594/PANGAEA.695935

Thatje S, Heilmayer O, Laudien J (2008) Climate variability and El Niño Southern Oscillation: implications for natural coastal resources and management. Helgol Mar Res 62(Suppl 1):5–14

Thiel M, Macaya EC, Arntz WE, Bastias H and others (2007) The Humboldt Current System of northern and central Chile. Oceanogr Mar Biol 45:195–345

Timmermann A, Oberhuber J, Bacher A, Esch M, Latif M, Roeckner E (1999) Increased El Niño frequency in a climate model forced by future greenhouse warming. Nature 398:694–697

Tsuchi R (1997) Marine climatic responses to Neogene tectonics of the Pacific Ocean seaways. Tectonophysics 281: 113–124

Urban HJ (1994) Upper temperature tolerance of ten bivalve species off Peru and Chile related to El Niño. Mar Ecol Prog Ser 107:139–145

von Ihering H (1907) Les mollusques fossils du tertiaire et du crétace supérieur de l'Argentine. An Mus Nac B Aires Ser III 7:1–611

Vörösmarty CJ, Routhier M, Wright A, Baker T, Fernandez-Jauregui CA, Donoso MC (1998) A regional hydrometeorological data network for South America, Central America, and the Caribbean (R-HydroNET V1.0). Institute for the Study of Earth, Oceans, and Space, University of New Hampshire, Durham, NH Available at: www.r-hydronet.sr. unh.edu/ (accessed on 20 February 2009)

Waylen PR, Caviedes CN (1990) Annual and seasonal streamflow fluctuations of precipitation and streamflow in the Aconcagua River basin, Chile. J Hydrol (Amst) 120: 79–102

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Englewood Cliffs, NJ

4 Discussion of Results and Conclusions

In this chapter the results of the thesis are summarized and discussed comprehensively. Further more detailed discussion is provided in the attached publications.

D

4.1 Taxonomic revision of *D. marincovichi* and *D. obesulus*

The shell morphology (W:H and H:L ratios) of *D. marincovichi* and *D. obesulus* exhibit significant differences between ten sampling sites (Publication I, Tab. 4). Nevertheless, the morphometric characteristics specified by Coan (1983) (Publication I, Fig. 2 and Tab. 2) frequently lead to contradictions concerning the identification of both species. When pooled, genetic results indicated that the divergences observed for both morphotypes (0-1.2%) are within typical intraspecific levels (Held 2000; Hebert *et al.* 2003; Cárdenas *et al.* 2009) (Publication I, Fig. 5). The lack of significant genetic differentiation between populations suggests an extensive and ongoing gene flow between geographically separated populations and between nominal *D. obesulus* and *D. marincovichi*.

Additionally, the examination of sperm morphology could discern no significant differences in morphology between the main components (acrosome, nucleus, midpiece and flagellum) of either species (Publication I). Comparison with sperm of previously described species of Donacidae showed differences in shape and size from those described here (Publication I, Fig. 4, Tab. 5) (van der Horst *et al.* 1986; Hodgson *et al.* 1990; Sousa & Oliveira 1994; Healy 1995; Matos *et al.* 1995, *D. hanleyanus*, M. Herrmann, unpublished). Thus, neither mitochondrial genetic differentiation nor differences in sperm ultrastructure were able to confirm the morphotypes defined by Coan (1983) as distinct species.

Maximal linear distance between populations within the study area is approximately 1900 km. Meroplanktonic larval development of *Donax* species takes about three to four weeks, but may also be delayed under unfavourable

conditions (Chanley 1969b). Considering the time of larval development and the speed of the Peruvian Coastal Current (Publication I, Fig. 1), approximately 0.54 km h^{-1}, links between the examined sub-populations may be achieved by stepping-stone larval exchange within a modest number of generations (Publication I). Similar long-distance interpopulational genetic connectivity has been recorded for different *Donax* species (Adamkewicz 1989; Murray-Jones & Ayre 1997; Laudien *et al.* 2003). Results of this study concur with numerous publications demonstrating the variability of shell shape in Donacidae and highlighting its unreliability as a taxonomic feature (Tab. 1).

Tab. 1: *Donax* species exhibit high uncertainty concerning taxonomic identification due to phenotypic plasticity. Where distribution range of species is not provided by the author, alternative literature was used (if available) to define distribution of species.

Species	Distribution (Author)	Author
D. striatus	21°09'N; 4°56'S	Wade (1967a)
D. denticulatus	10°38'N; 64°16'W (Vélez *et al.* 1985)	Wade (1967b)
D. variabilis	33°41'S; 78°53'W (Wilson 1999)	Adamkewicz (1989)
D. parvulus	Atlantic coast of Florida	Adamkewicz & Hara-
D. texasianus	North America to Caribbean	seewych (1994)
D. serra	22°7'S; 14°17'E	Donn (1990)
D. peruvianus	23°28'S; 0°27'S (Coan 1983)	Guzmán *et al.* (1998)
D. serra	24°38'S; 14°43'E	Laudien *et al.* (2003)
D. obesulus	3°30'S; 18°27'S	Publication I

In suggesting that variation in shell morphology may be due to phenotypic plasticity rather than an expression of genetic differentiation between populations. McLachlan *et al.* (1995) investigated the influence of beach morphodynamics on the shell shape of 12 bivalve species from four continents. The results indicated that reflective beaches (Dean's parameter 0-2; Defeo & McLachlan 2005) favor

smaller, uniform and more wedge-shaped shells, while dissipative beaches (Dean's parameter 5-10) favor larger valves with a broader spectrum of shapes. The 12 observed species fell into two groups: those with smaller shells (<40 mm) from reflective beaches and larger shelled clams (>60 mm) from more dissipative beaches. The shells of the current study, defined as both *D. marincovichi* and *D. obesulus* all fell squarely into the group of wedge-shaped shells typical of the Donacidae (Fig. 22).
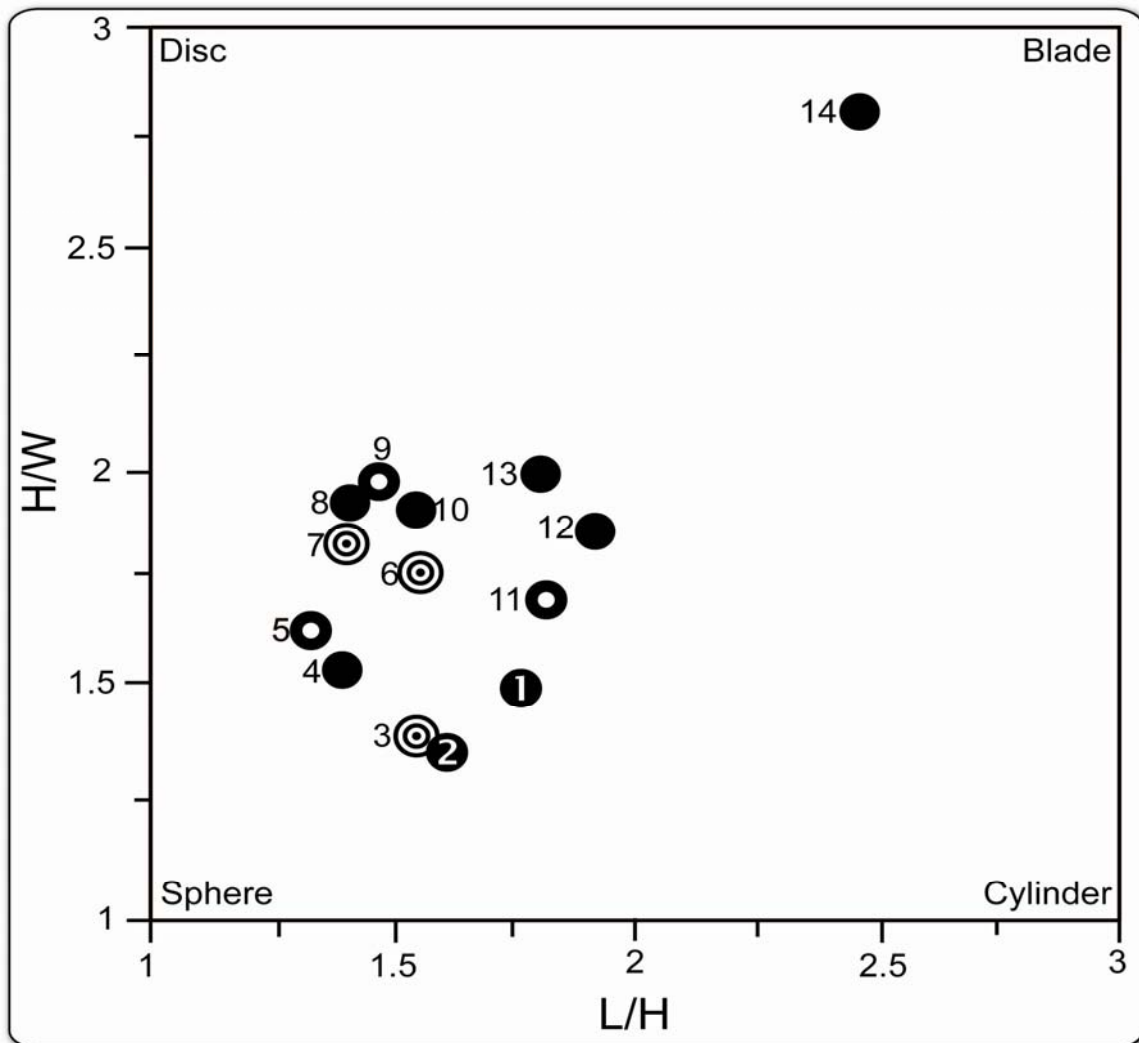

Fig. 22: Shell shapes of 14 bivalves based on length:height (L:H) and height:width (H:W) ratios: (1) *Donax marincovichi*, (2) *Donax obesulus*, (3) *Donax hanleyanus*, (4) *Tivela stultorum*, (5) *Atactodea striata*, (6) *Donax serra*, (7) *Donax sordidus*, (8) *Paphies donacina*, (9) *Donax faba*, (10) *Paphies subtriangulata*, (11) *Donacilla angusta*, (12) *Mesodesma donacium*, (13) *Mesodesma mactroides*, (14) *Siliqua patula*. Solid circles denote bivalves from dissipative beaches, open circles, species establishing adult populations on reflective beaches and 'target' symbols are species whose adult populations settle on intermediate beach types. Figure are modified after McLachlan *et al.* (1995).

Nevertheless, the *D. marincovichi* morphotype, with its flatter and less wedge-shaped form may be better suited to intermediate beaches (Publication I, Fig. 3), whereas the shorter and more wedge-shaped *D. obesulus* morphotype from northern Chile and Peru may be more adapted to reflective beaches (Publication I, Fig. 3) (McLachlan *et al.* 1995). However, this conclusion remains hypothetical as beach profiles of the respective locations were not recorded during sampling.

Based on the results presented here, *D. marincovichi* and *D. obesulus* are better characterized as different ecomorphs (*sensu* Nelson *et al.* 1993) of a single species *D. obesulus* Reeve, 1854. This means revising the total number of eastern Pacific Donacidae for Ecuador, Peru and Chile (Fig. 23). These totals show a pronounced decline from (sub-) tropical to temperate latitude (Fig. 23).
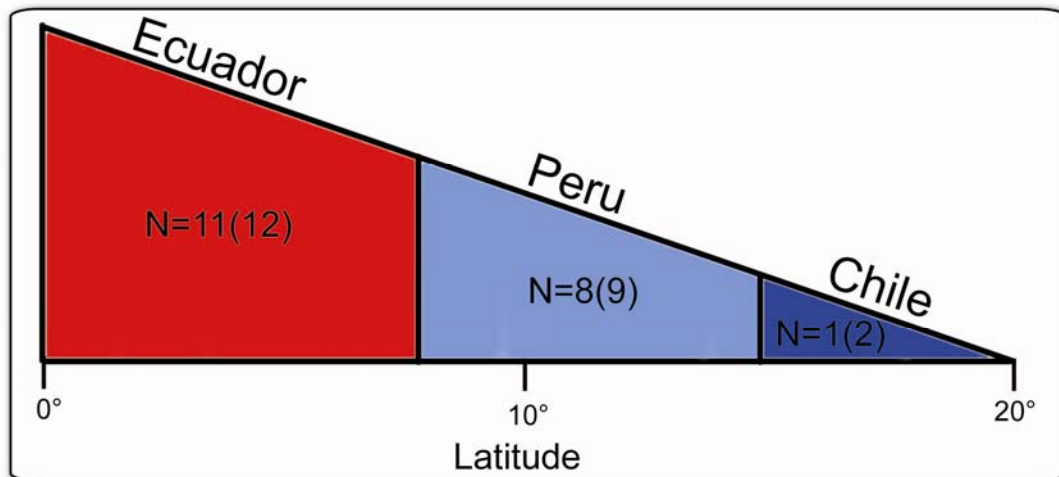
Fig. 23: Total number (N) of Donacidae for Ecuador, Peru and Chile after taxonomic revision of *D. marincovichi* and *D. obesulus* (Publication I), totals in brackets after Coan (1983). Number of species decreases strongly from (sub-) tropical (Ecuador) to temperate (Chile) latitudes.

Key Points

● The family Donacidae shows a large variability with respect to shell morphology, resulting in taxonomic ambiguity.

● W:H and H:L ratios of *D. marincovichi* and *D. obesulus* show significant differences, whereas identification based on several other shell characteristic features (Coan 1983) are highly uncertain.

● The CO1 gene analysis and sperm morphology do not support the differentiation

between species described by Coan (1983).

● The observed phenotypic plasticity (shape, dimensions, sculpture and color) may be adaptive to highly dynamic environment the species are living in, thus identification based exclusively on shell morphology is highly questionable.

● *D. marincovichi, D. peruvianus* and further synonyms describing the same species should be renamed as *D. obesulus* Reeve, 1854.

● The total number of *Donax* species for Eucador, Peru and Chile should be revised downwards from estimates based on the taxonomy of Coan (1983).

D

4.2 *D. obesulus*: *in vitro*-experiments on larvae

4.2.1 *D. obesulus*: early larval development under normal and El Niño
temperatures

Despite its importance in understanding the biology and population dynamics of bivalve species, larval biology of this group has not received much attention (Chicharo & Chicharo 2000a; b). The lack of knowledge is partly be explained by the effort involved in obtaining larvae. The distinct responses of different species to artificial spawning and the effort required to maintain systems for *in vitro*-experiments make the rearing of larvae under experimental conditions no easy task (Helm *et al.* 2004).

Artificial spawning of *D. obesulus* was induced in order to obtain larvae for *in vitro*-experiments and to describe the larval cycle. At a maximum adult length of ~35 mm, *D. obesulus* is a relatively small bivalve, and so a size independent method of induction, temperature shock method (+5°C), was applied (Publication II).

The method resulted in an excellent yield in quantity and quality of larvae. For the first time embryonic and early larval development of *D. obesulus* could be described (Publication II, Fig. 1) and temperature and salinity experiments were performed *in vitro* (Publication II). The larval cycle was found to follow a pattern

common to several previously described marine bivalve species (Chanley 1969b; Frenkiel & Moueza 1979).

Larval development of *D. obesulus* was observed over a period of 16 days under NTC and ENTC. Under ENTC larvae exhibited higher growth rates, faster development and a slightly higher mortality, although overall mortality was very low (<2%). Temperature has been considered as one of the main abiotic factors influencing larval growth (Tettelbach & Rhodes 1981; Devakie & Ali 2000). It is well documented that larval stages of different species have different optimal temperature conditions for maximal growth and that beyond optimal thresholds growth is lowered and mortality increased (Tettelbach & Rhodes 1981; His *et al.* 1989; Baba *et al.* 1999) (Fig. 24).
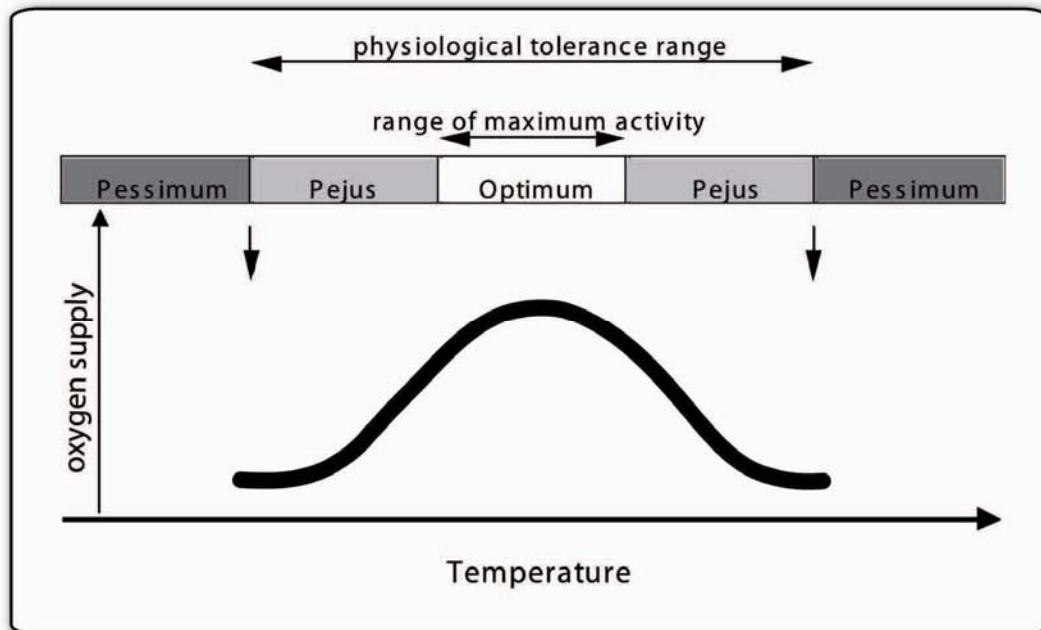
Fig. 24: Physiological tolerance in organisms according to Shefford´s law. Critical (sublethal) pejus temperatures (arrows) are reached when the organism loses its ability to maintain oxygen levels in tissues (stress thresholds).

The increased growth rates and faster development of *D. obesulus* larvae observed under ENTC may be explained by that the tendency of rising temperatures to intensify metabolic processes as long as the critical upper temperature threshold of the particular species is not exceeded (Heilmayer *et al.* 2008). Results of the *in vitro*-experiment indicated that ENTC remains within critical

temperature limit for larvae of *D. obesulus*, which are thus able to cope and divert surplus energy into growth.

Key Points

● Despite the importance of larval stages in species ecology understanding of their biology is limited, perhaps due to the challenges of artificial spawning and maintenance of larvae.

● Embryonic and early larval development of *D. obesulus* was described for the first time.

● Larvae showed faster development and increased growth rate under ENTC compared to NTC.

D

4.2.2 *D. obesulus*: early larvae exposed to El Niño temperature and lower salinity

Salinity is an important additional parameter influencing growth and mortality of larvae and thus the reproductive success of species (Davis & Calabrese 1964; Robert *et al.* 1988; His *et al.* 1989; Devakie & Ali 2000; Yaroslavtseva & Sergeeva 2006). As anomalous temperatures and reduced salinity are supposed to increase their effects when arising in concert, a 48 hour *in vitro*-experiment considering both parameters was designed and implemented (Publication II): *D. obesulus* larvae reared at NTC and ENTC were exposed to normal, medium, low and very low salinities (35, 25, 15 and 5 ±1) in order to evaluate the combined effect of the two parameters (Publication II). The results indicated that larvae reared under both NTC and ENTC were highly tolerant of medium and low salinity (25 and 15 ±1). However, very low salinity (5 ±1) resulted in 100% mortality within a short time period (16 to 32 h depending on temperature). Thus, there appears to be a lethal salinity threshold for *D. obesulus* larvae between 15 to 5 ±1 under NTC and ENTC. Goodbody (1961) previously documented a rapid increase in mortality of marine fauna when salinity tolerance limits were surpassed.

Highest larval activity levels were observed under medium salinity (25 ±1), which might be interpreted as a reaction to increase the chances of escaping unfavorable conditions. At low salinity (15 ±1), activity was reduced as larvae begin to be handicapped by the conditions (Publication II, Fig. 5). This is in agreement with the reduced activity reported in tropical oyster (*Crassostrea iredalei*) larvae when salinity dropped below 15 ±1 (Devakie & Ali 2000). Along the HCS, strong salinity changes are to be expected close to river mouths during and shortly after heavy rainfall. Highest rainfall intensities are reported during EN (Waylen & Caviedes 1990; Romero *et al.* 2007). Data relating to SSS changes during EN off northern Chile and Peru are scarce (Riascos *et al.* 2009), however, in the tropics strong salinity changes occur annually and are seen to be intensified during EN (Goodbody 1961; Wade 1968; Riascos 2002; 2006). In Jamaica, Goodbody (1961) described massive salinity drops (down to 5) close to river mouths during three rainy seasons, causing mass mortality events in the neighboring benthic community. In the same location, high mortalities were documented for adult *D. denticulatus* at salinities below 10, along with a strong reduction in the abundance of larvae and spat (Wade 1968).

Frequent significant reductions in salinity close to river mouths are to be expected off northern Chile as a result of heavy precipitation during EN (Waylen & Caviedes 1990; Houston 2006). Populations of donacid clams are often located in such areas (Donn 1987), as in the case of the population at Chinchorro beach in Arica, which is located just a few hundred metres from a river outlet.

Key Points

● Larvae of *D. obesulus* reared under NTC and ENTC are able to withstand medium and lower salinities (25 and 15 ±1), but exhibit 100% mortality after a short time at very low salinity (5 ±1) at both temperatures.
● Heavy rainfall during EN has the potential to cause high mortalities among larvae close to river outlets as SSS is significantly reduced.

4.3 *D. obesulus* and *M. donacium*: *in vitro*-experiments on juveniles and adults

4.3.1 *D. obesulus* and *M. donacium*: El Niño and La Niña temperature tolerance

Temperature has been considered the most significant abiotic factor controlling growth, reproduction, nutrition and other physiological processes in marine bivalves, (Laudien *et al.* 2001; Heilmayer *et al.* 2004; Miyaji *et al.* 2007; Riascos *et al.* 2009). Bivalves are poikilothermic organisms, whose temperature is directly linked to that of their surroundings. SST in the coastal HCS shows little seasonal variability and conditions are similar over a broad range of latitudes. As a result, the region's marine species are closely adapted to fairly constant thermal conditions and are sensitive to anomalous temperature changes (Urban 1994; Camus 2001; Riascos *et al.* 2009).

Adult *D. obesulus* showed reduced tolerance to LNTC compared to NTC but a relatively high tolerance to ENTC. The growth rate was highest under NTC, followed by ENTC. After three weeks, mortality was 100% under LNTC but remained below 15% under NTC and ENTC (Publication III, Figs. 2 and 4). Before and during EN 1982-1983 Arntz *et al.* (1987) followed recently-recruited cohorts of *D. obesulus* off Santa María del Mar, south of Lima (~12°S). The clams showed a significantly reduced growth rate when SST peaked in February and March 1983, but were nonetheless able to survive this strong warming event (Arntz & Fahrbach 1991). Indeed, a southward extension of the geographic distribution of *D. obesulus* has been reported following EN warming, enabling this bivalve to reach temporarily as far south as ~23°S (Tomicic 1985; Carbajal *et al.* 1995), about 800 km south of its current southern distribution limit.

Unlike their larvae, adult *D. obesulus* show a higher growth rate at NTC than at ENTC (Publication II and III, Fig. 2). Adults also exhibited higher mortality under ENTC than larvae (Publication II, Fig. 4 and Publication III, results) (Tab. 2). The ability of meroplanktonic larvae to deal with extreme changes in temperature better than adults may be explained by their biological function in dispersal. As a highly

mobile life stage lasting for several weeks, or months under suboptimal conditions, larvae are able to expand or maintain the biogeographic distribution of a species or population as directed by the prevailing currents. Shifts in temperature and salinity can therefore induce strong latitudinal and vertical changes within the water column. Adult surf clams, on the other hand, are semi-sessile, and the fact that they remain in shallow waters implies little change concerning these vital parameters. Therefore, the high tolerance of larvae to a changing environment has to be considered a crucial factor in species survival and ability to colonize new areas.

Key Points

● *D. obesulus* exhibits low tolerance to LNTC and higher tolerance to ENTC.

● Adult specimens reveal lower tolerance to ENTC than their larvae.

● The different tolerances to temperature and salinity observed between adults and larvae may reflect the dissimilar environmental conditions routinely encountered by each life stage.

M

4.3.2 *M. donacium*: El Niño temperature and low salinity

Juvenile and adult *M. donacium* showed variations in growth rate between different temperatures (Publication III, Fig. 2 and Publication IV, Fig. 3). Juveniles revealed no differences in growth rate when salinities were modified; whereas adults exhibited growth rates (ENTC) at salinities between 35 and 10 (Publication IV, Fig. 3 and Tab. 3). LNTC had no appreciable effect on mortality, whereas under ENTC mortality increased to 67% by the end of the experiment (Publication III, Fig. 4).

In the period before and during EN 1982-1983, Arntz *et al.* (1987) documented mass mortalities for *M. donacium* during peaks of SST (Publication III, Fig. 5). Interestingly, *M. donacium* were observed to opened their shells a few days before they died, behavior that was also observed under laboratory conditions. The

results (Publication III and IV) clearly indicate opposing sensitivities in the two experimental species: while *D. obesulus* shows higher tolerance to ENTC, *M. donacium* fares better under LNTC (Publication III and IV) (Tab. 2).

Tab. 2: Tolerance of *Donax obesulus* (larvae and adults) and *Mesodesma donacium* (juveniles and adults) to El Niño temperature conditions (ENTC), La Niña temperature conditions (LNTC) and reduced salinity. Mortality rate at certain salinities is given for whole experiment duration, for details see respective publications. Abbreviations: (▲) high tolerance, (▼) low tolerance, (-) tolerance not verified and (PI, PII, PIII and PIV) publications I to IV. (*) indicates higher mortality in adults at ENTC than in larvae (Publication II).

Species-stage	ENTC	LNTC	Salinity (Mortality %)
Donax-larvae (PII)	▲	-	15 (0) and 5 (100)
Donax-adult (PIII)	▲(*)	▼	-
Mesodesma-adult (PIII and IV)	▼	▲	20 (20) and 10 (>50)
Mesodesma-juvenile (PIV)	▼	-	-

Local extinction of *M. donacium* and survivorship of *D. obesulus* after strong EN events has been observed repeatedly off Peru and northern Chile (Tomicic 1985; Arntz *et al.* 1987; Carbajal *et al.* 1995; Aburto & Stotz 2003; Quiroz. *et al.* 2006; Thiel *et al.* 2007; Riascos *et al.* 2009). Indeed, a southward extension of the geographic distribution of *D. obesulus* has been reported following EN warming, enabling this species to temporarily reach ~23°S (Tomicic 1985; Carbajal *et al.* 1995), about 800 km south of its current southern distribution limit.

Key Points
● *D. obesulus* exhibits higher tolerance to ENTC and lower tolerance to LNTC, whereas *M. donacium* shows a higher tolerance to LNTC and lower tolerance to ENTC.
● Juvenile *M. donacium* reveal a higher tolerance to lower salinity than adults.

DM

4.4 Response of *D. obesulus* and *M. donacium* to ENSO

The results presented here concern responses to extreme values of just two factors (temperature and salinity) and they are spatially limited to one extreme of the current species distribution. Even so, they offer insights into the mechanisms of observed changes related to EN and LN events. In reality the SST and SSS anomalies occurring during such events may be far less extreme, but responses may be affected by additional factors, which may reduce or even increase the potential effects, depending on the physical characteristics of the locality. Nonetheless, this thesis provides insights into the responses of *D. obesulus* (larvae and adults) and *M. donacium* (juvenile and adults) to EN and LN, which have been only marginally addressed in previous studies.

One striking aspect of the results is that while *D. obesulus* and *M. donacium* show opposite responses to ENTC, they both appear sensitive to LNTC, showing slower growth in comparison to NTC. This is probably the reason why (i) *D. obesulus* has not been able to establish permanent viable populations within areas colonized during EN (Tomicic 1985), and (ii) remnants of *M. donacium* populations have not been able to rapidly recolonize northern beaches during LN (Arntz *et al.* 1987; 2006) as would be expected under a completely contrasting response pattern.

In general, the present results support the hypothesis that temperature is an important factor causing regime shifts during EN, and its effects can be enhanced by reductions in salinity. Despite the fact that *D. obesulus* and *M. donacium* are sympatrically distributed along large sections of the coastal HCS, their biogeography reflects differing environmental affinities, which in turn underlie their contrasting responses to the warm and cold episodes of ENSO. The family Donacidae primarily inhabits warm areas with more than 75% of living species restricted to the tropics (Ansell 1983). The greatest species richness of Donacidae is recorded in the Eastern Pacific, with 11 species listed for the Ecuadorian coast, which also seems to be the origin of *D. obesulus* (Olsson 1961). However, as with

other families of coastal bivalves, the diversity of Donacidae decreases drastically to the south (Fig. 23) (Carranza *et al.* 2009). A similar distribution can be observed for gastropods occurring along the HCS, reflecting the tropical and temperate origins of the various species (Publication III, Fig. 1 and Tab. 3). The southward decrease in diversity is such that *D. obesulus* is the only species able to thrive on the coast of northern Chile (Publication I), and it maybe a reflection of the cooling effect of the HCS at this latitude. *D. obesulus* most likely represents a species of tropical affinity adapted to cooler waters, able to survive during strong EN and to expand temporarily southwards, but hampered in its ability to maintain viable populations in those areas under LN conditions. In contrast, recent species of the family Mesodesmatidae generally inhabit cold and temperate waters (Marins & Levy 1999; Beu 2006). Presumably, *Mesodesma* arrived in South America in the middle or late Pliocene as part of a massive northward migration of Antarctic molluscan species and was able to migrate further northwards following the establishment of the HCS (von Ihering 1907; Tarifeño 1980; Marins & Levy 1999).

The distribution and population stability of *M. donacium* seems closely linked to the interannual thermal variability associated to the warm and cold episodes of ENSO in the HCS (Publication IV, Fig. 5). The disparate origins of the two species are reflected in their actual distribution centers, which are, approximately, central northern Peru for *D. obesulus* and central Chile for *M. donacium* (Aguirre & Mendo 2008; Riascos 2008 and personal observations). Although the results support the hypothesis of a strong link between temperature tolerances, anomalous temperature and distribution shifts in *D. obesulus* and *M. donacium*, those shifts are unlikely to be solely explained in terms of this relationship. Several other biotic and abiotic processes must to be taken into account in order to gain a proper understanding of the biological responses of these species to ENSO (Publication IV). A whole array of abiotic factors may be modified during ENSO, notably salinity, wave action, sediment load, upwelling strength and hence nutrient and food availability, any number of which may act simultaneously to produce an integrated effect on local species (e.g. Arntz *et al.* 2006). It is difficult to address the full range of possible factors in any one series of

experimental studies. Interspecific interactions may play an important role in defining population processes and community structure in sandy beach ecosystems (see review by Defeo & McLachlan 2005). Such interactions may be significantly modified under strong environmental stress and may modulate the responses of species to the extremes of ENSO. *D. obesulus* and *M. donacium* occupy much the same niche and play similar ecological roles (Tarazona *et al.* 1986; Beu 2006) therefore, strong competitive interactions are to be expected, as found between *D. hanleyanus* and *M. mactroides* on the Atlantic coast of South America (Defeo & de Alava 1995; Herrmann *et al.* 2009a). Competition infers the existence of a limiting resource. Within the HCS, limiting factor is unlikely to be nutrition, at least not in the case of EN 1982-1983, when population abundances were comparatively low due to mass mortalities. Interspecific interactions may be implicated in the low ability of *M. donacium* to recover its former abundance and distribution and in the inability of *D. obesulus* to thrive long term on southern beaches after EN.

Despite the present results, it is not clear if and how competition occurs between these clams. Even after the mass mortality of *M. donacium* in Peru during EN 1982-1983, *D. obesulus* and the mole crab *Emerita analoga* were not able to achieve comparable densities to that of the former of *M. donacium* population; instead, an impoverished community comprising mainly small opportunistic polychaetes became established, leading Arntz *et al.* (1987) to suggest that no competition existed between these commonly dominant species. Doubtless further parameters will need to be included in future studies in order to clarify the steering pattern of *D. obesulus* and *M. donacium* populations. The beach type, as well as ENSO-driven changes in parasite load of dominant sandy beach species may also affect population dynamics and species distribution in the HCS (Oliva *et al.* 2007; Riascos *et al.* 2008).

4.5 Future Perspectives

● Despite the useful results attained in this study, a lack of knowledge persists concerning the recruitment processes of *D. obesulus*. This contribution should stimulate further studies on the population dynamics of adult surf clams but also focus interest on the response of early life stages to different abiotic and biotic conditions. In particular the early embryonic stages (<48 h) of bivalve species are sensitive to changing abiotic parameters (e.g. temperature and salinity). Further *in vitro*-experiments could clarify the consequences of strong EN and LN for the early embryonic stages.

● To improve the understanding of species reproduction a description of the complete larval cycle of *D. obesulus* (including metamorphosis) is necessary. Larval requirements for a successful metamorphosis should be evaluated.

● There is a lack of knowledge concerning the capability of *D. obesulus* and *M. donacium* larvae to suppress metamorphosis under unfavorable conditions. Such mechanisms will be crucial during recolonization processes, and are thus worthy of greater emphasis.

● To test the relevance of different beach types on the phenotypic plasticity of species, larvae of the same gene pool should be exposed to dissipative and reflective beach conditions. Thereafter the shell morphology of the grown specimens should be analyzed.

● Concerning genetic analysis of *D. marincovichi* and *D. obesulus*, future research should include examination of faster-evolving nuclear markers (e.g. AFLP, microsatellites Held & Leese 2007) that could corroborate the current findings based on mitochondrial sequences, and resolve more recent evolutionary events and at a finer geographic resolution than has been possible in this thesis.

● Even if the tropical origin of *D. obesulus* is confirmed, knowledge of the genesis of biogeographic patterns is very poor. It is not known whether this species emigrated to the HCS from Pacific or from Atlantic tropical regions. Further palaeogenetic studies should be implemented to track the roots of the species.

● As both study species are fast-growing, more emphasis ought to be given to exploring possibilities for aquaculture cultivation. Although *D. obesulus* is a small species, the gained knowledge may be applied to larger Donacidae (i.e. the African species *D. serra*), which have considerable potential in for aquaculture.

● Studies on *M. donacium* have demonstrated the importance of parasitism (Publication IV), the mechanism and effects of which are generally little known for Donacidae. No information is available at all concerning parasitism of *D. obesulus*, and further investigation should be conducted to address this.

5 Appendix

5.1 List of abbreviations

Abbreviation	Description
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CENSOR	Climate variability and El Niño-Southern Oscillation: Implications for Natural coastal Resources and Management
CO1	Cytochrome oxidase 1
EN	El Niño
ENSO	El Niño-Southern Oscillation
ENTC	El Niño temperature conditions
ESU	Evolutionary Significant Units
HCS	Humboldt Current System
H:L	Height/length
IMARPE	Instituto del Mar del Perú
LN	La Niña
LNTC	La Niña temperature conditions
LT_{50}	Lethal time until 50% of an experimental population died
OGP	Overall growth performance
PCR	Poly chain reaction
SCH	Sistema de la corriente de Humboldt
SE	Standard error
SL	Shell length (maximum anterior-posterior)
SSS	Sea surface salinity
SST	Sea surface temperature (°C)
TEM	Transmission Electron Microscopy
VBGF	Van Bertalanffy growth function
W:H	Width/height

6 Acknowledgements

Constanze von Waldthausen, Prof. Dr. Matthias Wolff and to all the people who have participated but not been written down here.

At last I would like to thank Sofía for her daily encouragement and motivation, for allowing me to participate in her enthusiastic and lovely way of life each day. Finally I would like to express my deepest gratitude to my now German-Colombian family who stood behind me all the way and pushed me forward whenever I needed it.

7 References

Aburto, J. & Stotz, W. (2003). Una experiencia de co-manejo de bivalvos en el marco de una nueva herramienta de administración pesquera en Chile: las áreas de manejo. Policy Matters 12: 200-204.

Adamkewicz, S.L. (1989). Differences in the frequencies of several characters in the clam *Donax variabilis* around the Cape Hatteras, North Carolina. The Veliger 32: 21-28.

Adamkewicz, S.L. & Haraseewych, M.G. (1994). Use of random amplified polymorphic DNA (RAPD) markers to asses relationships among beach clams of the genus *Donax*. The Nautilus 2: 51-60.

Aguirre, A. & Mendo, J. (2008). Crecimiento y producción de *Donax obesulus* Reeve, 1854 (Bivalvia: Donacidae) en playa Sarapampa, Asia, Lima. Ecol. Apl. 7 (1-2): 63-70.

Ansell, A.D. (1983). The biology of the genus *Donax*. In: McLachlan, A., Erasmus, T. & Junk, W.E. (Eds.) Developments in Hydrobiology Vol. 19: Sandy Beaches as Ecosystems. Dr. W. Junk, The Hague, Netherlands, 19: 607-635.

Arntz, W.E. (1986). The two faces of El Niño 1982-83. Arch. Fish. Mar. Res. 31: 1-46.

Arntz, W.E., Brey, T., Tarazona, J. & Robles, A. (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. & Fahrbach, E. (1991). El Niño-Klimaexperiment der Natur: Physikalische Ursachen und biologische Folgen. Birkhäuser, Basel. 264 p.

Arntz, W.E., Gallardo, V.A., Gutiérrez, D., Isla, E., Levin, L.A., Mendo, J., Neira, C., Rowe, G.T., Tarazona, J. & Wolff, M. (2006). El Niño and similar perturbation effects on the benthos of the Humboldt, California, and Benguela Current upwelling ecosystems. Adv. Geosci. 6: 243-265.

Baba, K., Tada, M., Kawajiri, T. & Kuwahara, Y. (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.

Beu, A.G. (2006). Marine Mollusca of oxygen isotope stages of the last 2 million years in New Zealand. Part 2. Biostratigraphically useful and new Pliocene to recent bivalves. J. R. Soc. N. Z. 36 (4): 151-338.

Bonsdorff, E. & Nelson, W.G. (1992). The ecology of coquina clams *Donax variabilis* Say, 1822, and *Donax parvula* Philippi, 1849, on the east coast of Florida. The Veliger 35 (4): 358-365.

Brattström, H. & Johanssen, A. (1983). Ecological and regional zoogeography of the marine benthic fauna of Chile. In Sarsia (ed.): *Report No. 49 of the Lund University Chile Expedition 1948-49:* 287-339. Bergen: University of Bergen, Department of Marine Biology, N-5065 Blomsterdalen, Norway.

Camus, P.A. (2001). Biogeografía marina de Chile continental. Rev. Chil. Hist. Nat. 74: 587-617.

Canby, T.Y. (1984). El Niño: global weather disaster. National Geographic Magazine, (165).

Carbajal, W., Carrasco, F.D., Curo, P. & Zamora, J. (1995). Cambios en la estructura comunitaria de la macroinfauna de playas arenosas, después del fenómeno El Niño 1986-1987, en Lambayeque, Perú. Gayana Oceanol. 3 (1): 29-40.

Cárdenas, L., Castilla, J.C. & Viard, F. (2009). A phylogeographical analysis across three biogeographical provinces of the south-eastern Pacific: the case of the marine gastropod *Concholepas concholepas*. J. Biogeogr. 36 (5): 969-981.

Carranza, A., Defeo, O., Castilla, J.C. & Rangel, T.F.L.V.B. (2009). Latitudinal gradients in species richness for South American Mytilidae and Ostreidae: can alternative hypotheses be evaluated by a correlative approach? Mar. Biol. 156 (9): 1917-1928.

Castilla, J.C. & Camus, P.A. (1992). The Humboldt-El Niño scenario: coastal benthic resources and anthropogenic influences, with particular references to the 1982/83 ENSO. S. Afr. J. mar. Sci. 12: 703-712.

Chanley, P. (1969a). *Donax fossor*: a summer range extension of *Donax variabilis*. The Nautilus 83 (1): 1-14.

Chanley, P. (1969b). 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.

Chicharo, L.M.Z. & Chicharo, M.A. (2000a). Estimation of the life history parameters of *Mytilus galloprovincialis* (Lamarck) larvae in a coastal lagoon (Ria Formosa - south Portugal). J. Exp. Mar. Biol. Ecol. 243 (1): 81-94.

Chicharo, L.M.Z. & Chicharo, M.A. (2000b). Short-term fluctuations in bivalve larvae compared with some environmental factors in a coastal lagoon (South Portugal). Sci. Mar. 64 (4): 413-420.

Clement, M., Posada, D. & Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657–1659.

Coan, E. (1983). The Eastern Pacific Donacidae. The Veliger 25 (4): 273-298.

Coan, E.V., Scott, P. & Bernard, F.R. (2000). Bivalve seashells of western north America: marine bivalve mollusks from arctic Alaska to Baja California. Museum of Natural History Monographs, Santa Barbara. 764 p.

Coe, W.R. (1953). Resurgent populations of littoral marine invertebrates and their depends on ocean currents and tidal currents. Ecology 34: 225-229.

Coghlan, B. & Gosling, E. (2007). Genetic structure of hybrid mussel populations in the west of Ireland: two hypotheses revisited. Mar. Biol. 150 (5): 841-852.

Dávila, P.M., Figueroa, D. & Müller, E. (2002). Freshwater input into the coastal ocean and its relation with the salinity distribution off austral Chile (35–55°S). Cont. Shelf Res. 22: 521–534.

Davis, H.C. & Calabrese, A. (1964). Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. Fishery Bulletin of the U.S. Fish and Wildlife Service 63: 643-655.

Davis, J.D. (1967). Polydora infestation of artic wedge clams: a pattern of selective attack. Proc. Nat. Shellfish Assoc. 57: 67-72.

Defeo, O. & de Alava, A. (1995). Effects of human activities on long-term trends in sandy beach populations: the wedge clam *Donax hanleyanus* in Uruguay. Mar. Ecol. Prog. Ser. 123: 73-82.

Defeo, O., de Alava, A., Valdivieso, V. & Castilla, J.C. (1993). Historical landings and management options for the genus *Mesodesma* in coasts of South America. Biología Pesquera 22: 41-54.

Defeo, O. & McLachlan, A. (2005). Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. Mar. Ecol. Prog. Ser. 295: 1-20.

Devakie, M.N. & Ali, A.B. (2000). Salinity-temperature and nutritional effects on the setting rate of larvae of the tropical oyster, *Crassostrea iredalei* (Faustino). Aquaculture 184 (1-2): 105-114.

Díaz, A. & Ortlieb, L. (1993). El fenómeno 'El Niño' y los moluscos de la costa peruana. Bull. Inst. fr. éstudes andines 22 (1): 159-177.

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 (3): 217-222.

Donn, T.E.J. (1990). Morphometrics of *Donax serra* Röding (Bivalvia: Donacidae) populations with contrasting zonation patterns. J. Coast. Res. 6 (4): 893-901.

Edgar, R. (2004). Muscle: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids. Res. 32: 1792-1797.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5): 294-299.

Frenkiel, L. & Moueza, M. (1979). Développement larvaire de deux Tellinacea, *Scrobicularia plana* (Semelidae) et *Donax vittatus* (Donacidae). Mar. Biol. 55: 187-195.

Galtsoff, P.S. & Gallardo, C.S. (1960). Ultrastructure of the spermatozoon of the oyster *Crassotrea virginica*. J. Ultrastruct. Res. 3: 241-253.

Giménez, J., Healy, J.M., Hermida, G.N., Nostro, F.L. & Penchaszádeh, P.E. (2008). Ultrastructure and potential taxonomic importance of euspermatozoa and paraspermatozoa in the volutid gastropods *Zidona dufresnei* and *Provocator mirabilis* (Caenogastropoda, Mollusca). Zoomorphology 127 (3): 161-173.

Goodbody, I. (1961). Mass mortality of a fauna following tropical rains. Ecology 42 (1): 150-155.

Guzmán, N., Saá, S. & Ortlieb, L. (1998). Catálogo descriptivo de los moluscos litorales (Gastropoda y Pelecypoda) de la zona de Antofagasta 23°S (Chile). Estud. Oceanol. 17: 17-86.

Healy, J.M. (1995). Comparative spermatozoal ultrastructure and its taxonomic and phylogenetic significance in the bivalve order Veneroida. In: Jamieson, B.G.M., Ausio, J. & Justine, J.-L. (Eds.) Advances in Spermatozoal Phylogeny and Taxonomy. Mémoires du Muséum National D' Histoire Naturelle, Paris, 166: 155-166.

Healy, J.M., Keys, J.L. & Daddow, L.Y.M. (2000). Comparative sperm ultrastructure in pteriomorphian bivalves with special reference to phylogenetic and taxonomic implications. In: Harper, E.M., Taylor, J.D. & Crame, J.A. (Eds.) The Evolutionary Biology of the Bivalvia, 177: 169-190.

Hebert, P.D.N., Ratnasingham, S. & DeWaard, J.R. (2003). Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 270 (1): 96-99.

Heilmayer, O., Brey, T. & Pörtner, H.O. (2004). Growth efficiency and temperature in scallops: a comparative analysis of species adapted to different temperatures. Funct. Ecol. 18 (5): 641-647.

Heilmayer, O., Digialleonardo, J., Qian, L. & Roesijadi, G. (2008). Stress tolerance of a subtropical Crassostrea virginica population to the combined effects of temperature and salinity. Estuar. Coast. Shelf Sci. 79 (1): 179-185.

Held, C. (2000). Phylogeny and biogeography of serolid isopods (Crustacea, Isopoda, Serolidae) and the use of ribosomal expansion segments in molecular systematics. Molecular Phylogenetics and Evolution 15 (2): 165–178.

Held, C. & Leese, F. (2007). The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol. 30: 513–521.

Held, C. & Wägele, J.W. (2005). Cryptic speciation in the giant Antarctic isopod Glyptonotus antarcticus (Isopoda: Valvifera: Chaetiliidae). Sci. Mar. 69 (2): 175-181.

Helm, M.M., Bourne, N. & Lovatelli, A. (2004). Hatchery culture of bivalves. A practical manual. FAO Fisheries Technical Paper 471: 177 p.

Herrmann, M. (2008). Population dynamics of the surf clams *Donax hanleyanus* and *Mesodesma mactroides* from open-Atlantic beaches off Argentina. Ph.D. thesis, University Bremen 236 p.

Herrmann, M., Carstensen, D., Fischer, S., Laudien, J., Penchaszádeh, P.E. & Arntz, W.E. (2009a). Population structure, growth and production of the wedge clam *Donax hanleyanus* (Bivalvia: Donacidae) from northern Argentinean beaches. J. Shellfish Res. 28 (3): 1-16.

Herrmann, M., Lepore, M.L., Laudien, J., Arntz, W.E. & Penchaszádeh, P.E. (2009b). Growth estimations of the Argentinean wedge clam *Donax hanleyanus*: a comparison between length-frequency distribution and size-increment analysis. J. Exp. Mar. Biol. Ecol. 379: 8-15.

His, E., Robert, R. & Dinet, A. (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 (4): 455-463.

Hodgson, A.N., Bernard, R.T.F. & van der Horst, G. (1990). Comparative spermatology of three species of *Donax* (Bivalvia) from South Africa. J. Moll. Stud. 56: 257-265.

Houston, J. (2006). The great Atacama flood of 2001 and its implications for Andean hydrology. Hydrol. Process. 20 (3): 591-610.

Huaraz, F. & Ishiyama, V. (1980). Madurez sexual de la 'concha mariposa' (*Donax peruvianus*) de la playa de Jahuay, Ica, Perú. Revista de Ciencias U.N.M.S.M. 72 (1): 47-56.

Jacquet, J., Hocevar, J., Lai, S., Majluf, P., Pelletier, N., Pitcher, T., Sala, E., Sumaila, R. & Pauly, D. (2009). Conserving wild fish in a sea of market-based efforts. Oryx: doi:10.1017/S0030605309990470.

Jaramillo, E. (1994). Patterns of species richness in sandy beaches of South America. S. Afr. J. Zool. 29 (4): 227-234.

Jaramillo, E., Contreras, H., Duarte, C. & Quijon, P. (2001). Relationships between community structure of the intertidal macroinfauna and sandy beach characteristics along the Chilean coast. Mar. Ecol. 22 (4): 323-342.

Jaramillo, E., Pino, M., Filun, L. & Gonzalez, M. (1994). Longshore distribution of *Mesodesma donacium* (Bivalvia: Mesodesmatidae) on a sandy beach of the south of Chile. The Veliger 37 (2): 192-200.

Keen, A.M. (1971). Sea shells of tropical West America. 2. Standford University Press, Standford, California. 1064 p.

Kimura, M. & Weiss, G.H. (1964). The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561-576.

Koehn, R.K., Hall, J.G., Innes, D.J. & Zera, A.J. (1984). Genetic differentiation of *Mytilus edulis* in eastern North America. Mar. Biol. 79 (2): 117-126.

Kortum, G. (2002). Germania in Pacífico: Humboldt, Chamisso and other early german contributions to Pacific research; 1741–1876. In: Benson, K.R. & Rehbock, P.F. (Eds.) Oceanographic History: the Pacific and Beyond. University of Washington Press, Seattle: 107–117.

Laudien, J., Brey, T. & Arntz, W.E. (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., Flint, N.S. & Bank, F.H.v.d. (2003). Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches. Biochem. Soc. Ecol. 31: 751-772.

Laudien, J., Rojo, M.E., Oliva, M.E., Arntz, W.E. & Thatje, S. (2007). Sublittoral soft bottom communities and diversity of Mejillones Bay in northern Chile (Humboldt Current Upwelling System). Helgol. Mar. Res. 61 (2): 103-116.

Laudien, J., Schiedek, D., Brey, T., Pörtner, H.-O. & Arntz, W.E. (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.

Levinton, J.S. & Suchanek, T.H. (1978). Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. Mar. Biol. 49 (4): 363-375.

Loesch, H.C. (1957). Studies of the ecology of two species of *Donax* on Mustang Island, Texas. Publ. Inst. Mar. Sci., Univ. Tex. 4: 201-227.

Marin, V.H., Delgado, L.E. & Luna-Jorquera, G. (2003). S-chlorophyll squirts at 30 degree S off the Chilean coast (eastern South Pacific): feature-tracking analysis. J. Geophys. Res. 108 (12): doi:10.1029/2003JC001935.

Marins, L.F. & Levy, J.A. (1999). High genetic distance between marine bivalves of the genus *Mesodesma* inhabiting the Atlantic and Pacific coasts of South America. Comp. Biochem. Physiol. 124: 313-319.

Matos, E., Matos, P., Corral, L. & Azevedo, C. (1995). Estudo ultraestrutural da espermatogenese de *Donax striatus* Linnaeus (Mollusca, Bivalvia) do litoral norte do Brasil. Rev. Bras. Zool. 12 (1): 221-227.

McLachlan, A. & Brown, A.C. (2006). The ecology of sandy shores. Academic Press. 392 p.

McLachlan, A. & Dorvlo, A. (2005). Global patterns in sandy beach macrobenthic communities. J. Coast. Res. 21 (4): 674-687.

McLachlan, A., Dugan, J.E., Defeo, O., Ansell, A.D., Hubbard, D.M., Jaramillo, E. & Penchaszádeh, P.E. (1996). Beach clam fisheries. Oceanogr. Mar. Biol. 34: 163-232.

McLachlan, A., Erasmus, T., Dye, A.H., Woolridge, T., Van der Horst, G., Rossouw, G., Lasiak, T.A. & McGwynne, L.E. (1981). Sand beach energetics: an ecosystem approach towards a high enery interface. Estuar. Coast. Shelf Sci. 13: 11-25.

McLachlan, A., Jaramillo, E., Defeo, O., Dugan, J.E., Ruyck, A.d. & Coetzee, P. (1995). Adaptions of bivalves to different beach types. J. Exp. Mar. Biol. Ecol. 187: 147-160.

Meza, F.J. (2005). Variability of reference evapotranspiration and water demands. Association to ENSO in the Maipo river basin, Chile. Global Planet. Change 47: 212-220.

Miyaji, T., Tanabe, K. & Schöne, B.R. (2007). Environmental controls on daily shell growth of *Phacosoma japonicum* (Bivalvia: Veneridae) from Japan. Mar. Ecol. Prog. Ser. 336: 141-150.

Morrison, J.P.E. (1971). Western Atlantic *Donax*. Proceedings of the Biological Society of Washington 83 (48): 545-568.

Murray-Jones, S.E. & Ayre, D.J. (1997). High levels of gene flow in the surf bivalve *Donax deltoides* (Bivalvia: Donacidae) on the east coast of Australia. Mar. Biol. 128 (1): 83-89.

Narchi, W. (1983). *Donax hanleyanus* Philippi, 1847, proposed conservation and proposed suppression of *Donax hilairea* Guerin, 1832 (Mollusca, Bivalvia). Z.N. (S.) 2152. Bull. zool. Nom. 40 (3): 188-189.

Nelson, W.G., Bonsdorff, E. & Adamkewicz, L. (1993). Ecological, morphological, and genetic differences between the sympatric bivalves *Donax variabilis* Say, 1822, and *Donax parvula* Philippi, 1849. The Veliger 36 (4): 317-322.

NOAA (2008). El Niño theme page - access to distributed informaion on El Niño. Available from: http://www.pmel.noaa.gov/tao/elnino/ninohome.html.

Nylander, J. (2004). MrModeltest2.2. Computer software distributed by the University of Uppsala.

Oliva, M.E., Barrios, I., Thatje, S. & Laudien, J. (2007). Changes in prevalence and intensity of infection of *Profilicollis altmani* (Perry, 1942) cystacanth (Acanthocephala) parasitizing the mole crab *Emerita analoga* (Stimpson, 1857): an El Niño cascade effect? Helgol. Mar. Res. 62 (1): 57-62.

Olsson, A.A. (1961). Mollusks of the tropical eastern Pacific, panamic Pacific Pelecypoda. Paleontological Research Institute, Ithaca. 574 p.

Ortlieb, L., Diaz, A. & Guzmán, N. (1996). A warm interglacial episode during oxygen isotope stage 11 in northern Chile. Quaternary Science Reviews 15: 857-871.

Pal, S.G. (1996). Some observations on the testes of *Donax lubrica* (Bivalvia: Donacidae). Malacol. Rev. 6: 55-62.

Palumbi, S.R., Martin, A. & Romano, S. (1991). The simple fool's guide to PCR, Version 2.0. University of Hawaii, Honolulu.

Paredes, C. & Cardoso, F. (2001). El género *Donax* en la costa peruana (Bivalvia: Tellinoidea). Rev. Per. Biol. 8 (2): 1-13.

Peredo, S., Parada, E. & Valdebenito, I. (1987). Gametogenesis and reproductive cycle of the surf clam *Mesodesma donacium* (Lamarck, 1818) (Bivalvia: Mesodesmatidae) at Queule Beach, southern Chile. The Veliger 30 (1): 55-68.

Peres, J.M. (1982). Major benthic assemblages. Mar. Ecol. 5: 373-522.

Pörtner, H.O., Storch, D. & Heilmayer, O. (2005). Constraints and trade-offs in climate-dependent adaptation: energy budgets and growth in a latitudinal cline. Sci. Mar. 69 (2): 271-285.

Quiroz., M., Tejada, A., Gonzales, A. & Castillo, R. (2006). Estrategia para reforzar el repoblamiento natural del recurso macha (*Mesodesma donacium*, Lamarck 1818) en el litoral de la provincia de Islay, región Arequipa-Perú. Informe IMARPE CCM013: 33-34.

Rey, R. (2006). Prohíben la extracción, recepción, transporte, procesamiento y comercialización del recurso marucha en tallas inferiores a 22 milímetros de longitud valvar: *Resolución Ministerial Nº 298-2006-Produce:* 2. Perú: Instituto del Mar del Perú, Informe N° 300-2006-PRODUCE/DGEPP-Dch.

Rey, R. (2007). Prohíben la extracción, recepción, transporte, procesamiento y comercialización del recurso marucha en tallas inferiores a 22 milímetros de longitud valvar: *Resolución Ministerial N° 445-2007-Produce:* 2. Perú: Instituto del Mar del Perú, Informe N° 544-2007-PRODUCE/DGEPP-Dch.

Rey, R. (2008). Prohíben actividades de extracción, procesamiento, transporte y comercialización del recurso marucha o palabritas en el litoral y ámbito del departamento de Lambayeque: *Resolución Ministerial Nº 607-2008-Produce:* 2. Perú: Instituto del Mar del Perú, Informe N° 452-2008-PRODUCE/DGEPP-Dch.

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 (1): 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 (6): 1283-1293.

Riascos, J.M. (2008). Thriving and declining: population dynamics of the macha (*Mesodesma donacium*, Bivalvia, Mesodesmatidae) along a latitudinal gradient of the Humboldt Current Upwelling System Ph.D. thesis, University Bremen 124 p.

Riascos, J.M., Carstensen, D., Laudien, J., Arntz, W., Oliva, M.E., Güntner, A. & Heilmayer, O. (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., Guzmán, N., Laudien, J., Heilmayer, O. & Oliva, M.E. (2007). Suitability of three stains to mark shells of *Concholepas concholepas* (Gastropoda) and *Mesodesma donacium* (Bivalvia). J. Shellfish Res. 26: 1-7.

Riascos, J.M., Heilmayer, O., Oliva, M.E., Laudien, J. & Arntz, W.E. (2008). Infestation of the surf clam *Mesodesma donacium* by the spinoid polychaete *Polydora biocipitalis*. J. Sea Res. 59: 217-227.

Robert, R., His, E. & Dinet, A. (1988). Combined effects of temperature and salinity on fed and starved larvae of the European flat oyster *Ostrea edulis*. Mar. Biol. 97: 95-100.

Romero, C.C., Baigorria, G.A. & Stroosnijder, L. (2007). Changes of erosive rainfall for El Niño and La Niña years in the northern Andean highlands of Peru. Clim. Change 85 (3-4): 343-356.

Rosenberg, R., Arntz, W., de Flores, E.C., Flores, L.A., Carbajal, G., Finger, I. & Tarazona, J. (1983). Benthos biomass and oxygen in the upwelling system off Peru. J. Mar. Res. 41: 263-279.

Ruiz-Azcona, P., Rodríguez-Sierra, R. & Martín, J.B. (1996). Culture of coquina clam, *Donax trunculus*, larvae. Aquaculture 139: 151-155.

Rutllant, J.A., Fuenzalida, H. & Aceituno, P. (2003). Climate dynamics along the arid northern coast of Chile: the 1997-1998 dinamica del clima de la región de Antofagasta (DICLIMA) experiment. J. Geophys. Res. 108.

Short, A.D. & Wright, L.D. (1983). Physical variability of sandy beaches. In: McLachlan, A. & Erasmus, T. (Eds.) Sandy beaches as ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands: 133-144.

Soares, A.G. (2003). Sandy beach morphodynamics and macrobenthis communities in temperate, subtropical and tropical regions - A macroecological approach. Ph.D. thesis, Faculty of Science, University of Port Elizabeth, South Africa: 171 p.

Soto, R. (1985). Efectos del fenómeno El Niño 1982-83 en ecosistemas de la I Región. Invest. Pesq. 32: 199-206.

Sousa, M. & Oliveira, E. (1994). Ultrastructural and cytochemical study of spermatogenesis in *Donax trunculus* (Mollusca, Bivalvia). J. Submicrosc. Cytol. Pathol. 26 (3): 305-311.

Stotz, W., Lancellotti, D., Lohrmann, K., von Brand, E., Burto, J., Caillaux, L.M., Valdebenito, M., Rodríguez, L., Araya, P. & Cerda, C. (2003). Informe final proyecto FIP 2001-24 Repoblamiento de bancos de macha en playa 'las machas' de Arica, I Región*:* 207 p.: Universidad Católica del Norte, Grupo de Ecología y Manejo de Recursos.

Tarazona, J., Arntz, W.E., Canahuire, E., Ayala, Z. & Robles, A. (1985). Modificaciones producidas durante 'El Niño' en la infauna bentónica de áreas someras del ecosistema de afloramiento peruano. In: Arntz, W.E., Landa, A. & Tarazona, J. (Eds.) 'El Niño', su impacto en la fauna marina. Boln. Inst. Mar. Perú, Callao, Spec. Issue: 55-63.

Tarazona, J., Paredes, C. & Igreda, M. (1986). Estructura del macrobentos en las playas arenosas de la zona de Lima, Perú. Revista de Ciencias U.N.M.S.M. 74 (1): 103-116.

Tarifeño, E. (1980). Studies on the biology of the surf-clam *Mesodesma donacium* (Lamarck, 1818) (Bivalvia: Mesodesmatidae) from chilean sandy beaches. Ph.D. thesis, University of California, Los Angeles: 203 p.

Tettelbach, S.T. & Rhodes, E.W. (1981). Combined effects of temperature and salinity on embryos and larvae of the Northern Bay scallop *Argopecten irradians irradians*. Mar. Biol. 63 (3): 249-256.

Thatje, S., Heilmayer, O. & Laudien, J. (2008). Climate variability and El Niño Southern Oscillation: implications for natural coastal resources and management. Helgol. Mar. Res. 62 (1): 5-14.

Thiel, M., Macaya, E.C., Acuña, E., Arntz, W., Bastias, H. & Brokordt, K., Camus, P.A., Castilla, J.C., Castro, L.R., Cortés, M., Dumont, C.P., Escribano, R., Fernández, M., Gajardo, J.A., Gaymer, C.F.,

Gómez, I., González, A.E., González, H.E., Haye, P.A., Illanes, J.E., Iriarte, J.L., Lancellotti, D.A., Luna-Jorquera, G., Luxordo, C., Manríquez, P.H., Marin, V., Muñoz, P., Navarrete, S.A., Pérez, E., Poulin, E., Sellanes, J., Sepúlveda, H.H., Stotz, W., Tala, F., Thomas, A., Vargas, C.A., Vásquez, J.A., Vega, J.M.A. (2007). The Humboldt Current System of northern and central Chile, oceanographic processes, ecological interactions and socioeconomic feedback. Oceanogr. Mar. Biol. 45: 195-345.

Tomicic, J.J. (1985). El Niño 1982-83 effects on the littoral communities of the Peninsula de Mejillones. Invest. Pesq. 32: 209-213.

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.

Utter, F.M. (1991). Biochemical genetics and fisheries managment: a historical perspective. J. Fisch. Biol. 39: 1-20.

van der Horst, G., Hodgson, A.N., Bernard, R., Salie, W. & Maasdorp, L.J.W. (1986). Sperm ultrastructure in two *Donax* (Bivalvia) species. Proceedings of the Electron Microscopial Society of South Africa 16: 69-70.

Vélez, A., Venables, B.J. & Fitzpatrik, L.C. (1985). Growth production of the tropical beach clam *Donax denticulatus* (Tellinidae) in eastern Venezuela. Carib. J. Sci. 21 (1-2): 63-73.

Villegas, M.J., Stotz, W. & Laudien, J. (2005). First record of an epibiosis between the sand crab *Emerita analoga* (Stimpson, 1857) (Decapoda: Hippidae) and the mussel *Semimytilus algosus* (Gould, 1850) (Bivalvia, Mytilidae) in southern Peru. Helgol. Mar. Res. 60: 25-31.

von Ihering, H. (1907). Les mollusques fossiles du tertiaire et du cretacé supérieur de l´Argentine. An. Mus. Nac. Buenos Aires ser. II: 611 p.

Wade, B.A. (1967a). On the taxonomy, morphology, and ecology of the beach clam, *Donax striatus*. Bull. Mar. Sci. 17: 723-740.

Wade, B.A. (1967b). Studies on the biology of the west Indian beach clam, *Donax denticulatus* L. I. Ecology. Bull. Mar. Sci. 17: 149-174.

Wade, B.A. (1968). Studies on the biology of the west Indian beach clam, *Donax denticulatus* L. 2. Life-history. Bull. Mar. Sci. 18 (4): 876-901.

Wang, H.J., Zhang, R.H., Cole, J. & Chavez, F. (1999). El Niño and the related phenomenon Southern Oscillation (ENSO): the largest signal in interannual climate variation. Proc. Acad. Nat. Sci. 96: 11071-11072.

Wang, T. & Overgaard, J. (2007). The heartbreak of adapting to global warming. Science 315: 49-50.

Waylen, P.R. & Caviedes, C.N. (1990). Annual and seasonal fluctuations of precipitation and streamflow in the Aconcagua river basin, Chile. J. Hydrol. 120 (1-4): 79-102.

Wilson, J.G. (1999). Population dynamics and energy budget for a population of *Donax variabilis* (Say) on an exposed South Carolina beach. J. Exp. Mar. Biol. Ecol. 239: 61-83.

Witt, J.D.S., Threloff, D.L. & Hebert, P.D.N. (2006). DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. Mol. Ecol. 15 (10): 3073-3082.

Yaroslavtseva, L.M. & Sergeeva, E.P. (2006). Adaptivity of the bivalve *Mytilus trossulus* larvae to short-and long-term changes in water temperature and salinity. Russ. J. Mar. Biol. 32 (2): 82-87.

Zar, J.H. (1999). Biostatistical analysis. 4. ed. Upper Saddle River, Prentice-Hall Inc. 663 p.

Zeichen, M.M., Agnesi, S., Mariani, A., Maccaroni, A. & Ardizzone, G.D. (2002). Biology and Population Dynamics of *Donax trunculus* L. (Bivalvia: Donacidae) in the south adriatic coast (Italy). Estuar. Coast. Shelf Sci. 54: 971-982.

Die "Berichte zur Polar- und Meeresforschung" (ISSN 1866-3192) werden beginnend mit dem Heft Nr. 569 (2008) ausschließlich elektronisch als Open-Access-Publikation herausgegeben. Ein Verzeichnis aller Hefte einschließlich der Druckausgaben (Heft 377-568) sowie der früheren **"Berichte zur Polarforschung** (Heft 1-376, von 1982 bis 2000) befindet sich im Internet in der Ablage des electronic Information Center des AWI (**ePIC**) unter der URL http://epic.awi.de. Durch Auswahl "Reports on Polar- and Marine Research" auf der rechten Seite des Fensters wird eine Liste der Publikationen in alphabetischer Reihenfolge (nach Autoren) innerhalb der absteigenden chronologischen Reihenfolge der Jahrgänge erzeugt.

To generate a list of all Reports past issues, use the following URL: http://epic.awi.de and select the right frame to browse "Reports on Polar and Marine Research". A chronological list in declining order, author names alphabetical, will be produced, and pdf-icons shown for open access download.

Verzeichnis der zuletzt erschienenen Hefte:

Heft-Nr. 609/2010 — "Daten statt Sensationen - Der Weg zur internationalen Polarforschung aus einer deutschen Perspektive", by Reinhard A. Krause

Heft-Nr. 610/2010 — "Biology of meso- and bathypelagic chaetognaths in the Southern Ocean", by Svenja Kruse

Heft-Nr. 611/2010 — "Materialparameter zur Beschreibung des zeitabhängigen nichtlinearen Spannungs – Verformungsverhaltens von Firn in Abhängigkeit von seiner Dichte", by Karl-Heinz Bässler

Heft-Nr. 612/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Arctic in 2009 (ARK-XXIV/1)", edited by Gereon Budéus

Heft-Nr. 613/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Antarctic in 2009 (ANT-XXV/3 - LOHAFEX)", edited by Victor Smetacek and Syed Wajih A. Naqvi

Heft-Nr. 614/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Antarctic in 2009 (ANT-XXVI/1)", edited by Saad el Naggar and Andreas Macke

Heft-Nr. 615/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Arctic in 2009 (ARK-XXIV/3)", edited by Wilfried Jokat

Heft-Nr. 616/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Antarctic in 2009 (ANT-XXV/4)", edited by Christine Provost

Heft-Nr. 617/2010 — "The Expedition of the Research Vessel 'Polarstern' to the Amundsen Sea, Antarctica, in 2010 (ANT-XXVI/3)", edited by Karsten Gohl

Heft-Nr. 618/2010 — "Sozialhistorische Studie zur Polarforschung anhand von deutschen und österreich-ungarischen Polarexpeditionen zwischen 1868-1939", by Ursula Rack

Heft-Nr. 619/2010 — "Acoustic ecology of marine mammals in polar oceans", by Ilse Van Opzeeland

Heft-Nr. 620/2010 — "Cool Libraries in a Melting World - Proceedings of the 23rd Polar Libraries Colloquy 2010 June 13-18, 2010, Bremerhaven, Germany", edited by Marcel Brannemann and Daria O. Carle

Heft-Nr. 621/2010 — "The Expedition of the Research Vessel "Polarstern" to the Arctic in 2010 (ARK-XXV/3)", edited by Volkmar Damm

Heft-Nr. 622/2010 — "Environmentally induced responses of *Donax obesulus* and *Mesodesma donacium* (Bivalvia) inhabiting the Humboldt Current System", by Daniel Carstensen