Population dynamics and ecology of the surf clam *Donax serra* (Bivalvia, Donacidae) inhabiting beaches of the Benguela upwelling system

Populationsdynamik und Ökologie der Brandungsmuschel *Donax serra* (Bivalvia, Donacidae) von Stränden des Benguela Auftriebssystems

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Learning – an endless sea,
Sea – endless learning.

Chinese reversed proverb

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SUMMARY

The surf clam *Donax serra* (Röding, 1798) dominates macrobenthic communities of extended and exposed sandy beaches of those southern African biogeographical regions where phytoplankton production is high: the cold Benguela upwelling system and areas of the warm Agulhas current system, which experience occasional upwelling and estuarine input. It feeds on phytoplankton and detritus, serves as food item for marine and terrestrial predators and is exploited by recreational anglers. The overall aim of this study was to investigate the population dynamics and ecology of Namibian *D. serra* in order to contribute essential knowledge for the understanding of its role in the Benguela upwelling ecosystem as well as for its potential use in aquaculture and exploitation activities. In particular two populations of the central Namibian coast were studied during November 1997 and December 1999.

Histological examinations of *D. serra* gonads and the condition index indicated a seasonal reproductive cycle, coupled to the annual mean sea surface temperature cycle. Spawning started in August/September and lasted until February. Recruits, however, were present for only three months in the intertidal zone. The period when these juveniles are abundant is decoupled from the spawning period and therefore cannot be predicted. Starvation, hydrodynamic processes, chemical parameters and different release times during the spawning period are likely to cause spatial and temporal differences in settlement time and recruitment strength.

Individual growth is best described by a Von Bertalanffy growth function with an asymptotic length (L_∞) of 82 mm and a growth constant (K) of 0.274 y^{-1} in both intertidal populations. Growth performance of *D. serra* agrees with values of cold-temperate and upwelling donacids while temperate *Donax*-species have intermittent and tropical/subtropical congeners show lowest values. The intertidal biomass of the studied populations ranged between 141 g ash free dry mass (AFDM) m^{-2} and 546 g AFDM m^{-2}, which is much higher that in *D. serra* populations at warm temperate beaches and distinctly surmounts the range for several non-upwelling *Donax*-species. In line, annual production exceeds values calculated from populations of habitats without permanent upwelling as well as those of non-upwelling donacids. The present values ranged between 167 g AFDM m^{-2} y^{-1} and 637 g AFDM m^{-2} y^{-1}, resulting in productivity values between 1.167 y^{-1} and 1.589 y^{-1}. Individual production was maximal at 56.5 mm shell length (0.83 g AFDM $ind.^{-1}$ y^{-1}).

To investigate whether toxic hydrogen sulphide affects survivorship of juvenile *D. serra* and thus is a potential community structuring factor, the reaction of these clams to low oxygen concentrations and sulphide presence was examined. *In vitro* exposure experiments were conducted using an innovative gas-tight continuous flow system. Hydrogen sulphide was adjusted to a concentration (0.1 mmol l^{-1}) as regularly found during native "sulphide eruptions", which occur sporadically in the highly productive inshore regions of the central Namibian Benguela. During the first 2 h of hypoxic and hy-

poxic-sulphidic exposure test clams emerged to the sediment surface, which might support the drift to locations with more favourable conditions. Juvenile clams possess a high sulphide detoxification capacity and are adapted to sulphur events by their ability to oxidise the penetrating hydrogen sulphide to non-toxic thiosulphate. In addition, they are able to switch to anaerobic energy production, indicated by a significant accumulation of succinate and alanine. However, tested clams were not able to withstand long periods of exposure, the median survival time (LT_{50}) under hypoxic sulphide incubation was 80 h. Spatial and temporal extended sulphur events are assumed to be a potential community-structuring factor, owing to their negative impact on recruitment.

Shell size measurements confirmed that clams from the cold Benguela were significantly rounder, flatter and less wedge-shaped than clams from the warm Agulhas. A genetic comparison of four *D. serra* populations inhabiting both regimes aimed to clarify if populations are separate (sub-) species reflected in allelic variation. Genetic analysis of twenty-two protein-coding loci was carried out by starch-gel electrophoresis. Populations studied are conspecific and possess genetic variation in the range of most other marine bivalves, which allows for potential adaptation to environmental changes. Little to moderate genetic differentiation among sub-populations relative to the maximal differentiation under complete fixation (F_{ST} = 0.016 - 0.089), moderate differentiation of individuals relative to their sub-population (F_{IS} = 0.265 - 0.452), and comparably high differentiation of individuals relative to the compound population (F_{IT} = 0.300 - 0.473) were found. The effective number of individuals exchanged between populations in each generation is high enough (1.44 - 8.65) to counteract genetic drift. Therefore it is proposed that observed morphological differences represent phenotypic plasticity enabling this species to inhabit different biogeographic regions. Gene flow, balanced selective pressure and evolutionary inertia are proposed as explanations for similarities of the geographically most distant populations. The substantial differentiation of the two Namibian populations indicates a potential biotic barrier and requires separate studies of the population dynamics.

The results of this investigation, especially the high growth and production rates as well as the ability to inhabit substrates in high abundances are encouraging for future aquacultural use of this species. Work perspectives are identified for further support of culturing activities of *D. serra*, which will moreover contribute to a broader understanding of sandy beach ecology.

ZUSAMMENFASSUNG

Brandungsmuscheln der Art *Donax serra* (Röding, 1798) dominieren makrobenthische Lebensgemeinschaften ausgedehnter, exponierter Sandstrände. Sie besiedeln Küsten zweier biogeografischer Regionen des südlichen Afrikas mit hoher Phytoplanktonproduktion: Strände des kalten Benguela-Auftriebssystems und Estuare oder Gebiete des warmen Agulhasstrom-Systems mit sporadischen Auftriebsereignissen. Die Muscheln ernähren sich von Phytoplankton und Detritus, dienen marinen und terrestrischen Räubern als Nahrungsquelle und werden von Freizeitanglern als Köder gesammelt. Das Hauptziel dieser Studie war die Untersuchung der Populationsdynamik sowie der Ökologie namibischer *D. serra*. Diese Ergebnisse werden dazu beitragen, einerseits die Rolle der Muschel im Benguela-Auftriebssystem besser zu verstehen sowie ander-erseits ihre Nutzung im Freiland und in Aquakulturen zu ermöglichen. Insbesondere zwei an der zentralen Küste Namibias vorkommende Populationen wurden zwischen November 1997 und Dezember 1999 untersucht.

Histologische Präparate adulter *D. serra*-Gonaden sowie der Konditionsindex zeigten einen saisonalen Reproduktionszyklus, der an den Jahresgang der Meeresoberflächentemperaturen gekoppelt ist. Das Laichen setzte im August/September ein und dauerte bis Februar. Sehr kleine Jungmuscheln waren jedoch nur über drei Monate in der Gezeitenzone anzutreffen. Der Zeitpunkt hoher Jungmuschelabundanzen ist von der Laichzeit abgekoppelt und kann daher nicht vorhergesagt werden. Verhungern, ungünstige hydrografische und chemische Bedingungen und die Abgabe des Laichs zu unterschiedlichen Zeitpunkten innerhalb der Reproduktionszeit sind wahrscheinlich die Ursachen für die räumlichen und zeitlichen Unterschiede in der Rekrutierung und deren Ausmaß.

Das individuelle Wachstum beider Gezeitenzonen-Populationen wurde am besten durch eine von Bartalanffy-Wachstumsfunktion mit einer asymptotischen Länge (L_∞) von 82 mm und einer jährlichen Wachstumskonstante (K) von 0,274 beschrieben. Die Wachstumsleistung von *D. serra* stimmte gut mit Werten von Donaciden aus kalttemperierten Habitaten und Auftriebsgebieten überein, währen gemäßigte *Donax*-Arten mittlere und tropische/subtropische niedrigste Werte zeigten. Die in der Gezeitenzone vorhandene jährliche Biomasse der untersuchten Populationen lag zwischen 141 g aschefreier Trockenmasse (AFDM) m^{-2} und 546 g AFDM m^{-2} und ist damit viel höher als die von *D. serra*-Populationen warm-gemäßigter Strände. Sie übersteigt auch deutlich die Biomasse anderer *Donax*-Arten aus Gebieten ohne Auftriebsereignisse. Ebenso sind die jährlichen Produktionswerte gegenüber Populationen aus Gebieten ohne ständigen Auftrieb, sowie gegenüber Donaciden aus Gebieten ohne Auftrieb höher und lagen bei den untersuchten Populationen zwischen 167 g AFDM m^{-2} und 637 g AFDM m^{-2}. Die jährliche Produktion belief sich daher auf 1,167 bis 1,589. Ein maximaler individueller Produktionswert wurde bei einer Schalenlänge von 56,5 mm ermittelt (0,83 g AFDM pro Individuum und Jahr).

Zur Untersuchung der Frage, ob giftiger Schwefelwasserstoff die Lebensdauer junger *D. serra* vermindert und daher als strukturierender Faktor auf die Lebensgemeinschaft wirkt, wurden die Reaktionen der Muscheln unter Sauerstoffmangel und unter Schwefelwasserstoffbedingungen untersucht. *In vitro*-Expositionsexperimente fanden in einem neu entwickelten gasdichten Durchflusssystem statt. Die Schwefelwasserstoffkonzentration (0.1 mmol l^{-1}) wurde entsprechend den regelmäßig während Schwefelausbrüchen gefundenen Konzentrationen eingestellt. Diese Ausbrüche treten sporadisch in den hochproduktiven Küstenregionen des zentralen namibischen Benguelastroms auf. Testmuscheln kamen unter Sauerstoffmangel bzw. Sauerstoffmangel und Schwefelwasserstoffbedingungen während der ersten beiden Stunden an die Oberfläche. Dieses Verhalten begünstigt den passiven Transport in Gebiete mit besseren Umweltbedingungen. Jungmuscheln sind in der Lage, Schwefelwasserstoff zu entgiften, was als Anpassung an die Schwefelereignisse interpretiert werden kann. Sie oxidieren den eindringenden Schwefelwasserstoff zu ungiftigem Thiosulfat. Darüber hinaus vermögen sie anaerob Energie zu gewinnen, erkennbar durch eine signifikante Anreicherung von Sukzinat und Alanin. Die getesteten Muscheln waren jedoch nicht in der Lage, längere Expositionszeiten zu überleben. Dies führte bei der Hälterung unter sauerstoffarmen Schwefelwasserstoffbedingungen zu einer mittleren Überlebenszeit (LT_{50}) von 80 Stunden. Zeitlich und räumlich ausgedehnte Schwefelereignisse sind daher infolge ihres negativen Einflusses auf die Rekrutierung als mögliche strukturierende Faktoren für die Lebensgemeinschaft zu werten.

Durch Vermessungen der Muschelschalen wurde bestätigt, dass Tiere des kalten Benguelastroms signifikant runder, flacher und weniger keilförmig sind, als Muscheln aus dem warmen Agulhasstromgebiet. Ein genetischer Vergleich wurde durchgeführt, um zu zeigen, ob vier *D. serra* Populationen von beiden Küstengebieten zu verschiedenen (Unter-)Arten gehören. Untersucht wurde, ob sich die Morphologie der Muscheln in Allelevariationen widerspiegelt. Zur genetischen Analyse wurden 22 proteinkodierende Loci mit Hilfe von Stärkegel-Elektrophorese untersucht. Die Ergebnisse zeigen, dass die Populationen zur gleichen Art gehören und genetische Variationen besitzen, die im Bereich der meisten marinen Muscheln liegen. Potentiell sind Anpassungen an geänderte Umweltbedingungen daher möglich. Geringe bis mittlere genetische Differenzierung wurde zwischen Subpopulationen relativ zur höchstmöglichen Differenzierung unter Inzucht-Bedingungen festgestellt (F_{ST} = 0.016 - 0.089). Eine mittlere Differenzierung von Individuen in Bezug zu ihrer Unterpopulation (F_{IS} = 0.265 - 0.452) und eine vergleichsweise hohe Differenzierung von Individuen relativ zur Gesamtpopulation (F_{IT} = 0.300 - 0.473) wurden nachgewiesen. Die Anzahl an Tieren, die effektiv zwischen Populationen ausgetauscht werden (1.44 - 8.65), war hoch genug, um der genetischen Drift entgegen zu wirken. Daher sind die gefundenen morphologischen Unterschiede am ehesten als phänotypische Plastizität zu erklären, die es den Tieren ermöglicht, verschiedene biogeografische Regionen zu besiedeln. Genfluss, ausgewogener Selektionsdruck und evolutive Trägheit kommen als Erklärungsmöglichkeiten für die Ähnlichkeit der beiden am weitesten geographisch voneinander entfernten

Populationen in Betracht. Die deutliche Trennung der beiden namibischen Populationen weist auf eine mögliche biotische Grenze hin. Daher muss die Populationsdynamik dieser Populationen getrennt untersucht werden.

Die Ergebnisse dieses Forschungsprojekts ermutigen, *D. serra* in Kultur zu nehmen, vor allem vor dem Hintergrund, dass diese Muschel das Substrat in hohen Abundanzen besiedeln kann und die Produktionsrate hoch ist.

Zukünftige Projektthemen wurden erarbeitet, um an diese Arbeit anknüpfend Aquakulturaktivitäten mit *D. serra* zu unterstützen und die Ökologie von Sandstränden umfassender zu verstehen.

1 INTRODUCTION

1.1 COASTAL UPWELLING SYSTEMS

Western boundaries of continental slopes are characterized by strong currents directed towards the equator, i.e. the Humboldt and California currents of the Pacific and the Canary and Benguela currents of the Atlantic Ocean, which transport cold water masses to lower latitudes. Coast parallel winds in conjunction with the Coriolis force (Ekman transport) result in an offshore net water transport inducing coastal upwelling (Fig. 1) of cold and nutrient rich water from intermediate depths (mostly 50-100 m) into the euphotic zone (e.g. Arntz and Fahrbach 1991). This stimulates a remarkably high (Jarman and Carter 1981, Walsh 1981, Schulz 1982, Mitchell-Innes *et al.* 2001) and year-round (Schulz 1982, Weeks and Shillington 1994) primary production evoking a strong secondary production in the pelagial (Barber and Smith 1981, Painting *et al.* 1993, Hewitson and Cruickshank 1993).

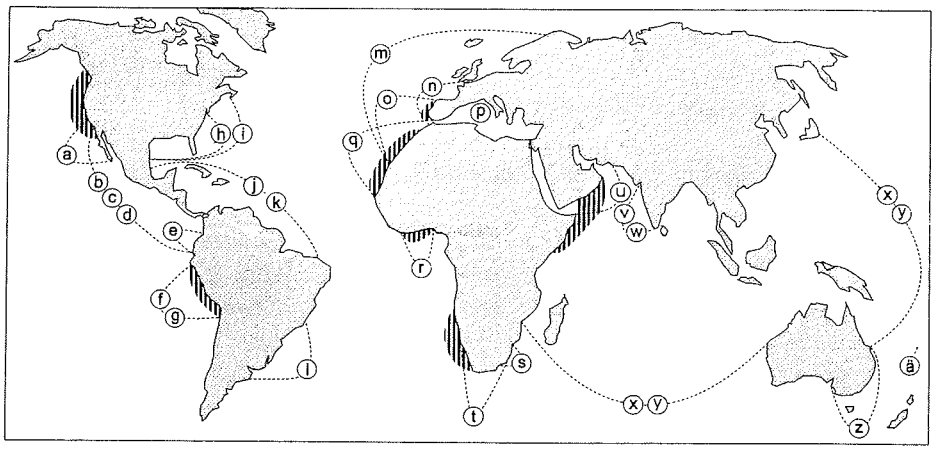

Fig. 1: Coastal upwelling areas (striped) of the World Ocean (modified from Mittelstaedt 1989) and the geographical distribution of selected donacid species (modified and extended from McLachlan *et al.* 1996): a: *D. gouldii*, b: *D. carinatus*, c: *D. panamensis*, d: *D. punctatostriatus*, e: *D. dentifer*, f: *D. marincovichi*, g: *D. obesulus*, h: *D. fosser*, i: *D. variabilis*, j: *D. denticulatus*, k: *D. striatus*, l: *D. hanleyanus*, m: *D. vittatus*, n: *D. variegatus*, o: *D. trunculus*, p: *D. semistriatus*, q: *D. venustus*, r: *D. oweni*, s: *D. sordidus*, **t: *D. serra***, u: *D. townsendi*, v: *D. incarnatus*, w: *D. spiculum*, x: *D. cuneatus*, y: *D. faba*, z: *D. deltoides*, ä: *D. pallidus*.

High pelagic primary production together with strong turnover rates in successive levels of the food web result in a high sedimentation rate followed by subsequent oxidative degeneration of the organic matter (van der Plas 1999). This causes the occurrence of extended oxygen minimum zones (Chapman and Shannon 1985, 1987, Arntz 1986, Bailey 1991, 1999, Erikson 1996). In consequence sublittoral benthic communities from the intermediate sublittoral (depending on the exposure of the coast between 15 m and 30 m) to the deep sea are characterized by low diversity, abundances and biomasses (off Peru: e.g. Rosenberg *et al.* 1983, Arntz *et al.* 1991; off Namibia: Eriksen 1996).

The intertidal and upper subtidal of upwelling ecosystems is typically well oxygenated due to the strong surf (McLachlan 1986, Arntz and Fahrbach 1991, Jaramillo 1994, Brazeiro and Defeo 1996). Oxygenation together with a huge food import from the pelagic system (Wulff and Field 1983, Cockcroft and McLachlan 1993) and kelp beds (Soares *et al.* 1997) enables macrozoobenthic species to form communities with high abundances and biomasses (e.g. Arntz *et al.* 1987, 1991, McLachlan 1990, Jaramillo 1994). High impact of breaking waves and rip currents generate a highly dynamic top sediment layer in which communities of low diversity predominate and therefore rather resemble those of temperate (Arntz and Arancibia 1989, Tarazona *et al* 1988, 1996) than those of tropical zones.

The driving force of the ecosystem, i.e. the upwelling of nutrient rich water is variable in time and space. Irregular meteorological changes can make upwelling inefficient due to sinking of the thermocline (Barber and Chávez 1983). On a large-scale such climatic anomalies are known as El Niño – Southern Oscillation (ENSO) from the Pacific (Philander 1990) and as "Benguela Niño" from Namibia (Shannon *et al.* 1990, Jury 1996).

1.2 THE NAMIBIAN UPWELLING SYSTEM

The Benguela current is unique in comparison to the other three major upwelling systems in that it is bounded at the equatorward and poleward ends by warm water regimes. The region experiences perennial upwelling of nutrient rich water (e.g. Lita *et al.* 2000) and the location and characteristics of the main upwelling cells have been documented and described by several authors (Nelson and Hutchings 1983, Lutjeharms and Meeuwis 1987, Hagen *et al.* 2001). Shannon (1985) identified six, Lutjeharms and Meeuwis (1987) seven upwelling cells (Publication III: Fig. 1). The two southern cells are more seasonal than those of the central Benguela. The principal upwelling cell is located in the vicinity of Lüderitz (27°S) with a typical westward extent of 250 km (Shannon and Nelson 1996, Hagen *et al.* 2001). A number of zonally orientated "fronts" tend to develop north of each major upwelling cell, a prominent one exists near 25°S, just north of the Lüderitz upwelling cell.

The surplus of organic material of the water-column sinks and is metabolized by bacteria which leads to extensive oxygen depletion in bottom waters (e.g. Hart and Currie 1960, Chapman and Shannon 1985, 1987, Truesdale and Bailey 2000). Off Namibia, the Benguela current is an oxygen-depleted ecosystem. Permanent features of hypoxic conditions may persist in inshore localities downstream of the major upwelling centres (Bailey 1991, 1999, Dingle and Nelson 1993). Further anaerobic degradation of organic matter due to sulphate reducing bacteria results in the production of hydrogen sulphide, which rises sporadically to the surface during "sulphide eruptions" (Bailey 1991). As a consequence, extremely low oxygen concentrations and high hydrogen sulphide concentrations were occasionally measured in the intertidal and upper subtidal (B. Currie, pers. comm.).

In contrast, the warm Agulhas current only experiences occasional upwelling at particular coastal areas. However, at these locations a combination of upwelling events, estuarine input, and possibly edge effects of the Agulhas current (Schoeman 1997) fuel vast surf-diatom populations, which provide the nutrition of rich intertidal suspension feeding populations.

1.3 THE GENUS *DONAX*

Exposed intertidal sandy beaches are commonly dominated by bivalves of the family Donacidae (superfamily Tellinacea) constituted by the genera *Donax*, *Egeria* and *Iphigenia*. On a world-wide basis, the well studied Donacidae form by far the largest group inhabiting such highly dynamic environments (for review: Ansell 1983). Although restricted by their specialization to beaches and subtidal high-energy habitats they show a strong adaptive radiation. As a major feature of this adaptive radiation tidal migration has been reported in many *Donax* species. Despite Ansell's (1983) assertion that members of the genus *Donax* are confined to distinct zoogeographical regions, several species show transient distribution across the subtropical-temperate border (Fig. 1). Only 5% of the 64 species are found in cold temperate areas (> 5°C, Bally 1986) with lowest species diversity on the West Coast of Africa (Ansell 1983). *Donax* species are the major primary consumers in sandy beach communities supported by high levels of phytoplankton production (e.g. Wade 1967, McLachlan and Lewin 1981). In turn, they are subject to predation by a wide variety of invertebrates, fish, birds and mammals (e.g. Luzzatto and Penchaszadeh 2001, Peterson *et al.* 2000, Salas *et al.* 2001) and are an important recreational and commercial resources in many areas (McLachlan *et al.* 1996).

1.4 *DONAX SERRA*

The surf clam *Donax serra* (Röding, 1798) is the largest species of the Donacidae. It inhabits extended and exposed sandy beaches from the northern boundary of Namibia to South Africa's Eastern Cape (Fig. 1, Donn 1990 b). Hence, its distribution covers two southern African biogeographical regions, the cold Benguela current and the warm Agulhas current system. The abundance of *D. serra* is locally variable and presumably related to food availability, sediment granulometry, wave exposure and beach morphology (Schoeman 1997). Clams form dense beds reaching biomasses of 8 500 g shell free dry mass (SFDM) per meter shoreline (Donn 1987, McLachlan 1996). They inhabit intermediate to dissipative beaches displaying characteristic surf circulation cells (Donn and Cockcroft 1989, McLachlan 1996). Populations differ in their size-class distribution across the beach faces: clams from the Southeast Coast and areas north of Henties Bay are restricted to the intertidal zone (Donn and Cockcroft 1989, Donn 1990 b) with larger clams performing an oscillatory migration from an upper intertidal position during spring tides through the juvenile belt to a position close to the water line

during neap tides and back (e.g. Porsch and McLachlan 1984, Schoeman 1997). In contrast, populations from the Western Cape up the southern African west coast as far Henties Bay are separated in intertidal (< 50 mm shell length) and subtidal (> 50 mm shell length) belts (de Villiers 1975 b, Donn 1990 b, McLachlan 1996, own unpubl. data). Intertidal clams of this region maintain a suitable position reacting apparently to changes in physical conditions like shifting sand substratum and changes in the ground water table (Donn *et al.* 1986). A novel technique not described in the enclosed publications allowed enhanced tracking of short term migration in the dynamic environment. The technique consisted of the tagging of 300 clams (22-35 mm shell length, 8+ months) with bar magnets. During 15 surveys (daily, from day 9 every 2 days) the net change in position of these clams was tracked with a Field-Force-Difference-Detector (MAGNEX 105, Ebinger, Germany). Variable positions along the beach profile were recorded within an area exceeding 5000 m^2 (Fig. 2). Further, a significant movement in current direction was evident, contributing to the dynamics of this system.

As surf clams do in general *D. serra* feeds on phytoplankton and detritus, is consumed by birds, fish and crabs and therefore is an important trophic link in surf zone food webs (McLachlan *et al.* 1980, 1996, Rossouw 1985). Clams are exploited for bait and their unique delicate taste makes them a potentially valuable food resource. Based on an economic evaluation Sims-Castley and Hosking (in review) calculated a possible price range of US$ 6.50 – 60.00/kg for export markets. Despite their potential value, many aspects of their population dynamics (reproduction, growth, production, mortality) in Namibia are still unknown.

Several studies on different aspects of the population dynamics were compiled elsewhere in southern Africa. Sexual differentiation of South African *D. serra* occurs at a mean length between 44 mm and 48 mm and sexes can only be distinguished histologically (de Villiers 1975 a, McLachlan and Hanekom 1979). Meroplanktonic larval life was estimated to last between one week (Lastra 1994) and four months (Birkett and Cook 1987). After settlement (1-1.5 mm shell length, Lastra 1994) in the subtidal, preferably at fine grain sizes (Lastra and McLachlan 1996), juveniles migrate to the upper intertidal (Donn 1987, Lastra and McLachlan 1996). Growth rates and population structure were concordantly only analysed for South African populations (de Villiers 1975 b, Donn 1986, Schoeman 1994, 1997). However, the unique "sulphur eruptions" along the Namibian coastline might have the potential to affect the structuring of populations as hydrogen sulphide can increase mortality rates of *D. serra* (Bailey 1999).

Knowledge of *D. serra* population dynamics is essential for the understanding of its ecology and crucial to support future aquaculture, exploitation activities and management. However, South African results cannot simply be transferred to Namibian populations. Morphological and the above mentioned behavioural dissimilarities of different South African populations have been investigated (Donn 1990 a, Donn and Els 1990, Soares *et al.* 1998). Hence, it is not clear if ecological and genetic differences separate the populations into different (sub-) species or stocks.

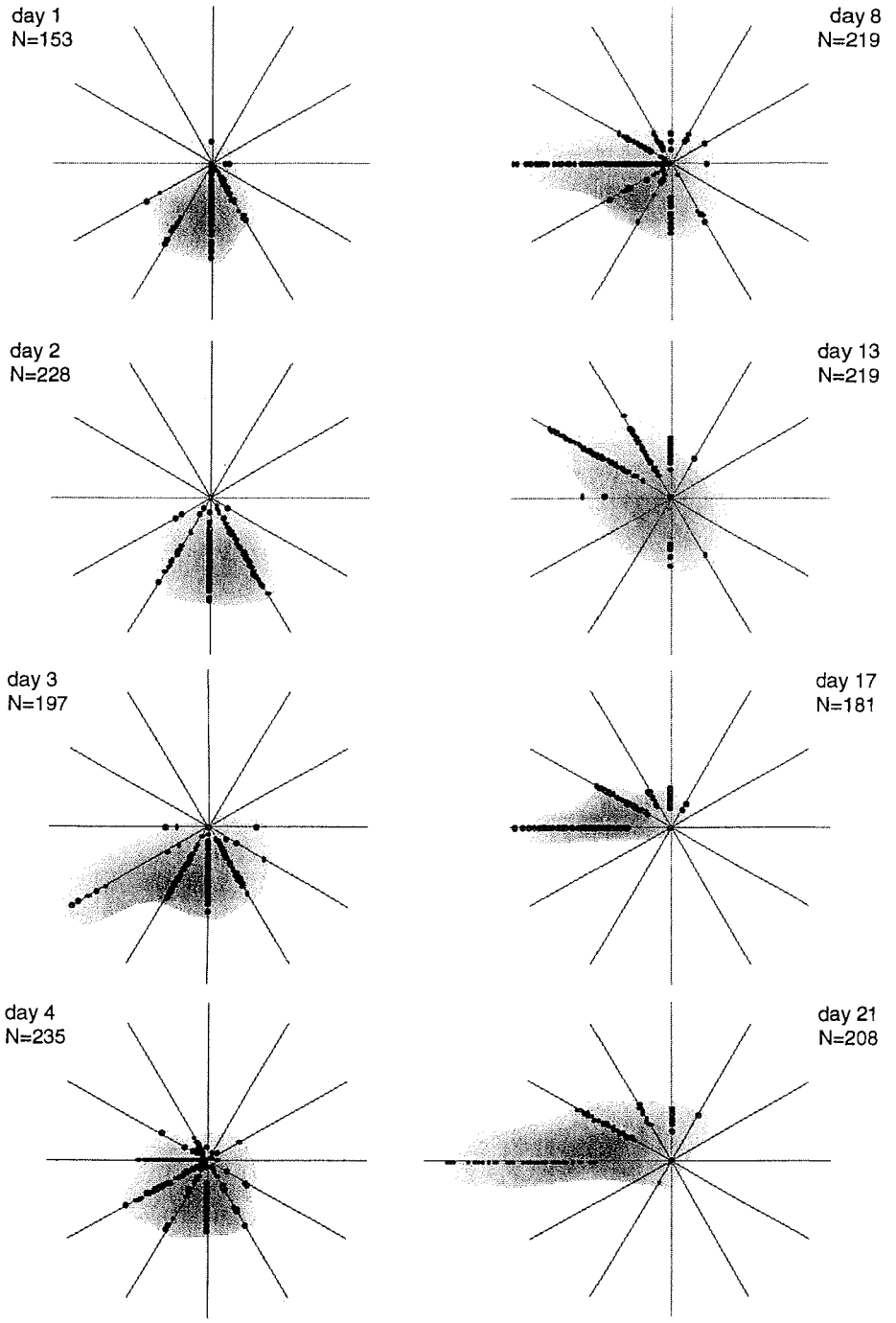

Fig. 2: Positions of intertidal tagged *D. serra* recovered on different days following release. Clams were liberated at a marked location represented by the centre point of each diagram and tracked within a round area (radius: 40 m = length of axis from centre, 60 m at day 21). Indicated clam positions within one of the 12 sectors were projected anticlockwise on the nearest transect and distributions are approximated by the shaded area. Horizontal axis is parallel to the shoreline.

1.5 AIM OF THIS STUDY

The aim of this study was to investigate the population dynamics and ecology of Namibian *D. serra*. In this context four topics were studied by testing the corresponding hypotheses:

1.) Gametogenic cycle and recruitment patterns:
Recruitment patterns are closely coupled to gametogenic activity at the same location.

2.) Growth and production:
Growth and production differ between *D. serra* of the cold (Namibia) and the warm water province (South Africa).

3.) Resistance of juvenile *D. serra* to hypoxia and sulphide exposure:
Juvenile *D. serra* are not resistant to hypoxia and sulphide exposure.

4.) Genetic comparison of *D. serra* populations along the southern African coastline:
D. serra populations from the cold water and the warm water province are separated genetically.

2. STUDY SITES

Sampling was conducted at two sandy beaches: Langstrand (22°47´S, 14°33´E; Site I) and Paaltjies IV (22°59´S, 14°24´E; Site IA) (Publication I and II: Fig. 1) during alternate spring tides between November 1997 and December 1999. To enable genetic and morphological comparisons beside Langstrand three additional beaches covering two different biogeographic provinces were selected: one Namibian (Meob Bay, 24°38´S, 14°43´E; Site II) and two South African beaches (Bloubergstrand, 33°51´S, 18°09´E; Site III; Maitlands 34°6´S, 25°13´E; Site IV) (Publication IV: Fig. 1).

Due to coastal upwelling at the west coast of southern Africa the average water temperature of the Benguela current is 13°C (Walker *et al.* 1984). Sea surface temperature (SST) for Site I and IA decreases to about 12°C in winter and rises to 23°C in summer with seasonal means at Walvis Bay of 16°C (summer/autumn) and 14°C (winter/spring) (Shannon 1985). SST at Site II is annually 13.5°C ranging between 10°C and 19.5°C (summer/autumn: 15°C, winter/spring: 12°C) (C. Bartholomae, unpubl. data) while Site III is exposed to even lower temperatures with an annual mean of 13°C (summer: 8 -14°C; winter: 11 - 17°C) (Walker *et al.* 1984). According to McLachlan's (1980) rating scale for exposure Site I and III are in a high energy intermediate morphodynamic state (Soares *et al.* 1996, Schoeman 1997), whereas Site IA and II are classified as reflective beaches with waves breaking almost directly in the intertidal zone (pers. obs.). All three are, however, open ocean beaches exposed to continuous wave action and subject to subequal semidiurnal tides with a maximum tide range of about 2 m (springs average 1.4 m, neaps 0.7 m) (McLachlan 1986).

Site IV is located at the southeast coast of southern Africa and influenced by the Agulhas current, which is deflected southwards at the Agulhas Bank (Brown and Jarmann 1978). The mean annual SST is 22°C (summer: 26°C; winter: 15 - 17°C) (Ansell and McLachlan 1980). Sporadically this region may however experience cold-water upwelling during strong eastern winds in summer (Goschen and Schumann 1995) albeit with a much lesser frequency than at the West Coast (Shannon 1985). The beach is in a high-energy intermediate to dissipative morphodynamic state (McLachlan 1990, Soares *et al.* 1998).

Further descriptions of the habitats can be found in McLachlan (1985) (Site I, IA), Holtzhausen (1999) (Site II), Soares *et al.* (1996) (Site III) and Schoeman (1997) (Site IV).

3. MATERIALS AND METHODS

In order to study population dynamics and ecology of the surf clam *D. serra* several methods were used including field observations and laboratory experiments. Methods used to achieve the specific objectives including the assigned study sites are given in Figure 3. The following chapter will summarize materials and methods used for this study, more detailed descriptions can be found in the publications (section 5, page 29).

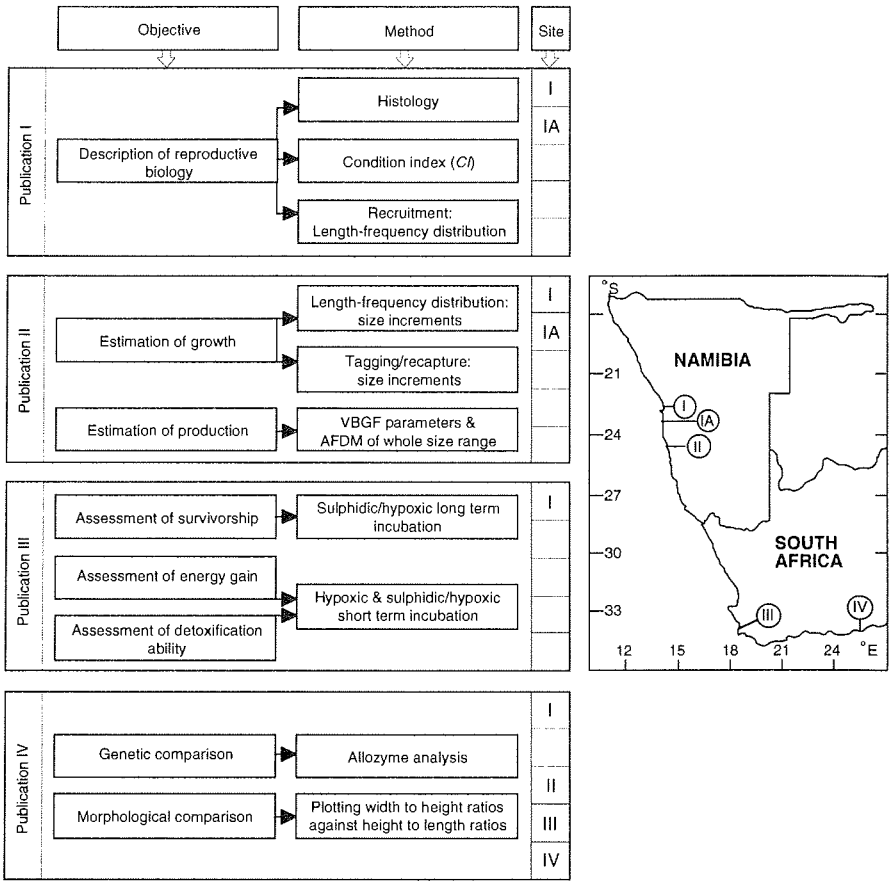

Fig. 3 Summary of methods used during this study for achieving a specific objective. Additionally, study sites and locations for the various projects and the respective publication number are given.

3.1 SAMPLING

The surf clam *D. serra* was quantitatively collected at monthly intervals (Site I: full moon, IA: new moon) from a series of stations (2 m intervals) along a transect perpendicular to the shore line. The transect extended from the spring tide high water mark to

1 m depth in the surf zone. Three replicates of 0.16 m^2 sand samples were excavated to 35 cm depth using a stirring box and the sediment was sieved on a 1-mm screen. All retained clams were immediately transferred to the laboratory for further investigations and thereafter released at the collection site with the exception of a sub-sample for determination of AFDM.

To study the gametogenic cycle an additional 50 subtidal adult *D. serra* (>54 mm) were collected from February 1998 to November 1999 for Site I and January 1998 to December 1999 for Site IA. These clams were dug out by hand in the surf zone at haphazard sampling locations during low tide. It was assured that no more than three clams inhabited the same spot were collected.

For *in vitro* experiments post-settled *D. serra* of the smallest cohort (2-6 mm anterior-posterior length, approx. 5.5 mg wet mass, WM) were collected (November and December 1999) at Site I by gently sieving the sediment (1 mm mesh).

An additional 32 adult *D. serra* were randomly collected within a 50 m stretch at the four sample sites (I, II, III, IV) during spring low water in March 1999 and transported alive to the laboratory for genetic and morphological comparison.

3.2 REPRODUCTION ANALYSES

Both sexes of *D. serra* possess a white gonad, which forms a sheath around the digestive gland (Publication I, Fig. 2). It shrinks and expands enormously into the foot cavity during the ripening process but cannot be separated from the visceral mass (de Villiers 1975 a, pers. obs.). Hence, monthly mass variation of the visceral mass from 30 live individuals was used to track changes in gonad mass by calculating a condition index (*CI*) (Site I: May 1998 - November 1999). As it was difficult to get enough adult bivalves at Site IA each spring tide, formalin-fixed *D. serra* were used between January 1998 and December 1999 excluding March/April 1999. Maximum shell length (anterior-posterior), height (ventral-dorsal) and shell width (left-right) were measured with vernier callipers (lower 1 mm) and total WM recorded (nearest 0.1 mg) immediately after collection and drying on absorbent paper. Thereafter animals were dissected, WM of the visceral mass recorded and *CI* calculated (for details see Publication I).

For histological gonad examination tissue samples (Publication I: Fig. 2) of 20 formalin-preserved (4%, Borax buffered) adults (Site I and IA) were sectioned according to standard methods. However, after dehydration and embedding in paraplast wax the blocks were frozen (-18°C) allowing histological sections (sledge microtome, Leica) of 1-3 μm in contrast to the common fines section width of 5-7 μm. Preparations of 914 individual gonads (I: 458; IA: 456) were GIEMSA stained (Giemsa 1907; Merck Art.-Nr. 1.09204) following standard procedures described in Romeis (1989). Preliminar inves-

tigations have shown that GIEMSA yielded the most satisfactory results (other stains tried: Harris' haematoxylin and eosin, azan, LADEWIG, toluidine blue). Gonads were classified into four stages of development (cytolysed, inactive, active and spawning) by microscopic examination based on de Villiers (1975 a), illustrated in Figure 4 and summarized in Publication I (p. 34).

3.3 GROWTH ANALYSES

All *D. serra* (Site I: n = 13 298, Site IA: n = 16 305) of the monthly surveys were measured (anterior-posterior length) with vernier callipers. From both beaches a sub-sample (n = 400) including the whole size range was used to obtain AFDM (g) by ignition of soft tissue at 550°C for 7 h (January and December 1998). The relationship between length and mass of *D. serra* was estimated ($M = a \times L^b$, M: AFDM, L: shell length, a and b: constants). Three different methods were used to analyse growth of *D. serra*: external and internal shell marks, tagging/recapture experiments and analyses of length-frequency distribution (LFD).

3.3.1 External and internal shell marks

The examination of external growth marks formed during distinct time intervals can be used to age bivalves (e.g. Rhoads and Lutz 1980). Therefore the dark lines of 60 individuals, visible at the surface of the anterior shell part were counted macroscopically (Publication II: Fig. 2). Additionally, microstructural shell deposits were analysed. The accretion commonly reflects tidal, daily or seasonal growth increments (e.g. Richardson 1989, Gaspar *et al.* 1999). Twenty embedded dry right valves (L = 56 - 82 mm) were sectioned, ground, polished and etched (details see Publication I). Acetate peel replicas were processed according to Richardson *et al.* (1979) and examined under a transmitted light-microscope.

3.3.2 Tagging/recapture experiments

Tagging/recapture experiments were conducted to observe readily comprehended individual growth. 7 215 *D. serra* (whole size range) were collected haphazardly at Site IA on three consecutive new moon spring tides (December 1998, January and February 1999). They were marked with two distinct parallel, shallow grooves from the ventral margin up onto the valve surface (e.g. Ropes and Merrill 1970, Ropes 1984, Publication II: Fig. 2) and released at the sample location. Care was taken at the time of release to ensure that the clams were not being carried away by strong swash. Marked clam recoveries were made in conjunction with monthly surveys. Length at time of release (L_1), reflected in a disturbance ring following the notch marks (Publication II: Fig. 2) and recapture length (L_2) were recorded. Obtained size increments were used for estimating growth parameters (see below).

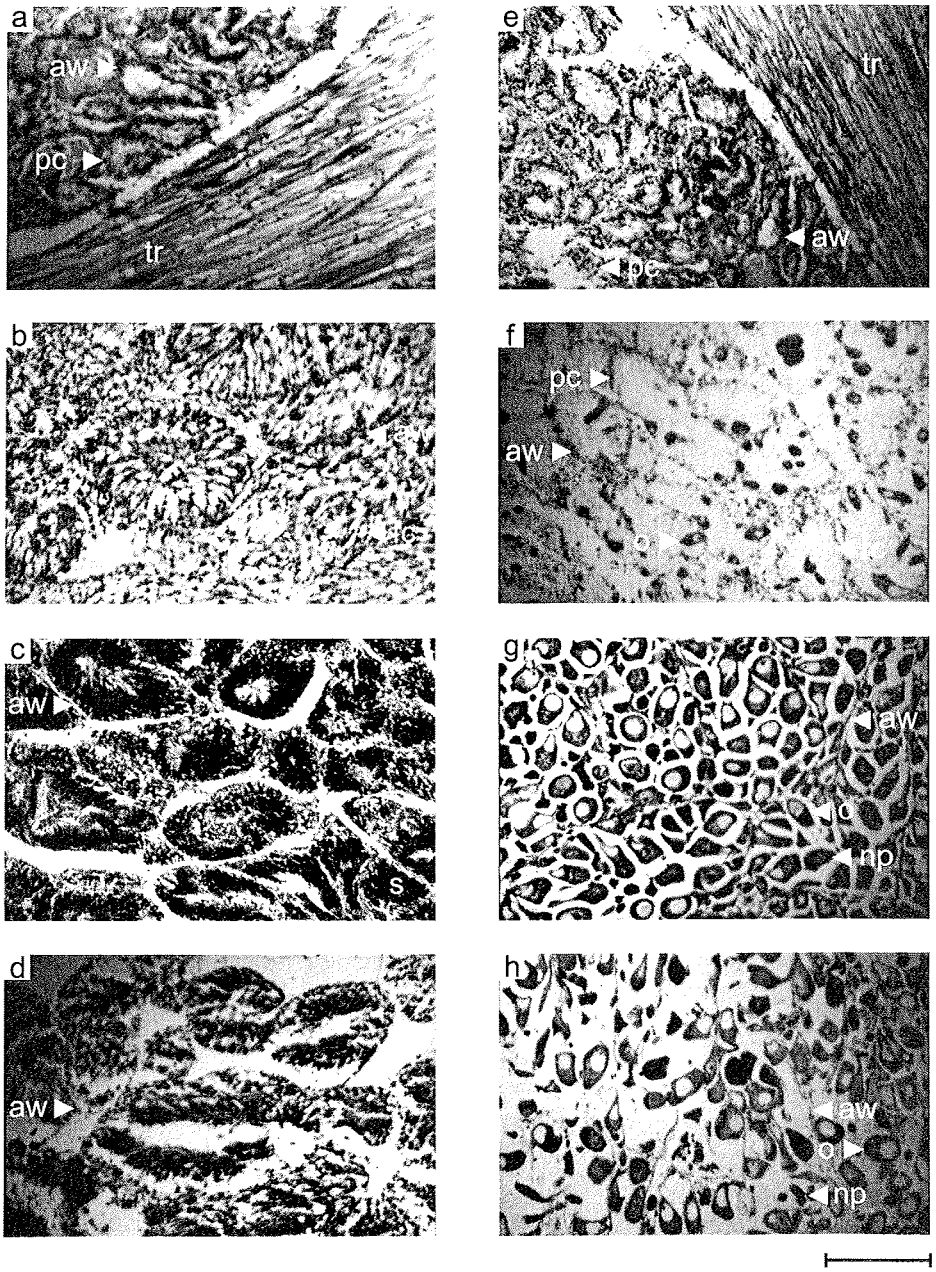

Fig. 4: Male (a-d) and female (e-h) gonad stages of *D. serra*: Cytolysed (a, e), inactive: pre-active (b, f), active (c, g) and spawning (d, h) (aw: alveolar wall, fc: follicle cell, np: nutritive particle, o: oocyte, pc: phagocytic cells, s: sperm, tr: transverse fibre fascicles). Light microscopical images (1000 ×), bar = 0.2 mm.

3.3.3 Analyses of length-frequency distributions (LFD)

A sequence of 25 (Site I) and 24 (Site IA) length-frequency histograms (2 mm size classes, monthly sampling) was used to estimate growth. Cohorts were distinguished by eye and mean individual cohort length computed by the weighted average length. Growth was described by a rearranged form of the von Bertalanffy growth function (VBGF, von Bertalanffy 1938) which was fitted to size increment data obtained from the LFD and tagging/recapture experiments using the non-linear Newton algorithm (see Publication I for equations).

The disadvantage of non-linear functions is the sensitivity to missing data at either end of the distribution (Pauly 1983, Wetherall *et al.* 1987). Since the population at Site I is exploited and the centre of adult individual distribution could not be sampled quantitatively, larger animals are poorly represented. Thus, data lacks size increments referring to large individuals. For this reason asymptotic length was not determined iteratively, but set to 82 mm according to the maximum length observed.

3.4 PRODUCTION

Total annual production, P (1998 and 1999) was calculated for the intertidal *D. serra* belt of Site I and IA by the mass-specific growth rate method (Crisp 1984, Brey 2001). P was estimated from the length-mass relation, the LFD obtained from all pooled samples and the VBGF. Mean annual biomass ($\overline{B}$) was estimated from abundance and mean individual AFDM of each length class (see Publication I for equations). From these data productivity values ($P/\overline{B}$) were estimated.

3.5 SEVERE HYPOXIA AND HYDROGEN SULPHIDE

3.5.1 Tolerance experiments

In order to investigate if "sulphur eruptions" along the central coast of the Benguela may explain the lack of recruiting cohorts and hence have the potential to impact the population structure, *in vitro* experiments were conducted. Post-settled *D. serra* were transferred into a gas-tight system with a continuous seawater flow (Publication III: Fig. 1) ameliorating the accumulation of released metabolites and the proliferation of anaerobic bacteria (de Zwaan *et al.* 2001, 2002) in the incubation water. Experiments were started after 3 h of acclimatization and survivorship investigated under normoxic and hypoxic conditions in (i) the absence and (ii) the presence of hydrogen sulphide. Experimental water (double filtered, salinity: 35, temperature: 16 ± 0.5°C) was running through six single 250-ml experimental chambers each containing 100 ml sterilised sand and one juvenile *D. serra*. A detailed description of the experimental set-up is given in Publication III (p. 56 - 58). The experiment was repeated four times always

using fresh individuals. In total 72 clams were tested, 24 under normoxic, 24 under hypoxic and 24 under hypoxic-sulphidic conditions, respectively. Deoxygenation was achieved by bubbling nitrogen gas for 4 h through preheated (30-35°C) water and confirmed titrometrically (Grasshoff 1983). Hypoxic-sulphidic water was prepared by adding a hydrogen sulphide stock solution (approx. 10 mmol) in N_2-saturated experimental water until a final sulphide concentration of 0.1 mmol l^{-1} was reached. Regular pH controls confirmed that sulphide did not change the milieu of the incubation medium significantly (see also: Hahlbeck *et al.* 2000, de Zwaan *et al.* 2002). The set concentration was monitored spectrophotometrically (Fonselius 1976) during the course of the incubation every 2 h. Again, at each refill of the water reservoir, as well as at the start and at the end of the experiment, oxygen concentration was checked titrometrically (Grasshoff *et al.* 1983). Mortality assessment was based on failure of the valve closure reflex (Jahn and Theede 1997) every 2 h over a period of seven days. It was given as median survival time (LT_{50}) using the probability relation between percent mortality and time (Litchfield 1949). In order to test the reduction of survival time a log-rank-test was performed.

3.5.2 Short term incubations in the presence of sulphide (0.1 mmol l^{-1})

In addition, three groups, with six juvenile *D. serra* each, were introduced into the above-mentioned experimental set-up for seven different time periods. A total of 21 groups of test animals (3 replicates × 7 time periods) were investigated. Incubation was terminated after 0, 1, 3, 6, 12, 24 and 48 h of exposure. Thereafter the tissues of the test animals were dissected quickly, blotted dry and the pooled sample of each replicate was immediately stored in liquid nitrogen until biochemical analysis.

3.5.3 Biochemical analyses

All biochemical analyses are described in detail in Publication III and are summarised here. Sulphide oxidation products (sulphite, cystein, thiosulphate, glutathione) were quantified from pooled soft tissue samples of six juvenile *D. serra*. High-Performance Liquid Chromatography (HPLC) after derivatisation with monobromobimane (e.g. Völkel and Grieshaber 1994, Jahn *et al.* 1996, Jahn 1997) was applied, as described in Schiedek *et al.* (1997). Additionally, the contents of succinate, alanine, aspartate and glutamate were measured as indicators for the onset of anaerobiosis. The free amino acids were separated and determined via HPLC from perchloric acid extracts of frozen tissue powder (Schiedek 1997). Succinate was analysed from trichloric acetic acid extracts of frozen tissue powder via capillary electrophoresis and detected with a Photo Diode Array. This is a new method modified after "Agilent" (T. Hirse and H.-O. Pörtner, unpubl.) as the enzyme which was commonly used for this analysis is no longer available. Thiosulphate, succinate and amino acid concentrations were tested for statistical significance using ANOVA at the 5% level. Prior to the test, data of thiosulphate were square-root transformed, data of succinate were logarithmically transformed in order to achieve normality and induce homogeneity of variance.

3.6 GENETIC AND MORPHOLOGICAL COMPARISON OF *D. SERRA*

3.6.1 Morphometric and analytic methods

Four morphological variables of *D. serra* were measured according to Soares *et al.* (1998): shell length (antero-posterior), height (ventro-dorsal) and width (left-right) (lower 0.01 cm) and WM (nearest 0.01 g). Thereafter extracts were prepared by homogenizing soft tissue (except stomachs in order to exclude interference from nutrition) in an equal volume of distilled water. Allozymes were analysed by starch-gel electrophoresis (12% potato starch) using electrophoretic procedures, the method of interpretation of gel banding patterns and locus nomenclature described in van der Bank *et al.* (1992). The five buffers used to separate enzymes are specified in Publication IV (p. 70). Gels were stained for allozymes listed in Publication IV: Table 1 (p. 70) and nine additional ones (p. 69) which showed insufficient activity for interpretation.

3.6.2 Statistics

Descriptive statistics including percentage polymorphic loci (*P*), mean number of alleles per locus (*A*) and average heterozygosity (*H*) (Nei 1978) were calculated from electrophoretic results using BIOSYS-1 (Swofford and Selander 1981). Further, allele classes among populations were compared for all polymorphic loci using contingency χ^2 analysis. Allele frequency differences were integrated across loci by calculating genetic distances for all pairs of populations (Nei 1978, Wright 1978) using BIOSYS-1. Different fixation indices, e.g. F_{ST} (measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation) were calculated (Wright 1978) and tested (Brown 1970, Workman and Niswander 1970, Waples 1987) to analyse genetic differentiation between populations (see Publication IV, page: 71). Thereby the four populations were considered to be a sub-sample of a hypothetical compound population resulting from the sum of all analysed specimens. For an essentially one-dimensional array of sampling sites, such as described in this study, Wright's (1969) "island model" may be used to express approximate equilibrium levels of gene flow (expressed as the effective number of migrants per generation, N_{EM}) within a structured population. Pairwise N_{EM} values were estimated from F_{ST} according to Takahata (1983). The genetic distances, *D* (standard: Nei 1972) and D_{78} (unbiased and adapted for small sample sizes: Nei 1978) were calculated between populations. Thereafter the distance Wagner procedure was applied, using the coefficient of Prevosti distance, which measures the distance for a single locus as half the sum of the absolute differences between the allelic frequencies of the populations (Wright 1978). Criterion 3 (see Swofford 1981) was used to determine addition sequence. An unrooted tree of genetic relationship was computed using D_{78} values and its reliability indicated by values of cophenetic correlation and bootstrapping support values (Efron 1982, Felsenstein 1985) via DISPAN (Ota 1993).

4 GENERAL DISCUSSION AND CONCLUSIONS

In the following chapter the results of this investigation will be summarised and discussed. A more detailed discussion can be found in the attached publications. Section 4.1 will focus on population dynamics of Namibian *D. serra*, while section 4.2 will emphasise the effects of hydrogen sulphide on juveniles. Section 4.3 considers whether the dynamics of the population studied here are representative for other southern African *D. serra* populations, or whether each stock must be studied separately. Finally some future perspectives will be outlined (4.4).

4.1 POPULATION DYNAMICS OF NAMIBIAN *D. SERRA*

4.1.1 Growth

Growth mark analyses, both from surface rings and microgrowth internal lines are not suitable to estimate growth in *D. serra* inhabiting exposed Namibian sand beaches (Publication II). However, growth parameters could be estimated by fitting a common VBGF ($K = 0.274y^{-1}$, fixed $L_\infty = 82$ mm) to size-increment data pairs resulting from monthly LFD data (Publication II: Fig. 4) and tagging-recapture data. The low recapture rate of 0.15% is assumed to be caused by drift with currents as marked clams were recovered up to 450 m north of the release area, similar to findings of Dugan and McLachlan (1999) and to my own observations on short-term migration (see Fig. 2).

An inter- and intra-specific comparison of growth parameters referring to non-linear growth functions is difficult due to the antagonism of VBGF parameters. Several authors (e.g. Pauly 1979, Munro and Pauly 1983, Moreau *et al.* 1986) have demonstrated the suitability of composite indices of overall growth performance (OGP) for comparison purposes. The index P is proportional to the maximum rate of body mass increase during lifetime, i.e. the mass increase at the inflexion point of the VBGF (see Publication II for further details). OGP of Namibian *D. serra* (P = 4.7) coincide well with results obtained from two West Coast populations (Elands Bay: P = 4.7, Melkbosstrand: P = 4.7, calculated from data of de Villiers 1975 b) and a Southeast Coast population (Maitlands: P = 4.7 - 5.2, calculated from data of Schoeman 1997) (Publication II: Fig. 7). Evidently Donacid-OGP values are habitat specific (Publication II: Fig. 7): Lowest P-values are found in species inhabiting tropical/subtropical regions (2.5 - 3.3, group A), intermittent values are found in temperate species (3.7 - 4.3, group B) and upwelling donacids have highest P-values (4.7 - 5.2, group C).

It has been shown that increased food availability is positively correlated with growth in suspension feeding clams (e.g. Jensen 1992, 1993, Nakaoka 1992). Nutrition can however become a limiting factor even at exposed sandy beaches (Defeo 1992, Defeo *et al.* 1992, Lima *et al.* 2000). Accordingly high (Jarman and Carter 1981, Walsh 1981) and year-round (Schulz 1982, Weeks and Shillington 1994) primary production might be the reason for the detected higher OGP of upwelling donacids.

A narrower annual temperature variation (about 10°C, compared to boreal regions of about 30°C) enables stenothermic species to inhabit coastal upwelling systems (e.g. Guillou and Bayed 1991). Pörtner *et al.* (2000) have shown, that costs of mitochondrial maintenance are lower in stenothermal than in eurythermal species, thus narrow temperature margins might favour growth performance of upwelling-donacids.

Conclusions

- From length-frequency distribution and tagging-recapture data pairs a van Bertalanffy growth function with a fixed asymptotic length of 82 mm and an annual growth constant of 0.274 was established.
- Overall growth performance of Namibian *D. serra* corresponds well to that of South African West Coast and South Coast populations, which leads to the acceptance of the first part of Hypothesis 2 stating that growth from both provinces coincides.
- Regarding overall growth performance of donacid, *D. serra* fits in a group of upwelling donacids with high performance presumably caused by constantly high food availability and/or a low temperature range.

4.1.2 Biomass and production

Intertidal biomass of Namibian *D. serra* ranged between 141 and 546 g AFDM m^{-2} (Publication II: Fig. 6). Since the aim of the study was to estimate the intertidal, e.g. for human exploitation, biomass, the present calculation has to be considered conservative with regard to the whole population as adult individuals preferably inhabit the subtidal (Donn 1990 b, Soares *et al.* 1998). However, the presented estimate exceeds former ones of *D. serra* from warm temperate South African beaches (27 g AFDM, McLachlan *et al.* 1981; 754 g AFDM per running meter beach line, McLachlan and Hanekom 1979; 1 731 g AFDM per running meter beach line, Schoeman 1997) and is significantly higher than results reported from other several *Donax*-species (0.1 – 2.0 g AFDM m^{-2}, Ansell *et al* 1978, Warwick *et al.* 1978, McLachlan and van der Horst 1979, McLachlan *et al.* 1981, Mazé 1990, Wilson 1999). From the Humboldt upwelling area 70 g AFDM (Talledo 1980, Tarazona *et al.* 1985) were reported for *D. marincovichi* (formerly called *D. peruvianus*) and remarkable 910 g AFDM m^{-2} for the surf clam *Mesodesma donacium* (Arntz *et al.* 1987), which resembles *D. serra* in morphology and ecological role.

The annual intertidal production ranged between 167 g and 637 g AFDM m^{-2} y^{-1} and exceeded estimates of several entire donacid populations from habitats without upwelling (P = 0.7 – 6.0 g AFDM m^{-2} y^{-1}; Ansell *et al.* 1978, Warwick *et al.* 1978, Mazé and Laborda 1988, Wilson 1999). There is a lack of information on production for Donacidae from permanent upwelling areas, but values of *M. donacium* inhabiting the Humboldt upwelling system (2 400 g AFDM m^{-2} y^{-1}, Arntz *et al.* 1987) exceed the presented *D. serra* values.

The calculated intertidal production/biomass ratios ($P/\overline{B}$: 1.167 y^{-1} - 1.589 y^{-1}) are slightly higher than $P/\overline{B}$ rates of an entire warm temperate South African *D. serra* population (0.63 - 1.06 y^{-1}, Schoeman 1997). This might be due to a bias (exploitation concentrates on adults, adults concentrate in the subtidal) of the LFD towards smaller individuals with high somatic productivity ratios (see also Urban and Campos 1994). However the obtained results are in the range of *D. sordidus* ratios (1.30 y^{-1} - 1.78 y^{-1}, McLachlan 1979, McLachlan and van der Horst 1979).

The ecological role of donacids ranges from dominating species with low production to species accounting for low benthic biomass but high mass-specific production rates (Warwick *et al.* 1978, Ansell 1983). South African warm temperated East Coast *D. serra* is responsible for 94% of macrobenthic production (McLachlan *et al.* 1981) and contributes significantly to the regeneration of dissolved and particular organic nitrogen (Cockcroft and McLachlan 1993). Biomass of *D. serra* is partially consumed by crabs, birds and benthos feeding fish (McLachlan *et al.* 1980, 1996, Rossouw 1985, own unpubl. data), characterizing this bivalve as an essential trophic link in the beach/surf ecosystem.

Conclusions
- Intertidal biomass and production of *D. serra* exceed that of donacids inhabiting areas without upwelling. Consequently the latter part of Hypothesis 2, which states that production does not differ between provinces is rejected.
- Intertidal productivity exceeds that of a warm temperate population including adults.
- As a community-characterizing species, secondary production by *D. serra* is essential for the beach/surf ecosystem.

4.1.3 Reproduction and potential triggers

Namibian *D. serra* from study site I and I A reproduced annually in summer and recovered during autumn and early winter. At both beaches ripening of germ cells and spawning was first observed in August/September and lasted until January/February (in 1998 at Site I even until April) (Publication I: Fig. 3 b, f). Unexpectedly the residual gametes do not always degenerate after spawning and the intervention of a typical cytolysed state, followed by a pre-active and active state is not obligatory (Publication I: Fig. 4 a, b). The gametogenetic cycle is negatively correlated with the *CI*, which consequently decreased in spring 1998, clearly marking the main spawning activity. At Site I this decrease was initially not that evident in 1999 because a high proportion of adult clams were still maturing, while the proportion of releasing individuals was low. During the main spawning season most of the individuals analysed had released their germ cells. This was reflected in a decrease of *CI* followed by an increase because of already recovering specimens. At Site I A *CI* started decreasing in April 1998, while the gonads were still undeveloped presumably caused by degeneration of residual gam-

etes (highest recorded percentage, see Publication I: Fig. 3 b) and therefore reflected in a mass loss of the visceral mass. A decline due to the spawning activity in summer 1999/2000 could not be identified, clearly indicating that histological analysis are necessary to clarify the reproductive cycle of *D. serra*.

Correspondingly South African *D. serra* from a beach close to Site IV show a distinct seasonal gametogenetic cycle, but with spawning in late summer (February to April, Hanekom 1975). From the same region McLachlan and Hanekom (1979) and van der Horst (1986) also reported more or less discrete spawning peaks but during summer and winter. Similar spawning events in summer were observed in related bivalves from the Humboldt Current upwelling system (Urban and Campos 1994). By contrast, gametogenetic cycles of *D. serra* on the west coast of South Africa are less distinct (de Villiers 1975 a, Birkett and Cook 1987).

The process of maturation and spawning in molluscs is commonly controlled by SST (e.g. Alagarswami 1966: *Crassostrea virginia, Ostrea edulis, Mya arenaria, Mercenaria mercenaria*, Urban and Campos 1994: *Gari solida, Semele solida, Protothaca thaca*, Robinson and Breese 1982: *Protothaca staminea*, Sasaki *et al* 1997: *Spisula sachalinensis*). Coincidentally, the SST measured near Site I shows a seasonality comparable to the gametogenetic cycle (Figure 3 a, e), although the mature gonad stage is reached earlier in the year than the SST maximum. The relatively constant winter temperature or, alternatively, some warmer days in August/September after a cold period may trigger the maturation of germ cells. *D. serra* spawns when SST rises, possibly favouring larval growth and metamorphic processes (Sprung 1992, de Severeyn *et al.* 2000). The dissimilarity in gametogenetic cycles of South African *D. serra* populations may be a consequence of different annual SST ranges (Southeast Coast: 6.5°C, West Coast: 4°C; Hanekom 1975). Monthly mean SST near Site I (January 1997 - December 1999) showed an annual range of 5.6°C. If the hypothesis is right, this range could be sufficient to cause a distinct synchronous reproductive cycle as opposed to year-round spawning at a SST range of 4°C. The length of the spawning period is negatively correlated to the SST difference between summer and winter (Publication I, Table 1).

Conclusions

- Namibian *D. serra* show a discontinuous annual reproductive cycle (spawning in summer) related to the mean sea surface temperature.
- The gametogenetic cycle is negatively correlated with the condition index. Histological validation is needed.
- The length of the spawning period is negatively correlated to sea surface temperature.

4.1.4 Recruitment

Post-settled *D. serra* (2-6 mm) are abundant in the intertidal during distinct time periods, but recruitment may vary from year to year (Publication II, Fig. 4). This variability is in agreement with findings of other authors (e.g. Arntz *et al.* 1987). In contrast to Southeast Coast beaches juveniles were apparently uniformly distributed at the two Namibian sites (I, IA). There was no evidence that recruitment events may have been missed, since all tracked cohorts were already observed at the respective size range (Publication II, Fig. 4). Further, longshore movements of juveniles (see Fig. 2) favour a mingled distribution. In line with Schoeman (1997), monitoring showed that the *D. serra* belt is inhabited by similar size-classes. The zone inhabited by recruits varied between a minimal (12 m) and maximal breadth (40 m) (see Fig. 2) according to the beach slope (Publication I: Fig. 5).

The recruitment pattern at Site I in March and April 1999 (Publication I: Fig. 3 d) reflects the spawning event observed between September 1998 and February 1999 (Publication I: Fig. 3 b). Recruits appeared in the intertidal zone during two months only although it is evident that spawning took place over a period three times longer. The duration of the meroplanktonic larval time of upwelling donacids and subsequent growth until 2 mm shell length is still unknown (McLachlan *et al.* 1996) and the present results remain inconclusive for *D. serra*. A larval phase of at least two months is supported by the abundance of recruits in April 1999 at Site I. Larval periods exceeding this estimate are evident from abundances during winter 1998 and 1999 at Site IA (Publication I: Fig. 3 h). The appearance of juveniles at Site I in September/October 1998 may have different explanations including: (i) recruits correspond to the actual spawning, indicating a larval period shorter than four weeks, which is in line with Donn's (1987) estimates, (ii) planktonic residuals from the previous summer spawning event (1997/98) with delayed metamorphosis supporting the postulation of a 3-4 months meroplanktonic period (Birkett and Cook 1987) and (iii) recruits settled in the deeper subtidal due to substrate stability, hydrodynamic processes or geotaxis (Jackson 1986, Coon *et al.* 1985) and subsequently migrated to the intertidal (Bally 1983, Donn 1987, Lastra and McLachlan 1996) in spring after a period of growth cessation.

Despite the simultaneous gametogenetic cycle, recruitment patterns varied considerably in time and space, which is in accordance with observations from rocky shore mussels of the same region (Harris *et al.* 1998, B. Currie pers. comm.). Only in January 1999 were recruits found at both sites synchronously. Owing to the specific current patterns of the Benguela, larvae may have been spawned south of the collection sites and thereafter drifted northwards as indicated by gene flow (Publication IV: Fig. 3). If this is the case for the majority of larvae recruited from one sub-population, observed abundance patterns would not be related to the spawning events that took place at the collection sites, but would be more related to the oceanographic processes at any point in time.

Besides the above-mentioned explanations, other factors may be responsible for the observed patterns. Batch spawning reflected in low frequencies of empty gonadal alveoli (Publication I) in connection with the exposure of sibling larvae to dissimilar environmental conditions may cause variation (Strathmann 1974, Olson and Olson 1989). Unfavourable hydrodynamic processes such as currents and wave action may prolong the larval period and cause spatial and temporal variation in larval concentration and settlement patterns (e.g. Kingsford 1990, Zimmerman and Pechenik 1991, Ebert *et al.* 1994, Harris *et al.* 1998). Moreover, food limitation may extend larval time and decrease abundance because of longer exposure to predation and starvation (Langdon 1983, Olson and Olson 1989, Sale 1990). The prolonged larval development may further result in an extended exposure to unfavourable chemical conditions, e.g. hydrogen sulphide, which influences larval and juvenile survival (Publication III, McArthur 1998, reviews: Vismann 1991 b, Diaz and Rosenberg 1995). Hydrogen sulphide events are very variable in extension (some hundred meters to kilometres, own. observ.) and thus may affect areas differently.

Conclusions
- Intertidal juvenile *D. serra* are abundant only during distinct time periods which vary between years.
- The period when juveniles are abundant appears to be decoupled from the spawning period and cannot be predicted clearly. Therefore Hypothesis 1 affirming that recruitment and gametogenic activity are closely coupled is rejected.
- Variations in biotic and abiotic conditions along with dissimilar release times within the spawning period may explain temporal and spatial differences in recruitment.

4.1.5 Mortality

Mortality rates could not be calculated for the studied *D. serra* populations since older individuals could not be included in the assessment. It is apparent, that estimates on sub-populations excluding the adult proportion are inaccurate: Results of immature clams ($Z = 4.26\ y^{-1}$, McLachlan and Hanekom 1979) are in discrepancy with significantly lower values ($Z = 0.59$, calculated from Donn 1993; $Z = 0.6\text{-}1.1\ y^{-1}$, Schoeman 1997) for the same population (Eastern Cape). These estimates may not be applicable for Namibian populations, as apart from senescence, predation is a significant mortality source and predators differ between coasts. In the Eastern Cape McLachlan *et al.* (1980) found extensive predation on *D. serra* by shore birds but results cannot be transferred to West Coast beaches because of differences in bird composition and abundance. For instance the mean number of Kelp Gulls observed at 128 days during the present study period at Site I was significantly higher (27.3 ind./km shoreline, max. 167 ind./km shoreline, own unpubl. data) than at the Southeast Coast beaches (4.74 - 12.01, McLachlan *et al.* 1980). Further, *D. serra* constituted 33% of Kelp Gulls' (*Larus dominicanus*) diet in the Eastern Cape ($N = 17$, McLachlan *et al.* 1980) while it ac-

counted for 50% at Site I and IA (N = 20, unpubl. data). Whitefronted Sandplover (*Charadrius marginatus*) did not ingest the tips of *D. serra* siphons at the present study sites (N = 20, unpubl. data) and Chondrichthyes feeding on *D. serra* (Rossouw 1985, du Preez *et al.* 1990) differ significantly in abundances between both coasts (J.A. Holtzhausen, pers. comm.).

Processes in the abiotic environment, e.g. low oxygen or hydrogen sulphide events have the potential to dissimilarly reduce survivorship of *D. serra* (Bailey 1999, Publication III). Additional to the natural mortality, human induced reduction of the stock may differ significantly between coasts.

Conclusions
- Mortality rates of Namibian *D. serra* were not estimated due to inadequate assessment of adults.
- Mortality rates of Eastern Cape populations are not representative for Namibian populations, as biotic and abiotic mortality sources are variable between coasts.

4.2 HYPOXIA AND HYDROGEN SULPHIDE

4.2.1 Survival under severe hypoxia and hydrogen sulphide

Spawning of ripe *D. serra* could not be induced in the laboratory during this 27-months study. Identification of larvae in plankton hauls was also not successful. Since the susceptibility for hypoxia and hydrogen sulphide theoretically decreases with increasing size due to a lower surface to volume ratio (Jahn *et al.* 1997) which was also experimentally proven in bivalves (e.g. *Theora lubrica*, Imabayashi 1986; *Mytilus edulis*: Wang and Widdows 1991, C. Bittkau unpubl. data), juveniles of the smallest size class were tested. Under severe hypoxia all test animals survived for more than four days (LT_{50}: 110 hours, Publication III: Fig. 2), whereas hypoxic-sulphidic conditions reduced survivorship significantly (LT_{50}: 80 hours). Non-dissociated hydrogen sulphide diffuses easily through biochemical membranes (Powell 1989, Julian and Arp 1992) and inhibits reversibly the last enzymatic reaction of the respiratory chain by forming a stable complex with cytochrome c oxidase. In the presence of sulphide, aerobic respiration is therefore impossible (Nicholls 1975, Nicholls and Kim 1981, 1982).

Conclusions
- Under severe hypoxia all juvenile *D. serra* survived for more than four days.
- Survival time is significantly reduced under hydrogen sulphide exposure.

4.2.2 · Anaerobic energy production

To survive under long term hypoxic conditions many euryoxic marine bivalves and other invertebrates use alternative pathways of energy production (e.g. Storey and Storey 1990, de Zwaan 1991, Grieshaber *et al.* 1994). Frequently the anaerobic product succinate is accumulated in soft tissue (e.g. Demers and Guderley 1994, Sukhotin and Pörtner 1999). Accordingly, post-settled *D. serra* switched to anaerobiosis almost directly after the onset of hypoxic-sulphidic conditions, evoking a nine-fold increase in succinate levels (Publication III: Fig. 3 b). Starting from a relatively high concentration, alanine values also became higher, further indicating anaerobiosis (Publication III: Fig. 3 c). This energy production might be a regular strategy used by intertidal species during low tide since the penetration of oxygen into the sediment is not sufficient to support aerobiosis (reviews: Schöttler and Bennet 1991, Grieshaber *et al.* 1994). Prior to acclimation alanine might already have been accumulated to an elevated concentration due to air exposure (low tide and subsequent transport) explaining the high initial level. The anaerobic metabolism includes a significant energy saving strategy leading Cockcroft (1990) to assume that this might explain the obvious success of *D. serra* on southern African coasts. However, anaerobiosis leads to a faster breakdown of glycogen, which is known to be the major energy resource during long-term anaerobiosis (Schöttler and Bennet 1991) and thus may limit survival upon its depletion. The glycogen content was not measured in the tested bivalves because of limited amount of tissue, but juvenile stores are likely to be lower than adults as is the case in other marine invertebrates (e.g. Schiedek and Schöttler 1990). Besides the limiting energy reserves the proliferation of anaerobic pathogenic bacteria, firmly associated with bivalves, can also be a prominent cause of death under hypoxic conditions (de Zwaan *et al.* 2001, 2002).

Conclusions
- Juvenile *D. serra* switch to anaerobic metabolism when exposed to hypoxic-sulphidic conditions which leads to the rejection of Hypothesis 3 stating that juveniles are not resistant.
- Increased mortality is thought to be due to the depletion of glycogen and proliferation of anaerobic pathogenic bacteria.

4.2.3 Detoxification of sulphur compounds

Marine bivalves regularly exposed to hydrogen sulphide have acquired a variety of biochemical adaptations including the detoxification of sulphur compounds to less or non-toxic oxidation products (Cary *et al.* 1989, O'Brien and Vetter 1990). These products can also be found in other marine invertebrates (Vismann 1991 a, Völkel and Grieshaber 1995). In juvenile *D. serra* non-toxic thiosulphate, the only product of mitochondrial sulphide oxidation, accumulated immediately from the onset of exposure (af-

ter 24 h: 0.148 μmol g^{-1} wet mass, WM; Publication III: Fig: 3 a). This suggests that test animals used oxygen, which was stored in body fluids to detoxify penetrating hydrogen sulphide while they had already switched to anaerobiosis. Additionally, oxygen remains in the incubation water (< 0.3 ml l^{-1}, due to the remaining oxygen in the nitrogen gas corresponding to the purity grade) and might be utilised. Accordingly, the observed decreasing thiosulphate concentration (> 24 h) is most likely indicating a lower production, caused by the diminishing oxygen tension, along with outward diffusion. As the substance is highly soluble, it can not be accumulated to infinite concentrations within tissues. Grieshaber and Völkel (1998) concluded that it is generally not further metabolised and no thiosulphate transport system has been identified in invertebrates (Hauschild *et al.* 1999).

The observed maximal thiosulphate concentration is in the same range detected in the Baltic clam *Macoma balthica* (0.177 μmol g^{-1} WM, Jahn and Theede 1997) but lower than found in other marine invertebrates (*Marenzelleria* cf. *wireni*: 1 μmol g^{-1} WM, Schiedek *et al.* 1997, *Arenicola marina* 30 μmol g^{-1} WM, Grieshaber *et al.* 1995, *Hediste diversicolor*: 100 μmol g^{-1} DM, Hahlbeck *et al.* 2000).

Remarkably, after hypoxic-sulphidic exposure no sulphide could be detected in the tested bivalves' tissue. Therefore it is assumed, that detoxification is very efficient for a period of about 24 hours when exposed to 0.1 mmol l^{-1} hydrogen sulphide. This concentration was measured in the coastal area during sulphide eruptions (A. van der Plas, pers. comm.).

Conclusions
- Juvenile *D. serra* oxidise penetrating hydrogen sulphide to non-toxic thiosulphate.
- Tested clams have a high sulphide detoxification capacity within the initial 24 hours.

4.2.4 Behavioural adaptation under hypoxic-sulphidic exposure

In contrast to epibenthic mussels, which close their shells to avoid the contact with toxic hydrogen sulphide (*Mytilus edulis*: Jørgensen 1980, *Perna perna* Schiedek and Currie, unpubl. data), juvenile *D. serra* migrated to the sediment surface under hypoxic- and hypoxic/sulphidic conditions and extended their siphons into the water column. This behaviour was also observed from other infaunal bivalves during experimental (*Mulinia lateralis*: Shumway *et al.* 1983, *Abra alba*: Rosenberg *et al.* 1991, *Scrobicularia plana*: Oeschger and Pedersen 1994) and field studies (*Mya arenaria*, *Cerastoderma edule* and *Abra alba*: Jørgensen 1980). The permanent siphon expansion may reflect a strategy to monitor the water for better conditions without being coupled to high ventilation activity as observed for *S. plana* (Oeschger and Pedersen 1994).

The induced surfacing of juvenile *D. serra* along with the ability to deal with toxic hydrogen sulphide for one day appears to be a behavioural adaptation to the specific conditions prevailing under coastal "sulphide eruptions" within the Benguela current system. These events often impact only a restricted surface area of some 100 m^2 (Currie 1999, pers. observ.). Therefore, migration to the sediment surface favours the drift to unaffected areas which seems to compensate for disadvantages like a possible wash up followed by overheating and/or predation (McLachlan *et al.* 1980, Phil *et al.* 1992, unpubl. data). However, moving to the surface and thus facilitating along and across shore movements are even common under normoxic conditions (see Fig. 2, Donn *et al.* 1986).

Conclusions
- Under hypoxic- and hypoxic/sulphidic conditions *D. serra* migrates to the sediment surface and extends its siphons.
- Surfacing increases the possibility of drift to areas with more favourable conditions.

4.3 INTERPOPULATIONAL COMPARISONS OF *D. SERRA*

4.3.1 Morphological comparison

Morphological comparisons were conducted to relate morphological differences to genetic variability of four *D. serra* populations separated by up to 2 500 km of shoreline. West Coast *D. serra* were significantly different (rounder, flatter and less wedge-shaped) from Southeast Coast clams (Publication IV: Fig. 2) supporting previous studies by Donn (1990 a) on adults and Soares *et al.* (1996) on juveniles. Habitus characteristics may be exclusively determined genetically (Rothwell 1993), or by the interaction between genes and the environment (Pigliucci 1996, Soares *et al.* 1999). Environmentally determined plasticity may buffer evolutionary changes (Grant 1991) while directional selection pressure acting upon a diverse, genetically determined phenotype will initiate it (Soares *et al.* 1999). Thus more wedge shaped, elongate clams would be selected for in the intertidal (Southeast Coast) because they swash-ride more efficiently, burrow faster and consequently are less exposed to predators. In contrast flat, disc shaped shells are selected for on the West Coast due to increased stability in the subtidal habitats (Donn 1990 b).

Genetic determination of habitus differences appears unlikely as analysis of allozymes group Site I and Site IV together. Thus it is much more probable that different shell shapes are the result of environmental impacts resulting from phenotypic plasticity. Transformation experiments of marked spat could verify this hypothesis.

Conclusions
- *D. serra* from the cold province are significantly rounder, flatter and less wedge-shaped than clams from the warm province.
- Shell shapes are likely to be the result of environmental impacts resulting from phenotypic plasticity.

4.3.2 Genetic comparison

The genetic analyses of twenty-two protein-coding loci from the four *D. serra* populations (I, II, III, IV) revealed genetic variation in the range commonly found in marine bivalves (Publication IV: Table 4) while no distinct fixed allele patterns were observed. Genetic differentiation (D_{78} = 0.003 - 0.044, Publication IV: Table 3) was in the margin for conspecific populations (0 - 0.05, Ferguson 1980) and compared well with *Mytilus galloprovincialis* (*D*: 0.032 ± 0.001, Quesada *et al.* 1995) and *Macoma balthica* (*D*: 0.025 - 0.029, Meehan 1985). Moreover, the obtained results were expectedly lower than those of sympatric *D. variabilis* and *D. parvula* populations (Nelson *et al.* 1993) and values between *Mytilus* species (D_{78}: 0.16 - 0.28 based on 16 - 23 loci, Skibinski *et al.* 1980, Grant and Cherry 1985, Väinölä and Hvilson 1991).

The low genetic dissimilarity of the studied populations (in discrepancy to the morphological differences) is supported by relatively low *F*-values of differentiation among subpopulations (F_{ST} = 0.016 - 0.089), i.e. less than one tenth of the allozyme diversity was explained by dissimilarities between samples, and more than 90% by variation within local populations. This supports the idea of Brown and McLachlan (1990) who postulated that invertebrates inhabiting exposed sandy beaches are generalists and possess co-adapted genomes supporting high plasticity. This allows them to withstand and rapidly adapt to the dynamic of such habitats and may override stochastic processes (e.g. random genetic drift and mutation) which promote divergence of populations (Kreitman and Akashi 1995). If different phenotypes are fit for different environmental conditions (Sultan 1995), the species may theoretically survive long geological periods without genetic alteration.

Nevertheless, the observed low allozyme differences revealed that five loci (EST-2, PEP-B2, PEP-C2, PGM-1, PGM-2) show sufficient demarcating variation for two conspecific groups (Publication IV: Fig. 1). Allozymes influence growth rates and overall fitness (Koehn and Gaffney 1984, Singh and Green 1984), thus selection for the most advantageous enzyme related to environmental factors can result in allozymic heterogeneity of differently exposed populations. As analysed loci within subpopulations were affected variably, I postulate different sensitivity of the enzyme systems to various environmental selective forces. In accordance Gartner-Kepkay *et al.* (1980) observed that very variable environments were reflected in variable genomes of *M. edulis* populations.

Specific activities of enzymes may be induced by temperature (Lombard and Grant 1986). Selection in the studied *D. serra* populations is apparently related to SST combining Population II and III, which inhabit the cold bases of upwelling cells and the two outlying populations with only 3°C temperature difference during summer (Shannon and Nelson 1996). The observed repeated trend in the parameters *A*, *H*, and *P* supports this hypothesis. Further *D*-values are highest, when a cold and a warm site are paired.

Although gene flow (N_{EM} = 1.44 - 8.65; N_{EM} > 1 counteracts differentiation: Slatkin 1987) can explain the overall similarity, it is unlikely to explain grouping of the outlying populations: Gene import to Population IV is much lower than to Population I (Publication IV: Fig. 3) and by-passed intermediate populations are less similar. Therefore an analogous trend again related to SST is more likely to explain the combination than natural gene flow.

The analyses further revealed a geographic separation of the two Namibian populations (I and II), which is consistent with a postulated biological discontinuity in the vicinity of Meob Bay (e.g. van der Bank and Holtzhausen 1998/99). This biotic barrier could be caused by a) a constant cold water filament of the major upwelling cell (e.g. Shannon 1989, Publication IV: Fig. 1), b) a deflection of the poleward under-current located in the vicinity of Meob Bay (*cf.* Monteiro 1999) or c) the combination of changes in circulation, turbulence and stratification (Agenbag and Shannon 1988). Moreover, the present genetic distribution may also reflect historical patterns of gene flow influenced by large-scale climatic changes ascertained for the Atlantic over the Pleistocene (e.g. Thunell and Belyea 1982). However, the distinction of the two Namibian stocks requires separate analyses of population dynamics. If the parameters are not similar, proper management of future commercial *D. serra* harvests must be adjusted respectively.

Conclusions

- *D. serra* populations are conspecific and possess genetic variation in the range of most other marine bivalves, thus Hypothesis 4 stating that populations from both provinces are not separated genetically is accepted.
- A discrepancy between morphological differences and genetic similarity exists most likely due to phenotypic plasticity.
- Selection in the studied *D. serra* populations is apparently related to sea surface temperature.
- The substantial subdivision of two Namibian populations requires separate analyses of population dynamics.

4.4 FUTURE PERSPECTIVES

The human population is growing at an exponential rate (e.g. Cohen 1996) and coastal regions are attracting increasing numbers of residents (Sahrhage and Lundbeck 1992). This places enormous demands not only on food resources, but also on sources of employment. *D. serra* might be a valuable species for future aquaculture due to its high abundance, production rate, use as bait and its potential economic value for export markets (Sims-Castley and Hosking, in review). Therefore, some consideration and propositions promoting its aquacultural use along with the additional possibility to close gaps in our knowledge of its ecology will be specified:

- There is a substantial lack of information on recruitment processes of *D. serra*. Future research should focus on early life history and the possibility to culture larvae under different abiotic and biotic conditions. This information is particularly needed to produce spat for bivalve cultures, but additionally will confirm the strength of larval connections between sub-populations. In the latter regard studies may involve surveying genetic variation in newly settled wild larvae and juveniles in particular.

- The genetic analyses revealed allelic diversity particularly at the peptidase (PEP) locus which is in agreement with findings for *M. edulis* and *Geukensia demissa* (Young *et al.* 1979, Garthwaite 1986). Different PEP-E genotypes provide differences in growth rate and tissue weight (Koehn and Gaffney 1984, Garthwaite 1989). In consequence this is a useful locus for selective breeding.

- Growth of suspension feeding bivalves is related to food availability (e.g. Defeo *et al.* 1992, Jensen 1993, Lima *et al.* 2000). *D. serra* appears to feed on a wide variety of particles, with respect to both origin and size (< 40 μm) (Stenton-Dozey 1989, Matthews *et al.* 1989). Analyses of the digestive tract content in combination with the composition of trophic markers (lipids and fatty acids: e.g. Graeve *et al.* 2001, pigments: e.g. Abele-Oeschger *et al.* 1992) can help to clarify the feeding mode and detect prey items of Namibian *D. serra*. Further *in-situ* experiments should be conducted to estimate assimilation efficiency and clearance rates under diets with varying nutritional values (Brown *et al.* 1989, Stenton-Dozey 1989), such as different laboratory cultured algae species, to get prerequisite information for nutrition under rearing conditions.

- During this study *D. serra* was successfully maintained in natural sediment using a flume tank with additional flow of unfiltered seawater. Future work should focus to rear this clam by keeping it in sediment filled boxes, which can be placed in the channel system of the local austere farms (owners are interested to participate in a practical approach). The channels provide flow conditions as they connect a higher levelled and continuously filled seawater pond with the shore. The warming of the nutrient rich upwelling water during its stay in the pond results in high primary production evoking perfect feeding conditions for the austeres and presumably for *D. serra* as well.

• Many aquaculture systems suffer from anaerobic degradation of surplus organic material by sulphate reducing bacteria (e.g. Stenton-Dozey *et al.* 2000, Mitra and Patra 2001). This leads to the production of hydrogen sulphide. Presented *in-situ* experiments revealed significant effects of sulphide on juvenile *D. serra*. A follow up on present knowledge should stress the impact on different larval stages as well as on adult populations. Moreover, the experimental *set-up* provides the opportunity to confirm a community structuring effect of naturally occurring hydrogen sulphide: Namibian macrozoobenthos is separated into two zoogeographical provinces, but species of the southern province reappear further north in a small pocket at the vicinity of Möwe Bay (Currie 1999). This site is apparently not associated with the post-upwelling cell and thus not impacted by hypoxia and hydrogen sulphide. Therefore it seems reasonable to test the capacity of different taxa from both zoogeographical provinces to deal with hydrogen sulphide exposure and demonstrate the role of hydrogen sulphide as a structuring factor.

• *D. serra* tolerates intertidal habitats with wide temperature ranges. *In-situ* experiments have revealed that even in aerated water oxygen limitation at cold as well as warm critical temperatures sets in prior to functional failure in several marine invertebrates (Zielinski and Pörtner 1996, Sommer *et al.* 1997, Sommer and Pörtner 1999, Frederich and Pörtner 2000). Therefore, future work should define the optimal temperature window of settlement, growth and reproduction that is required for successful maintenance of an aquacultural stock. Furthermore, pejus (pejus = getting worse) temperatures have been shown to closely match temperature limits of species' geographical distribution in different habitats (*cf.* Frederich and Pörtner 2000, Pörtner 2001). Thus pejus temperatures should be correlated with the zoogeographical distribution.

• During the present histological examination sporocysts and redia of a trematode were recorded in *D. serra* for the first time. It is not clear whether and to what extent the parasites may be harmful to the natural stock. Trematodes may render clam individuals sterile (Coe 1953), but the observed infection rate of 1% (Site I) does not seem to be crucial for the populations. However a bivalve culture may be endangered considering high clam densities. Trematode infections have also been observed from other *Donax* species (*D. gouldi:* Coe 1953, *D. vittatus*: Pelseneer 1928, *D. trunculus*: Ramón *et al.* 1999) and most infestation occurred in advance of mass mortality, albeit authors stressed that these parasites were not necessarily responsible. For future aquaculture activities a precise knowledge of possible impacts, the life cycle of this parasite and opportunities to protect the culture is essential.

5 PUBLICATIONS

This thesis includes four publications listed below. Additionally, my contribution of each study is explained.

Publication I

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

I developed the conceptual approach and the sampling design to analyse reproduction and recruitment of Namibian *D. serra*. The laboratory work, processing of the data, analyses and interpretation were done by myself and discussed with the second author. After writing the manuscript, it was discussed and improved with the co-authors.

Publication II

Laudien, J., Brey, T. and Arntz, W.E.
Population structure, growth and production of the surf clam *Donax serra* (Bivalvia, Donacidae) on two Namibian sandy beaches.
Estuarine, Coastal and Shelf Science.

The concept and initial idea of this article to analyse the population structure, growth and production of Namibian surf clams was elaborated by the three authors. I conducted all the practical work and the data processing. Thereafter the data analysis procedure for the growth estimates was developed in co-operation with the second author. My manuscript draft was discussed and revised with both co-authors.

Publication III

Laudien, J, Schiedek, D., Brey, T., Pörtner, H.-O. and Arntz W.E., 2002
Survivorship of juvenile surf clams *Donax serra* (Bivalvia, Donacidae) exposed to severe hypoxia and hydrogen sulphide. *Journal of Experimental Marine Biology and Ecology, 271, 9-23.*

Myself developed the original idea for this publication. I developed the experimental set up and design and carried out the exposure experiments. Biochemical analyses were discussed with the second and the second last author. I analysed the data and discussed the concept of the manuscript with the second author. The final version was achieved with the joint work of the co-authors.

Publication IV

Laudien, J., Flint, N.S., van der Bank, F.H. and Brey, T. Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches.
Biochemical Systematics and Ecology.

The idea and the original conceptual approach to study the genetic and morphological variation in populatioins of *D. serra* were elaborated by myself. I conducted under aid of the second author all the practical work and the data analysis. The third author assisted while interpreting the results. My first version of the manuscript was improved by the comments of the three co-authors.

PUBLICATION I

REPRODUCTION AND RECRUITMENT PATTERN OF THE SURF CLAM *DONAX SERRA* (BIVALVIA, DONACIDAE) FROM TWO NAMIBIAN SANDY BEACHES.

Laudien, J., Brey, T. and Arntz, W.E. (2001)

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ABSTRACT

Reproduction and recruitment of the surf clam *Donax serra* on two Namibian beaches were studied over a period of two years. Histological examination of the gonads indicated a discontinous annual reproductive cycle, related to monthly mean sea surface temperatures. The spawning season lasted from August/September until February but juveniles (2-6 mm anterior-posterior shell length) were only present for three months in the intertidal zone. The condition index indicates that the species spawns during autumn and summer, but histological validation is needed. The period when juveniles are abundant is decoupled from the spawning period and therefore cannot be predicted clearly, even if the spawning time is known. Starvation, hydrodynamic processes, chemical parameters and different release times during the spawning period are thought to cause the differences in settlement time and in recruitment strength between locations.

Keywords: condition index, *Donax serra*, histology, Namibia, recruitment, reproduction, sandy beach ecology

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INTRODUCTION

Fuelled by upwelling, the Benguela ecosystem off Namibia is one of the most productive marine systems in the world (Jarman and Carter 1981, Schulz 1982). The macrozoobenthos of the intertidal and shallow subtidal of large exposed Namibian sandy beaches is typically dominated by the surf clam *Donax serra* (Röding, 1798) (McLachlan 1985, 1996, McLachlan *et al.* 1996, Donn and Cockcroft 1989), but despite its abundance many aspects of its population dynamics in Namibian are unknown.

The reproductive biology of *D. serra* elsewhere in southern Africa is documented in other studies, but it is summarized here. Sexual differentiation occurs at a mean length of 44 mm in the Eastern Cape (McLachlan and Hanekom 1979) and 48 mm on the South African west coast (de Villiers 1975 a). Sexes can only be distinguished histologically (de Villiers 1975 a). The exact duration of the meroplanktonic larval phase is not known exactly and is therefore subject to some speculation. Whereas Lastra (1994) speculated on a value of 1-2 weeks, Birkett and Cook (1987) estimated 3-44 months, both for South African west coast populations. Early post-larval *D. serra* settle subtidally after metamorphosis and subsequently migrate to the upper intertidal (Donn 1987, Lastra and McLachlan 1996). The factors that promote settlement and habitat selection are unknown, but grain size is significantly correlated with the abundance of recruits (Lastra and McLachlan 1996). At the time of settlement, spat are approximately 1-1.5 mm in shell length and resemble adults closely (Lastra 1994).

In this paper, some information is given on the reproductive biology of *D. serra* populations that inhabit two Namibian sandy beaches subject to different levels of exposure to wave action. Gametogenic activity and recruitment are compared.

MATERIALS AND METHODS

Research area and sampling

The study was carried out at two Namibian sandy beaches (Fig. 1), Langstrand (22°47′S, 14°33′E) and Paaltjies IV (22°59′S, 14°24′E). According to McLachlan's (1980) rating scale for exposure, Paaltjies IV can be characterized as very exposed and reflective and Langstrand as exposed and of an intermediate type, being partly recessed behind the sand peninsula known as Pelican Point (Fig. 1).

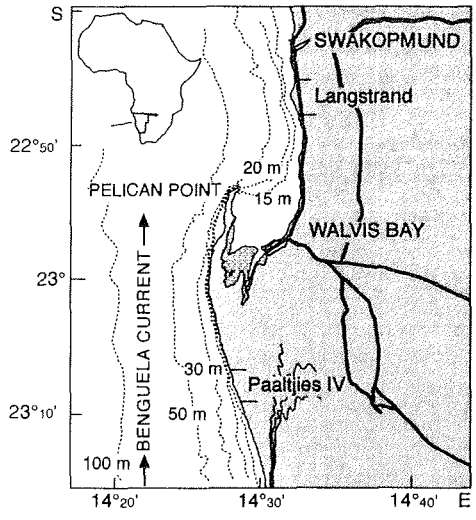

Fig. 1: Map showing the sampling sites Langstrand and Paaltjies IV and the nearby location in Swakopmund where sea surface temperature measurements were taken.

The area is subject to subequal semidiurnal tides with a maximum tide range of 2 m; spring tides average 1.4 m and neap tides 0.7 m. Sea surface temperature (SST) varies between 11°C in winter and 23°C in summer. A summary of the main abiotic features of the two beaches is given in McLachlan (1985).

A total of 50 adult *D. serra* (> 54 mm) was collected at monthly intervals (Langstrand: February 1998 to November 1999, Paaltjies IV, January 1998 to December 1999). Between sites, sampling time was moved two weeks between full (at Langstrand) and new moon (at Paaltjies IV). Surf clams were dug out by hand in the surf zone at haphazard sampling sites during low tide. No more than three clams were dug out from the same site. In addition, recruits (here defined as clams ranging from 2 to 6 mm shell length) were sampled quantitatively at a series of stations (2 m intervals) along a transect transverse to the shoreline. At each station, three replicates of a 0.16 m^2 sample were excavated to 35 cm depth using a stirring box and the sand sieved on a 1-mm screen. All clams were taken to the laboratory immediately for further investigation. A subsample of 20 adult individuals was preserved in buffered formalin for histological sectioning.

Gametogenic cycle

Tissue samples (Fig. 2) of formalin-preserved adult *D. serra* were embedded in paraplast wax. Histological sections (1-3 µm, GIEMSA stained) were produced according to standard methods (Romeis 1989). Gonads (458 from Langstrand and 456 from Paalt-

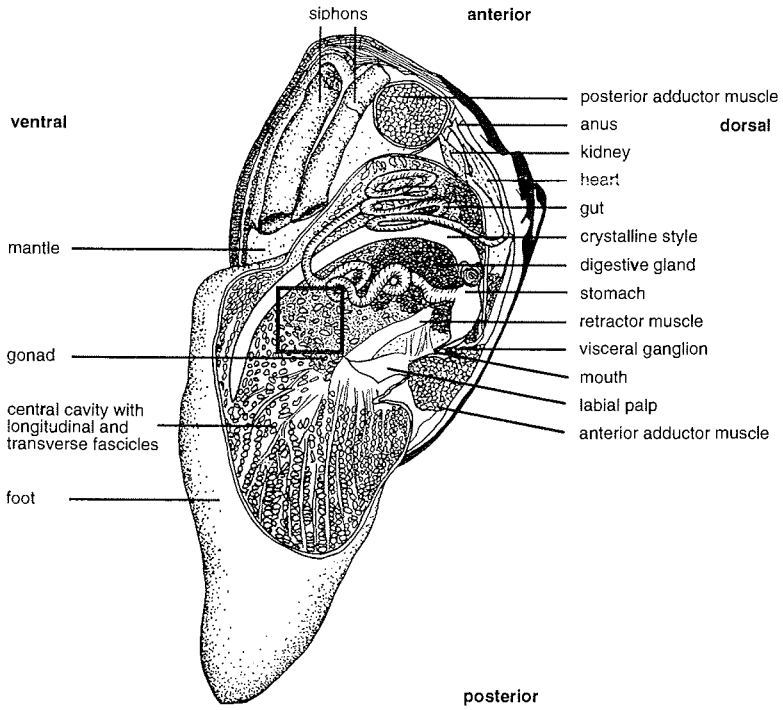

Fig. 2: Anatomy of *Donax serra*. The square shows the location of the tissue sample used for histological examination

jies IV) were classified into four stages of development (cytolysed, inactive, active, spawning) by microscopic examination, based on the description of de Villiers (1975 a). The stages are summarized below.

In the *cytolysed* stage, reproductive material appears completely degenerated, and alveoli are very small and wide apart. Phagocytic cells are present in massive numbers. Before gametogenesis at the *inactive stage*, the reproductive material is typically scant and intersected by broad, continuous transverse muscular fascicles. Alveoli are well formed, small, sometimes separated and usually filled with follicle cells. After spawning the quantity of reproductive material in inactive forms varies. Alveoli appear loosely arranged, scattered with a prominent portion of transverse muscular fascicles. Phagocytes are common. At the *active stage*, germ cells are in various stages of development and fill the alveoli. Alveoli are large and their walls are always complete and close together. There are few or no phagocytes or follicle cells. *Spawning stage* animals show clear signs of a recent loss of gametes. The reproductive material varies in quantity, but it is fairly abundant. The alveolar pattern is disturbed, walls are broken and alveoli appear flattened.

Condition index and recruitment

Seasonal variation in the weight of the total visceral mass was used to track changes in gonad weight by calculating a condition index (*CI*). Total wet weight was recorded to the nearest 0.1 mg immediately after collection and drying on absorbent paper. Thereafter valves, mantle, siphons, retractor and adductor muscles (Fig. 2) were removed. The wet weight of the resulting standard compact unit consisting of the visceral mass and foot, was recorded. The *CI* was calculated according to the equation of de Villiers (1975 a), namely $CI = 100W_v / (W_t - W_v)$, where W_v is the wet weight of the visceral mass (including the foot) and W_t is the total wet weight.

Recruits in the sampling transect were counted and measured (anterior-posterior shell length) at each sampling date on both beaches.

RESULTS

Monthly mean SST displayed seasonality, with values highest in January/February and lowest temperatures between May and October. The mean annual range was 5.6°C (Fig. 3 a, e). The morphology of D. serra was assessed macroscopically to define the region in which most of the gonad was located; thereafter, tissue samples were taken (Fig. 2). The percentage of mature *D. serra* gonads (both sexes) in each gonad stage is shown in Figure 4. Sex ratios did not deviate significantly from unity (Langstrand, 225 males and 220 females, $\chi^2 = 0.086$, $df = 1$, $p>0.01$; Paaltjies IV, 220 males and 235 females, $\chi^2 = 0.43$, $df = 1$, $p>0.01$). No case of hermaphroditism was found at either beach. The gonads of both populations were mature in summer and inactive during autumn and early winter, without the intervention of a typical cytolysed state (Fig. 4).

The gametogenic cycle at Langstrand (Fig. 3b) was correlated with the mean *CI*, which decreased significantly three times (Fig. 3 c): from June to August 1998 (ANOVA, $p<0.05$), from October 1998 to January 1999 (ANOVA, $p<0.05$) and from July to Sep-

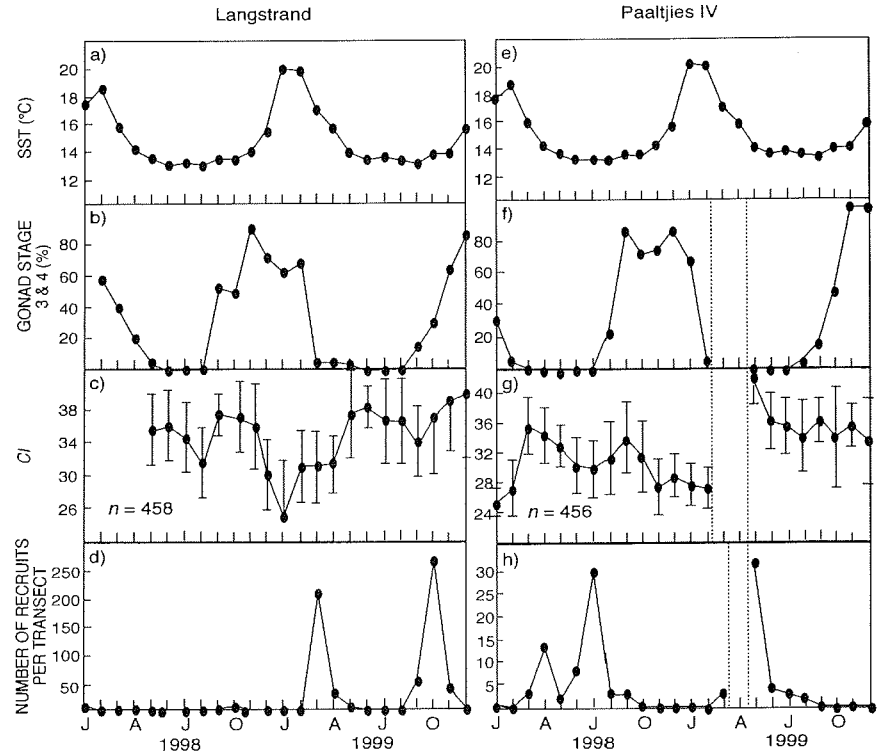

Fig. 3: (a) and (e) Mean SST measured daily (07:00) at Swakopmund, January 1998 to December 1999, the percentage of gonad stages ripe (Stage 3) and spawning (Stage 4) at (b) Langstrand and (f) Paaltjies IV, the mean condition index (*CI*) for monthly samples of adult *D. serra* at (c) Langstrand and (f) Paaltjies IV, and the number of *D. serra* recruits (2–6 mm) recorded per monthly transect at (d) Langstrand and (h) Paaltjies IV. Times when no collections were made are indicated.

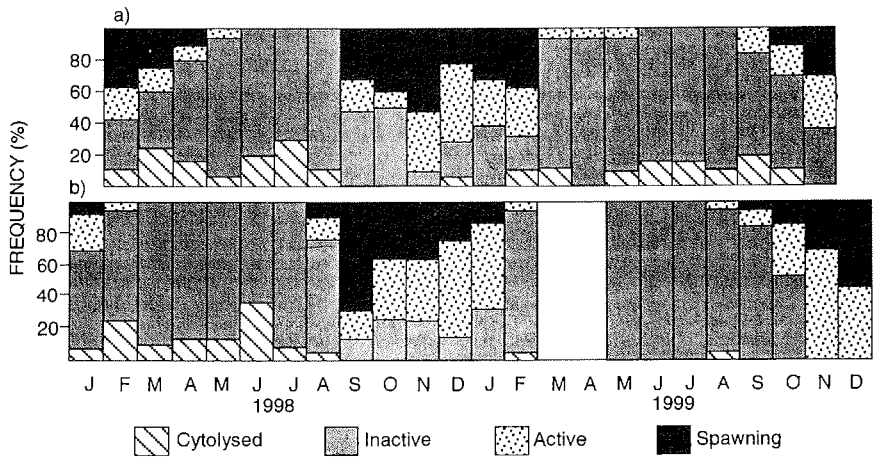

Fig. 4: Distribution of gonad states in mature *D. serra* gonads (both sexes; after De Villiers 1975) sampled at (a) Langstrand and (b) Paaltjies IV. Note sampling time between locations was shifted two weeks between full moon (Langstrand) and new moon (Paaltjies IV).

tember 1999 (ANOVA, $p < 0.05$). In between and thereafter, *CI* increased significant (January - February 1999, ANOVA, $p < 0.05$; September - November 1999, ANOVA, $p < 0.05$). The settling period of recruits did not exceed three months: December 1997, September - October 1998, March - May 1999 and September - November 1999 (Fig. 3d).

The gametogenic cycle at Paaltjies IV had a similar seasonal pattern (Fig. 3b), except that the six-month spawning period started a month earlier. *CI* decreased from March to June 1998 (ANOVA, $p < 0.05$), increased from July to September (ANOVA, $p < 0.05$), then decreased a second time between October 1998 and early 1999 (October - January, ANOVA, $p < 0.05$). No adult *D. serra* were collected at Paaltjies IV during March and April 1999. Recruits were abundant at Paaltjies IV during autumn and winter, peaking in July 1998 and May 1998, and were found for a longer period than at Langstrand (March - September 1998, March - August 1999; Fig. 3h). To preclude possible methodological error, populations were sampled at 300 m intervals; the distribution was apparently uniform at each sampling site and date.

DISCUSSION

Seasonality

The annual reproductive cycle of Namibian *D. serra* is negatively correlated with the *CI*. The decrease of the *CI* in spring 1998 clearly marked the main period of spawning activity. As a consequence of the prolonged spawning period of the population all reproductive stages were present (Fig. 4 a, b). At Langstrand the decrease was not that evident because a high proportion of the clams were still maturing. During the main spawning season most of the clams analysed had released their germ cells, resulting initially in a low *CI* but then an increase as some animals began recovering towards the end of the spawning period (January - February 1999; Fig. 3b, c). At Paaltjies IV, the *CI* decrease during autumn 1998 despite the fact that the gonads were still undeveloped; this may have been caused by the degeneration of residual gametes (highest recorded percentage, see Fig. 4b) and a corresponding weight loss of the visceral mass. A decrease as a result of spawning activity in summer 1999/2000 could not be identified, clearly showing that histological analysis is the only way to clarify the spawning cycle of the species.

Hanekom (1975) studied the reproductive cycle of *D. serra* in South Africa's Eastern Cape on the basis of histological sections. In his one-year study he observed a distinct cycle, but with spawning in late summer (February - April). In contrast, McLachlan and Hanekom (1979) and van der Horst (1986) found more-or-less discrete spawning peaks during summer and winter in the same region. Similar distinct reproductive cycles and spawning events in summer were reported for clams of the Humboldt Current upwelling system by Urban and Campos (1994). In contrast gametogenic cycles of *D. serra* are less distinct in South Africa's Western Cape region (de Villiers 1975 a, Birkett and Cook 1987).

The recruitment pattern seen here provides clear evidence that juvenile surf clams occur only sporadically and that recruitment varies from year to year, agreeing with the findings of other authors (e.g. Arntz *et al.* 1987). In contrast to the observations of Las-

tra and McLachlan (1996), who worked on the beaches of South Africa's Eastern Cape, the 2 - 6 mm cohort was apparently uniformly distributed at the Namibian study sites. There was also no evidence that recruitment events may have been missed, because each new cohort was subsequently tracked (unpublished data). The present study showed that the *D. serra* belt is inhabited by similar size-classes in accord with the findings of Schoeman (1997), who also worked on beaches of South Africa's Eastern Cape. The zone occupied by recruits varies, according to the slope of the beach, between a minimum breadth of 12 m and a maximum of 40 m (Fig. 5). The larger juveniles populate a narrower belt in the intertidal.

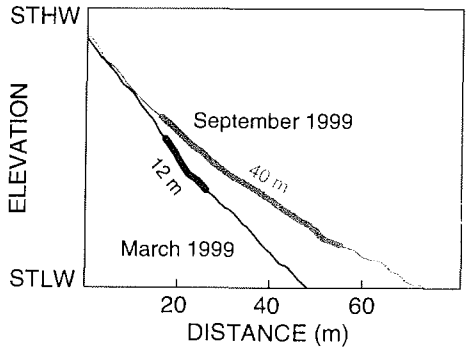

Fig. 5: Beach profile from the spring tide high water mark (STHW) to the spring tide low water mark (STLW) and the related distribution of *D. serra* recruits for March 1999 and September 1999 at Langstrand. With a steeper beach slope the width of the recruit belt becomes narrower.

The juvenile abundance pattern (Fig. 3d) found at Langstrand in March and April 1999 was the result of the spawning event that took place between September 1998 and February 1999. Recruits appeared in the intertidal zone during two months only, even though it is likely that they were released over a period of six months. The duration of the planktonic period of donacids inhabiting upwelling regions is still unknown (McLachlan *et al.* 1996) and the current results regrettably remain inconclusive in terms of *D. serra*. The presence of recruits during March 1999 may indicate that the larval phase lasts at least two months, but there are several possible explanations for the appearance of juveniles in September/October 1998 including:

- Settlement could be of the very first recruits of a spawning, indicating a larval period of <4 weeks (in agreement with the suggestion of Donn 1987);
- Planktonic remnants of the pool of larvae spawned in summer 1997/98 (molluscan larvae may delay metamorphosis and settlement under unfavourable conditions – see Pechenik 1985, Coon *et al.* 1990). It may therefore be possible for the larvae to delay their settlement until after the four winter months (*circa* Birkett and Cook 1987);
- Additionally, recruits from 1997/98 could have settled deeper (Donn 1987, Lastra and McLachlan 1996) as a result of hydrodynamic processes, greater stability of the substratum or geotaxis (Coon *et al.* 1985, Jackson 1986). Insignificant growth of such recruits during winter is possible (as observed for juveniles, unpublished data), so migration to the intertidal may have taken place during spring 1998.

Unfortunately, neither the hydrodynamics of the sample sites nor larval behaviour are sufficiently well known to discuss these possibilities authoritatively. At Paaltjies IV (Fig. 3h) the abundant recruits found between March and September 1998 are likely to be the offspring from summer 1997/98. Juveniles observed there between March and August 1999 were presumably spawned between August 1998 until January 1999.

Although the adult populations showed a nearly synchronous gametogenic cycle, the temporal settlement pattern of recruits varied considerably between sites. Only in January 1999 were recruits found in the intertidal of both beaches simultaneously. Such variation agrees with observations on the behaviour of mussels in the same region (Harris *et al.* 1998). Lastra and McLachlan (1996) also reported temporal variations in *D. serra* spat distribution, although they found recruits throughout the year.

Duration of the larval period and recruitment success are strongly influenced by the actual time of release and the associated environmental conditions (Olson and Olson 1989), probably explaining the observed asynchronous pattern and temporal differences in recruitment strength. Food limitation may cause starvation (Langdon 1983, Olson and Olson 1989) or prolonged development, resulting in a longer exposure to possibly unfavourable conditions in the plankton (e.g. predators, hydrogen sulphide; e.g. Sale 1990, Diaz and Rosenberg 1995). Further, unfavourable hydrodynamic processes (currents, wave action) may increase the meroplanktic period and influence dispersal and settlement patterns (e.g. Roughgarden *et al.* 1988, Zimmerman and Pechenik 1991, Harris *et al.* 1998). Owing to the hydrodynamics (specifically the current patterns) of the Benguela, larvae may have been spawned south of the collection sites followed and transported northwards. In such a case, recruitment would not be expected to be related to the condition of the adults inhabiting the collection sites, but more related to the oceanographic processes pertaining at any point in time. What is clear, however, is that future research should focus on the larval biology of *D. serra*, specifically trying determine the duration of the planktonic stage.

De Villiers (1975 a) reported on the results of a 28-month study of South African west coast populations of *D. serra*. He failed to find a repeated pattern of settlement, but noted that juveniles were most abundant in spring and early summer, but were found all year round. De Villiers' findings (1975 a) agree with those of Lastra and McLachlan (1996) who observed sporadic recruitment events in Eastern Cape populations of the same species, with periods of maximum spat density during summer. Berry (1978) and Harris *et al.* (1998) also noted that rocky shore bivalves around southern Africa recruit in summer.

Potential spawning triggers

Maturation and spawning of bivalves are triggered by changes in SST (Urban and Campos 1994, Sasaki *et al.* 1997). During winter, SST off Namibia is relatively stable, in contrast to the dynamic temperature variations of spring and summer. Therefore, maturation of germ cells in *D. serra* may be triggered by a long period of stable but cold SST and/or some warmer days in late winter/early spring, i.e. before the temperature starts to rise appreciably and consistently. Spawning would then take place when the temperature is higher, possibly favouring larval growth and metamorphosis (e.g. Roosenburg *et al.* 1984, Walker *et al.* 1995). Hanekom (1975) hypothesized that the wider annual range in SST on South Africa's southeast coast (6.5°C) compared to the West Coast (4°C) could be a determining factor for the distinctly different gametogenic cycles in South African *D. serra* populations. Table 1 compares spawning periods at four southern African sandy beaches, with emphasis on the annual range of SST. The results presented here are in line with earlier findings: a SST range of 4.0°C is associated with a pattern of continuous reproduction, a range of 5.6°C with a spawning

period of >6 months, and a range of 6.5°C with a spawning period of 4 months. Therefore our study clearly supports the hypothesis that the reproduction cycle of *D. serra* is related to annual SST.

Table 1: Comparison of spawning periods at four southern African sandy beaches sorted by increasing SST difference between winter and summer. The lack of complete overlap in the spawning period of the two Namibian populations under study can be explained by the two-week shift in sampling time.

Location	Latitude	Spawning period	SST (°C)	SST difference (°C) between summer and winter	Reference
Melkbosstrand	33°41'S	No distinct seasonal cycle	12-16	4.0	de Villiers (1975a)
Paaltjies IV	22°59'S	Aug. - Jan. 1998/99	12-19	5.6	This study
Langstrand	22°47'S	Sep. - Feb. 1998/99	12-20	5.6	This study
St Francis Bay	33°59'S	Feb. - May 1974	12-25	6.5	Hanekom (1975)

ACKNOWLEDGEMENTS

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PUBLICATION II:

POPULATION STRUCTURE, GROWTH AND PRODUCTION OF THE SURF CLAM *DONAX SERRA* (BIVALVIA, DONACIDAE) ON TWO NAMIBIAN SANDY BEACHES.

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ABSTRACT

Population structure, growth and production of the surf clam *Donax serra* (Bivalvia, Donacidae) inhabiting highly exposed sandy beaches of Namibia, were investigated between November 1997 and December 1999. From length-frequency distribution and tagging-recapture data a von Bertalanffy growth function with an asymptotic length (L_∞) of 82 mm and a growth constant (K) of 0.274 y^{-1} was established. Regarding growth performance of Donacidae, *D. serra* fits in a group of species inhabiting cold-temperate and upwelling regions. The intertidal biomass of the studied population ranged between 141 g ash free dry mass (AFDM) m^{-2} and 546 g AFDM m^{-2}. Individual production was maximal at 56.5 mm shell length (0.83 g AFDM $ind.^{-1}$ y^{-1}) and annual production ranged between 167 g AFDM m^{-2} y^{-1} and 637 g AFDM m^{-2} y^{-1}, resulting in productivity values ($P/\overline{B}$) between 1.167 y^{-1} and 1.589 y^{-1}. This data underlines the importance of *D. serra* for the beach/surf ecosystem. Further the findings of this study are crucial to support future aquaculture or exploitation activities and management.

Keywords: *Donax serra*, bivalves, population dynamics, growth, production, intertidal sand habitat, upwelling, Africa West Coast.

INTRODUCTION

Bivalves of the family Donacidae inhabit exposed intertidal sandy beaches and form world-wide by far the largest group living in such highly dynamic environments (for review: Ansell, 1983). Members of the genus *Donax* are commonly the main primary consumers in soft bottom communities, while they are in turn subject to predation by a wide variety of invertebrates, fish, birds and mammals (e.g. Luzzatto & Penchaszadeh, 2001; Peterson *et al.*, 2000; Salas *et al.*, 2001). Moreover, these clams are important recreational and commercial resources in many countries (McLachlan *et al.*, 1996). Although the abundance is limited by their specialisation to coastal high-energy habitats *Donax* species show a strong adaptive radiation with tidal migration as a common feature. Notwithstanding Ansell's (1983, p.608) assertion that donacids are restricted to distinct zoogeographical domains, some species inhabit regions with an overlap of subtropical and temperate zones. Only 5% of the 64 species are found in cold temperate areas (> 5 °C; Bally, 1986) with lowest species diversity on the West Coast of Africa (Ansell, 1983, p.608).

The largest-sized donacid *Donax serra* (Röding, 1798) inhabits the macrozoobenthic community of extended exposed sandy beaches along the Namibian coast and forms dense beds (Donn & Cockcroft, 1989; McLachlan, 1996). As surf clams in general *D. serra* feeds on phytoplankton and detritus, is consumed by birds, fish and crabs and therefore this clam is an important trophic link in surf zone food webs (McLachlan *et al.*, 1980, 1996, p.201; Rossouw, 1985; own unpubl. data). Furthermore, it is exploited for angling bait, and is of potential value for human consumption. Sims-Castley and Hosking (in review) calculated a possible price range of US$ 6.50 - 60.00/kg for export markets. Despite its significant ecological role and its potential commercial value few aspects of the population dynamics of the cold-temperate Namibian stocks have been investigated, e.g. the reproductive biology (Laudien *et al.*, 2001). Growth rates and population structure were studied elsewhere in southern Africa (de Villiers, 1975; Donn 1986; Schoeman, 1994, 1997), but these results cannot simply be transferred to Namibian populations since it was reported that populations from the Southeast and West Coast of southern Africa differ in behaviour and morphometrics (Donn, 1990; Soares *et al.*, 1998; Laudien *et al.*, in revision). Further genetic differentiation exists between Namibian sub-populations (Laudien *et al.*, in revision). Thus knowledge of growth parameters and production are essential for the understanding of the ecology and productivity of *D. serra* inhabiting sandy bottoms of the central Benguela upwelling region. Further, it is crucial to support future aquaculture or exploitation activities and management.

MATERIAL AND METHODS

Study site and sampling

D. serra were collected during alternate spring tides from two Namibian sandy beaches (Figure 1), Langstrand (22°47´S, 14°33´E) and Paaltjies IV (22°59´S, 14°24´E). According to McLachlan's (1980) rating scale for exposure, Paaltjies IV can be characterised as very exposed and reflective (15.5, mean slope 1/24) and Langstrand as exposed and of an intermediate type (13, mean slope 1/10), being partly recessed behind the sand peninsula Pelican Point (Figure 1).

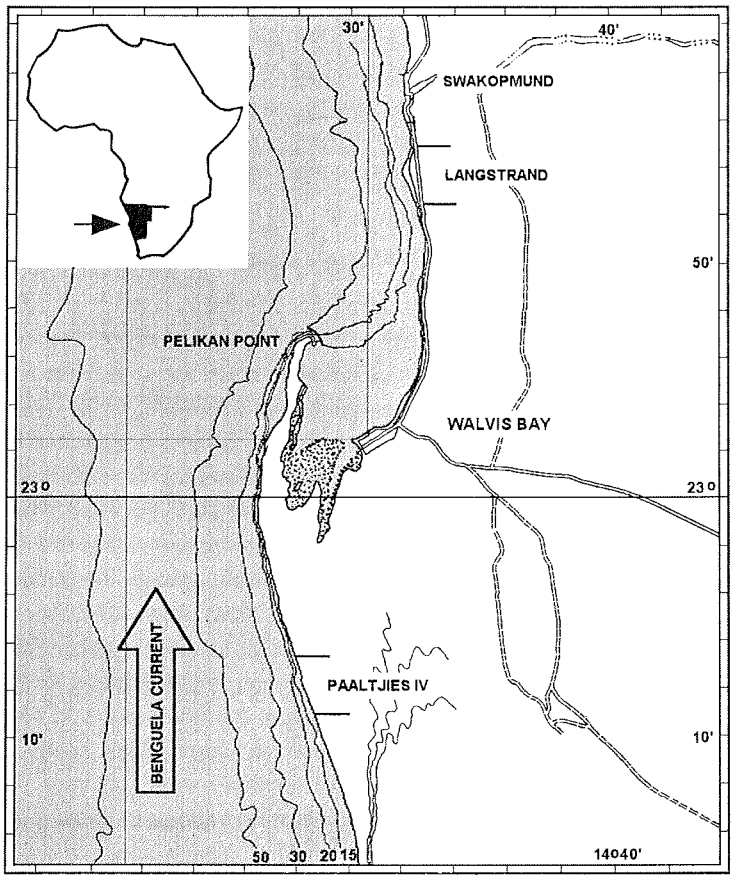


Figure 1: Map of the study sites "Langstrand" and "Paaltjies IV".

Both are open ocean beaches receiving continuous wave action. The area is subject to subequal semidiurnal tides with a maximum tide range of 2 m; spring tides average 1.4 m and neap tides 0.7 m. The sea surface temperature varies between 11 °C in winter and 23 °C in summer. Both beaches are composed of well-sorted medium sand with mean particle diameters ranging at Langstrand between 254 μm (low-shore) and 291 μm (mid-shore) and at Paaltjies IV between 398 μm (low-shore) and 255 μm (mid-

shore), respectively. There is no freshwater seepage at either beach thus salinity is 35. Both beaches are well-drained and oxygenated. McLachlan (1985, p.157) gives a summary including additional features of both beaches.

D. serra were sampled quantitatively at monthly intervals (Langstrand: November 1997 to November 1999; Paaltjies IV: November 1997 to December 1999) from a series of stations (2 m intervals) along a transect transverse to the shoreline from the spring tide high water mark to the subtidal of 1 m water depth. At each station, three replicates of a 0.16 m^2 sand sample were excavated to 35 cm depth using a stirring box and the sand was sieved on a 1-mm screen. All surf clams were transferred to the laboratory immediately for further investigation. The anterior-posterior length of each individual was measured to the lower 0.1 mm with vernier callipers. In total 13298 clams were measured at Langstrand and 16305 at Paaltjies IV. Parameters of the relationship between length and mass of *D. serra* were estimated by regression analysis:

$$M = a \times L^b \quad (1)$$

where M is ash-free dry mass, AFDM (g), obtained by ignition of soft tissue at 550 °C for 7 hours, L is the shell length (mm) and a and b are constants. AFDM was determined for 400 specimens of all size classes between January and December 1998. For comparisons, all published values were converted to AFDM according to Brey *et al.* (1988).

Growth

External and internal shell marks

Dark lines at the anterior end of the valves of *D. serra* (Figure 2) were counted macroscopically. Microstructural shell deposits commonly reflect tidal, daily or seasonal growth increments and vary consistently in width according to exposure time and temperature (e.g. Richardson, 1989; Gaspar *et al.*, 1999, p.311). Therefore internal shell marks may be suitable for growth analyses. Twenty embedded right valves (between 56 and 82 mm; resin: Metset, Type SW, Buehler 95-B130007) were analysed according to Richardson *et al.* (1979). Plane sec-

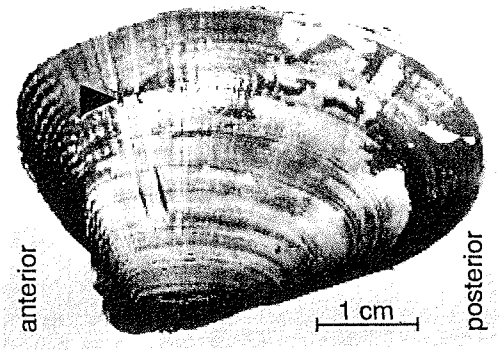


Figure 2: Right valve of *D. serra* recovered 13 June 1999 six months after marking and release. The notch marks (arrow) and growth thereafter show clearly at the valve margin. Estimated growth was 17.6 mm. Additionally typical dark lines at the anterior edge of the valve can be observed.

tions along the axis of maximum growth were prepared and thereafter grounded on wet grinding paper (P120 followed by P1200). Sections were polished (Mecapol 200 with Meraprex 3 μm; PRESI, France) and etched in 0,5% DE-CAL™ (National Diagnostics, Atlanta, Georgia 30336) for one minute. Acetate peel replicas were prepared according to Richardson *et al.* (1979) and examined under a transmitted light microscope (e.g. Richardson, 1989; Gaspar *et al.*, 1999).

Tagging/recapture experiments

7215 individuals of *D. serra* covering the whole size range were collected at Paaltjies IV on three consecutive new moon spring tides in December 1998, January and February 1999. The animals were transported to the laboratory while burrowed in wet sand. Two 0.7 mm thick carborundum discs spaced 2 mm apart and mounted in the mandrel of an electric grinder were used to produce distinct parallel, shallow grooves from the ventral margin up onto the valve surface (e.g. Ropes & Merrill, 1970; Ropes, 1984, p.30). The marked clams were released at Paaltjies IV within a rectangular area (7 × 5 m). Each individual was put into a 15 cm deep hole and covered with sediment in order to prevent the clams being carried away by the strong swash. The recapture length and the length at the time of release reflected in a disturbance ring following the notch marks (Figure 2) were measured. The obtained size increments were used for the estimation of growth parameters (see below).

Length-frequency distribution and analyses

A series of 25 (Langstrand) and 24 (Paaltjies IV) length-frequency distributions (2 mm size classes, monthly) were determined. ELEFAN (Electronoc Length-Frequency Analysis, Pauly & David, 1981; Gayanilo *et al.*, 1989) was not applicable in the present analysis as it is currently not capable of simultaneously estimating more than one annual growth curve (e.g. Schoeman 1997, p.77, p.90). Cohorts were identified by eye and mean individual length in each identified cohort was computed by the weighted average $Lmean_{c,t}$:

$$Lmean_{c,t} = \frac{(L_{j-1} \times N_j) + (L_j \times N_j) + (L_{j+1} \times N_{j+1})}{N_{j-1} + N_j + N_{j+1}} \quad (2)$$

where N_i and L_i are number of specimens and midlength of size class j and j is the size class with the highest number of individuals; c and t are indices of cohort and month, respectively.

Growth was described by the von Bertalanffy growth function (VBGF; von Bertalanffy, 1938):

$$L_t = L_\infty(1 - e^{-K(t-t_0)}) \quad (3)$$

where L_t is length at age t, L_∞ is the asymptotic length (mm), t is the age (y) and t_0 is the age at zero length. A rearranged form of the VBGF

$$L_2 = L_1 + (L_\infty - L_1)(1 - e^{-K(t_2 - t_1)}) \quad (4)$$

was fitted to size increment data (length L_1 at t_1 and L_2 at t_2) obtained from length frequency data and tagging/recapture data using the non-linear Newton algorithm.

Non-linear functions are sensitive to missing data at either end of the distribution (Pauly, 1983; Wetherall *et al.*, 1987). As the Langstrand population is exploited and the centre of adult individual distribution is subtidal, larger animals are poorly represented and our samples and data lack size increment data referring to larger individuals. Therefore the parameter $L\infty$ was not determined iteratively, but set to 82 mm according to the maximum length observed.

Production

Total annual production (January – December 1998 and January – December 1999) was calculated for the intertidal *D. serra* belt of both beaches by the mass-specific growth rate method (Crisp, 1984; Brey, 2001) from the size-mass relation, the size-frequency distribution obtained from all pooled samples and the VBGF:

$$P = \sum N_i \times M_i \times G_i \qquad [\text{g AFDM m}^{-2}\,\text{y}^{-1}] \quad (5)$$

N_i and M_i are the average number of animals (N m^{-2}) and mean individual AFDM in length class i, and G_i is the mass-specific growth rate:

$$G_i = b \times K \times (L_\infty / L_i) - 1) \qquad [\text{y}^{-1}] \quad (6)$$

where b is the exponent of the size-mass relation, K, L_∞ are VBGF parameters and L_i is the mean size in class i.

Mean annual biomass was computed by:

$$\bar{B} = \sum N_i \times M_i \qquad [\text{g AFDM m}^{-2}] \quad (7)$$

and annual $P/\bar{B}$ ratios of the *D. serra* populations were calculated from annual total production P and annual mean biomass $\bar{B}$.

RESULTS

Growth

External and internal shell marks

The number of macroscopic shell marks at the anterior end of the valves was linearly correlated with shell length ($y = 0.5x + 4.6$, $r^2 = 0.93$, $n = 30$). The analyses of micro-growth structures revealed a fine meandric growth line pattern in the outer of three shell layers (outer prismatic, middle crossed lamellar and inner homogeneous and complex crossed lamellar layer) (Figure 3). The microstructural deposit pattern was neither detectable as discreet increments throughout the shell nor did it show consistent cycles as to be expected from tidal, lunar or annual rhythms.

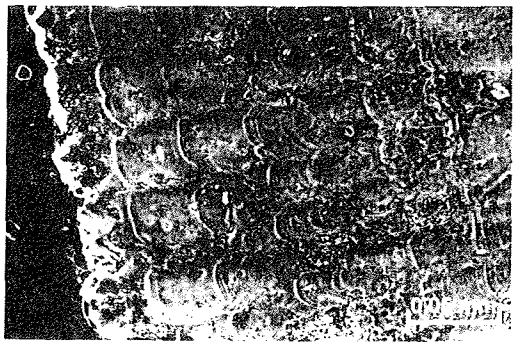


Figure 3: REM picture of a sectioned valve showing microgrowth bands.

Length-frequency distribution and tagging-recapture

New cohorts were detected at Langstrand in December 1997, September 1998, May 1999 and August 1999. In 1997 two additional cohorts were evident: September/October (extrapolated) and around April (D. Louw, unpubl. data). At Paaltjies IV one single new cohort was observed during both years in March. Defined cohorts could be tracked up to 13 months resulting in 72 size-increment data pairs (Langstrand 45 pairs, Paaltjies IV 27 pairs; Figure 4). During the first year of life, a mean length of 35 mm was reached at both beaches. Only eleven (0.15%) of the 7215 marked *D. serra* were recaptured. A common VBGF with a growth constant $K = 0.274\ y^{-1}$ and fixed $L_\infty = 82$ mm was fitted to the combined data set ($r^2 = 0.97$; Figure 5) as the comparison of residuals from size-increment data and tagging-recapture data of both populations and among each other revealed no significant differences.

Biomass and production

Mean annual clam abundance at Langstrand was 96.4 ind. m^{-2} (1998) and 95.5 ind. m^{-2} (1999) and at Paaltjies IV 141.4 ind. m^{-2} (1998) and 54.9 ind. m^{-2} (1999), respectively. This represents a mean annual biomass $\overline{B}$ at Langstrand of 288.8 g AFDM m^{-2} (1998) and 171.9 g AFDM m^{-2} (1999) and at Paaltjies IV of 545.9 g AFDM m^{-2} (1998) and 141.2 g AFDM m^{-2} (1999) (Figure 6 b - e).

The observed relation between length and AFDM of *D. serra* $y = 4 \times 10^{-6} \times x^{3.2576}$ ($r^2 = 0.96$, $n = 965$) was used for production estimates. Individual production increased to its highest value at 56.5 mm length (0.83 g AFDM ind.$^{-1}$ y^{-1}) and decreased thereafter (Figure 6 a). The distribution of total annual production P and the abundance among the size classes are illustrated in Figure 6 b - e. Annual production ranged between 167 and 637 g AFDM m^{-2} y^{-1}, depending on beach and year and $P/\overline{B}$ ratios were between 1.2 and 1.6. In order to convert AFDM to wet mass (WM) the empirical relationship WM = 13.318 × AFDM (n = 400) can be used.

DISCUSSION

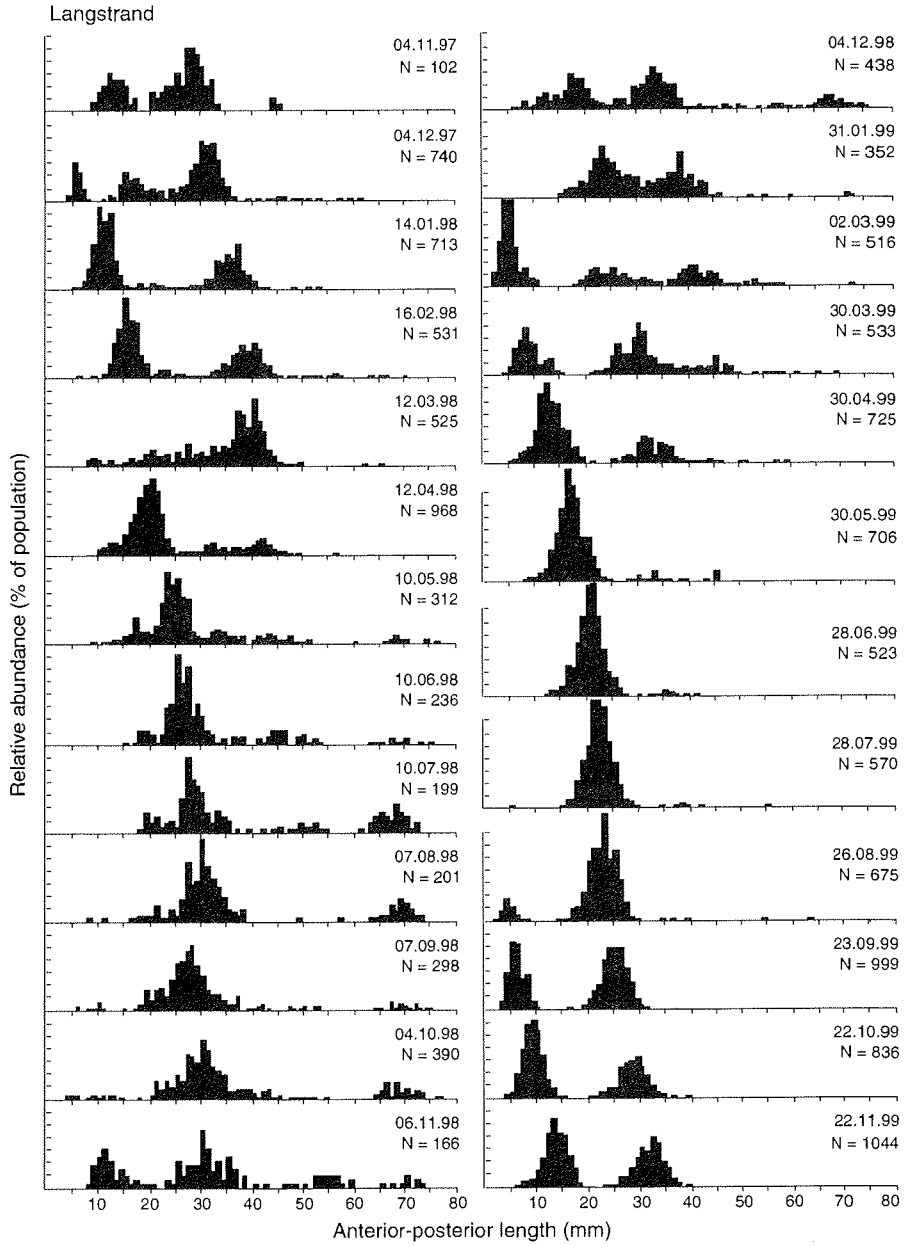
Growth data and growth

The analyses of macroscopic lines at the anterior end of the *D. serra* valve (Figure 2) revealed that these structures are linearly correlated with shell length. Previous studies (de Villiers, 1975, p.11; McLachlan & Hanekom, 1979, p.189; Schoeman, 1997, p.77) are consistent with our findings that growth of *D. serra* follows a non-linear growth function. Thus, the external stripe pattern are not likely to reflect a temporal pattern.

Microgrowth analyses of Namibian *D. serra* are unsuitable to estimate growth. The amplitudes of cyclic growth patterns as observed in Donacidae inhabiting sheltered environments (Nayar, 1955; Wade, 1968, p.890; Ramón & Richardson, 1992, p.19) is too small to be detected in the random pattern caused by disturbance events (e.g. continuous strong wave action) for donacids inhabiting exposed habitats. Consequently uninterpretable microgrowth patterns have been reported for surf-zone *D. trunculus* (Ramón *et al.*, 1995, p.667; Gaspar *et al.*, 1999, p.311) and *D. variabilis* (Wilson, 1999, p.69). The microgrowth pattern of upwelling donacids is only usable when a prominent disturbance ring can be detected which follows a strong event (e.g. Benguela Niño, river run-off) and is reflected in the shell structure of all individuals within the population (de Villiers, 1975, p.12).

The low recapture rate of 0.15% in the tagging-recapture experiment is most likely due to natural along-shore migration. All marked animals were found in flow direction of the Benguela current up to 450 m north of the release area. A pilot study with tagged *D. serra* revealed as well a significant daily longshore migration (J. Laudien, unpubl. data; see also Dugan & McLachlan, 1999).

Non-linear growth functions are difficult to compare, whereas several authors (e.g. Pauly, 1979; Munro & Pauly, 1983; Moreau *et al.*, 1986) demonstrated the suitability of composite indices of overall growth performance (OGP) for inter- and intra-


Langstrand
04.11.97
N = 102
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14.01.98
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16.02.98
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07.08.98
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N = 298
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N = 390
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N = 725
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28.06.99
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28.07.99
N = 570
26.08.99
N = 675
23.09.99
N = 999
22.10.99
N = 836
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N = 1044
Relative abundance (% of population)
10 20 30 40 50 60 70 80
Anterior-posterior length (mm)

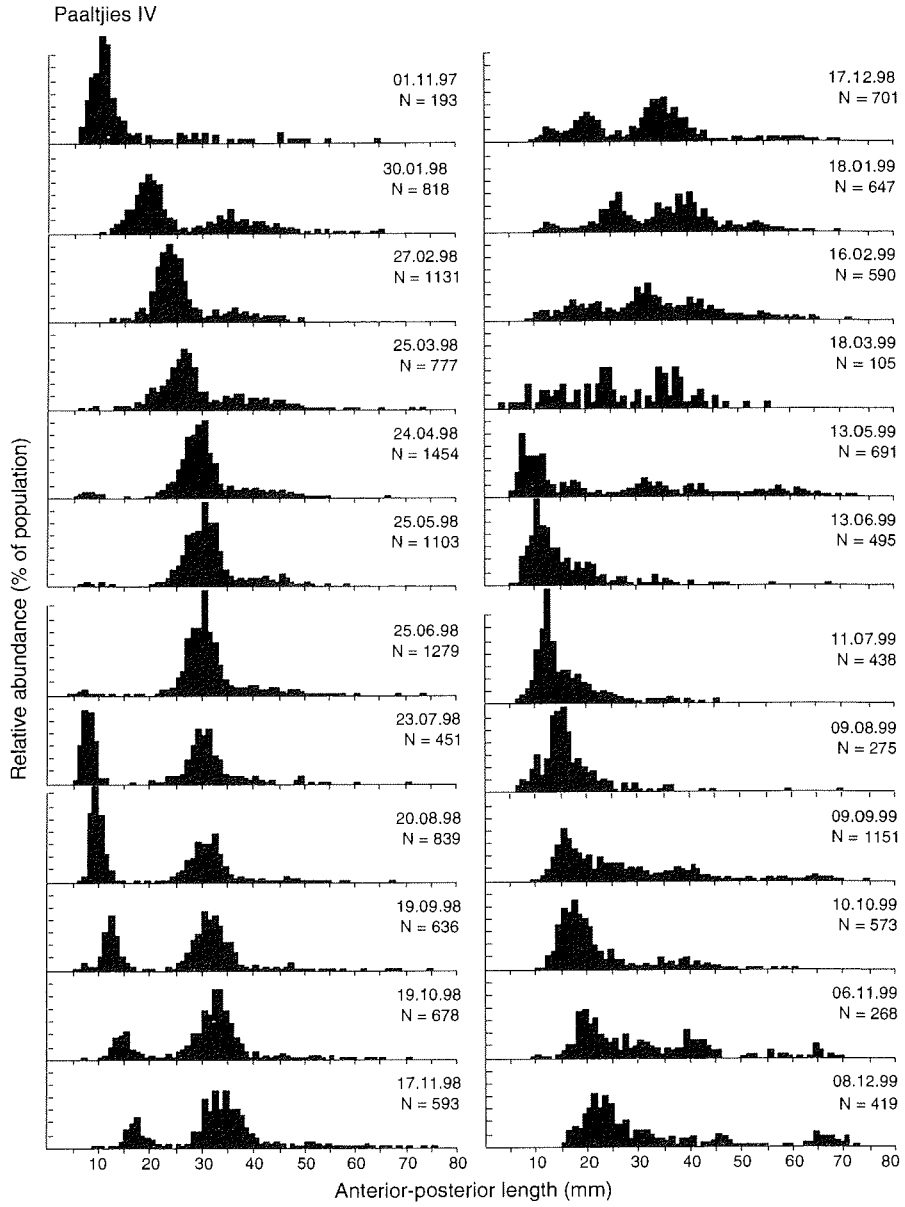

Figure 4: Monthly length-frequency distribution of *D. serra* collected (a) at Langstrand (November 1997 – November 1999) and (b) at Paaltjies (November 1997 – December 1999 except April 1999). Each scale unit on the y-axis is equivalent to 2% of population.

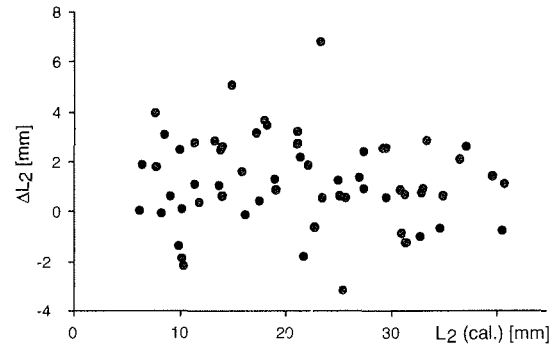

Figure 5: Residuals of best-fitting von Bertalanffy growth curve of *D. serra*; $\Delta L_2 = L_2$ (measured) - L_2 (calculated).

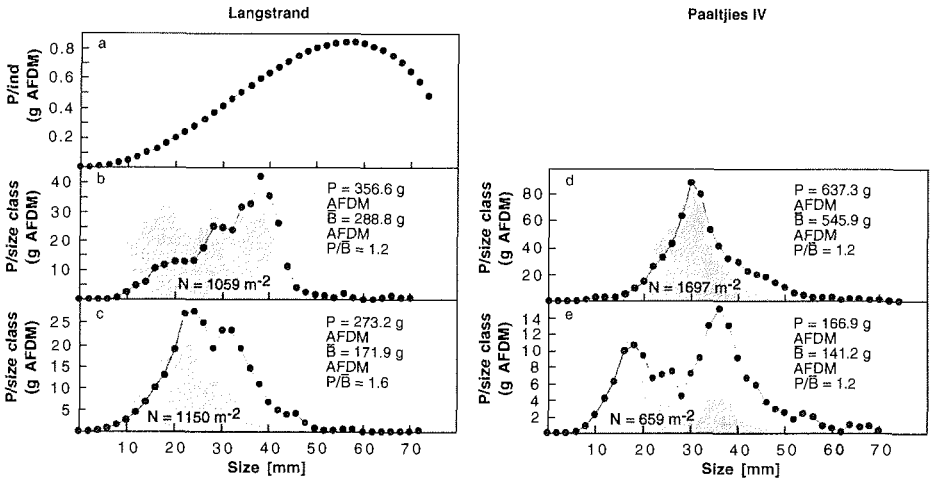

Figure 6: Distribution of annual somatic individual (a) and population production at Langstrand for 1998 (b) and 1999 (c) and at Paaltjies IV for 1998 (d) and 1999 (e). Additionally the mean abundance (grey area = 100 %) for different length classes of *D. serra* is included.

specific comparisons. The index *P* is proportional to the maximum rate of body mass increase during lifetime, i.e. the mass increase at the inflexion point of the VBGF. Since few values of maximum body mass can be found in the literature and maximal mass is proportional to L_∞. *P* was calculated by:

$$P = \log(K \times [L_\infty]^3) \qquad (8)$$

OGP of Namibian *D. serra* (*P* = 4.7) corresponds well with values calculated from a data set of de Villiers (1975) for two West Coast populations (Elands Bay: *P* = 4.7, Melkbosstrand: *P* = 4.7) (Figure 7). Our values are also in line with P values computed from data of Schoeman (1997) for a Southeast Coast population (Maitlands: *P* = 4.7 - 5.2). A compilation of donacid OGP data indicated that OGP is habitat specific (Figure 7): Species inhabiting tropical/subtropical regions show lowest OGP (2.5 - 3.3, group A),

temperate species have intermittent OGP (3.7 - 4.3, group B) while species of upwelling regions show the highest OGP (4.7 - 5.2, group C). Growth of suspension feeding bivalves is related to food availability (Wade, 1968, p.891; Nair *et al.*, 1978; Peterson, 1982; Sastre, 1984; Jensen, 1992, 1993; Nakaoka, 1992) which can get limited at exposed sandy beaches (Defeo, 1992; Defeo *et al.*, 1992; Lima *et al.*, 2000). Consequently the high (Jarman & Carter, 1981; Walsh, 1981) and year-round (Schulz, 1982, p.203; Weeks & Shillington, 1994) primary production in upwelling areas might be the major cause for the observed higher OGP of upwelling donacids.

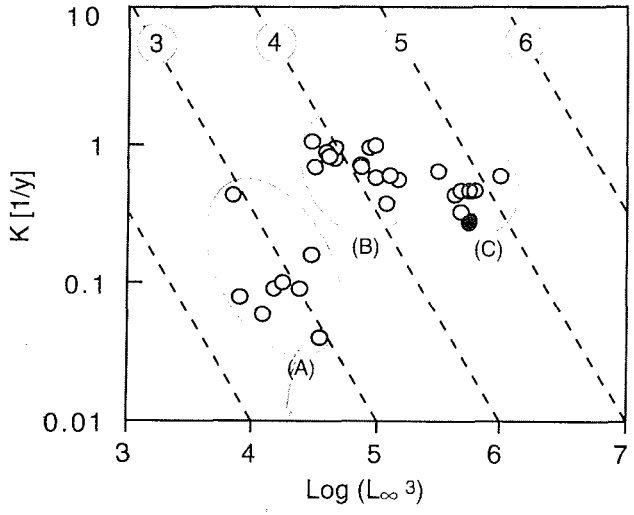

Figure 7: Auximetric grid (according to Pauly 1979) comparing overall growth performance index $P = \log (K \times L_\infty^3)$ of several Donacidae (O) with Namibian *D. serra* (●). Plot indicates three groups (A) tropical/subtropical, (B) temperate and (C) upwelling species. Diagonal lines indicate equal values of P (numbers in circles). Data: (A): *D. cuneatus*: Nayar (1955), Talikhedkar *et al.* (1976); *D. incarnatus*: Ansell *et al.* (1972), Nair *et al.* (1978), Thippeswamy & Mohan Joseph (1991); *D. faba*: Alagarswami (1966); *D. denticulatus*: Vélez *et al.* (1985); (B): *D. trunculus*: Ansell and Lagardère (1980), Guillou and Le Moal (1980), Bodoy (1982), Fernández *et al.* (1984), Mazé and Laborda (1988), Ramón *et al.* (1995), Voliani *et al.* (1997); *D. vittatus*: Ansell and Lagardère (1980); *D. hanleyanus*, Defeo (1996); (C) *D. marincovichi*: Arntz *et al.* (1987); *D. serra*: de Villiers (1975), Farquhar (1996), Schoeman (1997), present study.

Another reason may be the narrower annual temperature range of permanent coastal upwelling areas (about 10 °C) compared to boreal regions (about 30 °C) which facilitate settlement of stenothermic species (e.g. Guillou & Bayed, 1991, p.297). There is evidence that costs of mitochondrial maintenance are lower in stenothermal than in eurythermal species (Pörtner *et al.*, 2000). Therefore low temperature variations might favour growth performance of upwelling donacids.

Biomass and production

D. serra was the only bivalve inhabiting the studied Namibian beaches. The intertidal biomass of the population ranged between 141 and 546 g AFDM m^{-2} (Figure 6). Taking into account that the centre of the adult specimen distribution is likely to be situated in the subtidal (Donn, 1990; Soares *et al.*, 1998), our estimate of intertidal biomass has to be considered conservative regarding the entire population. However, our value is much higher than in *D. serra* at warm temperate (South Africa) beaches (27 g AFDM, McLachlan *et al.*, 1981, p.16; 754 g AFDM per meter beach line,

McLachlan & Hanekom 1979, p.190). Schoeman's (1997) value of 1731 g shell free dry mass per meter beach line corresponds to 48 g AFDM m^{-2} in the *Donax*-belt (30 m belt width, D. Schoeman, pers. comm.; AFDM = 0.831 × DM, Brey, 2001). Apparently the biomass reached by *D. serra* inhabiting the upwelling system distinctly exceeds the range reported for several non upwelling *Donax*-species (0.1 – 2.0 g AFDM m^{-2}; Ansell *et al,* 1978, p.276-277; Warwick *et al.*, 1978, p.222; McLachlan & van der Horst, 1979, p.200; McLachlan *et al.*, 1981 p16; Mazé, 1990, p.160; Wilson, 1999, p.72). From the South American Humboldt upwelling system again higher values are feasible: 70 g AFDM for *D. marincovichi* (Talledo, 1980; Tarazona *et al.*, 1985) formerly called *D. peruvianus*. High biomass was also reported for the surf clam *Mesodesma donacium* (910 g AFDM m^{-2}; Arntz *et al.*, 1987, p.651) which is very similar in shape and size to *D. serra* and plays a comparable ecological role in the Humboldt ecosystem.

The annual intertidal production of *D. serra* ranged between 167 g and 637 g AFDM m^{-2} y^{-1} at Paaltjies IV and between 273 g and 357 g AFDM m^{-2} y^{-1} at Langstrand. These values are significantly higher than values of 34 - 46 g AFDM m^{-2} y^{-1} calculated from habitats without permanent upwelling at the Eastern Cape of South Africa (Schoeman, 1997) and converted to g AFDM m^{-2} y^{-1} (see above). The presented values also distinctly exceed those of non-upwelling donacids. Ansell *et al.* (1978, p.276-277) found production values of 2.9 g AFDM m^{-2} y^{-1} and 3.3 g AFDM m^{-2} y^{-1} for tropical (India) *D. incarnatus* and *D. spiculum,* respectively. Warm temperate *D. variabilis* produced 6.0 g AFDM m^{-2} y^{-1} (Wilson, 1999, p.72), *D. trunculus* produced between 1.8 and 3.7 g AFDW m^{-2} y^{-1} (Mazé, 1990, p.160) and production of temperate *D. vittatus* was 0.7 g AFDM m^{-2} y^{-1} (Warwick *et al.*, 1978, p.222). To our knowledge, there is currently no information available on production for Donacidae from permanent upwelling areas, but *M. donacium* from the Humboldt upwelling system has even higher production rates (2400 g AFDM m^{-2} y^{-1}, Arntz *et al.*, 1987, p.651). Therefore upwelling habitats seem to favour higher production rates presumably due to year-round food availability, high food quality and low temperature ranges around the optimal temperature, on which clams are adapted (Pörtner *et al.* 2000).

Production/biomass ($P/\overline{B}$) ratios of *D. serra* ranged between 1.167 y^{-1} and 1.589 y^{-1}. These values are slightly higher than those of warm temperate South African *D. serra* (0.63 – 1.06 y^{-1}) (Schoeman, 1997) but correspond to *D. sordidus* (1.30 y^{-1} – 1.78 y^{-1}; McLachlan, 1979, p.64; McLachlan & van der Horst, 1979, p.200) and *D. trunculus* (1.37 y^{-1} – 2.26 y^{-1}; Mazé, 1990, p.160). As the subtidal adults are not accounted for in our calculation and, additionally, exploitation concentrates on large clams, the size-frequency distribution is biased towards smaller individuals with high somatic productivity ratios (see also Urban and Campos 1994, p.95).

Donacids play different roles in different habitats. On tropical beaches (Venezuela) *D. denticulatus* dominates benthic biomass but only accounts for a comparatively low portion (5%) of the total production (Ansell, 1983, p.622). In comparison the combined

contribution of Indian *D. incarnatus* and *D. spiculum* to macrobenthic production ranged between 56% and 61% (Ansell *et al.*, 1978). In temperated shallow water habitats of the Bristol Channel (U.K.) *D. vittatus* only accounts for 0.75% of the benthic biomass, a high mass-specific production rate, however, ensures that it ranks amongst the top five secondary producers in the community (Warwick *et al.*, 1978, p.239). At the warm temperated South African East Coast *D. serra* is responsible for 94% of macrobenthic production while *D. sordidus* contributes only 2.5% (McLachlan *et al.*, 1981, p.16). The role of *D. serra* in the beach/surf zone ecosystems is important as it significantly contributes to the regeneration of dissolved and particulate organic nitrogen (Cockcroft & McLachlan, 1993). Part of the secondary production by this clam is consumed by crabs, birds and benthos feeding fish (McLachlan *et al.*, 1980, 1996; Rossouw, 1985; own unpubl. data), which makes *D. serra* an essential trophic link in the coastal upwelling ecosystem. Along with high abundance and production rate, its use as bait and potential economic value for export markets (Sims-Castley & Hosking, in review) *D. serra* is apparently be a valuable species for aquaculture. Future research should evaluate the possibility to rear *D. serra.* Further, ageing methods, which are independent of variable environmental factors (e.g. isotopic age determination) should be focussed on.

ACKNOWLEDGEMENTS

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PUBLICATION III:

SURVIVORSHIP OF JUVENILE SURF CLAMS *DONAX SERRA* (BIVALVIA, DONACIDAE) EXPOSED TO SEVERE HYPOXIA AND HYDROGEN SULPHIDE.

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ABSTRACT

Toxic "sulphide eruptions" sporadically occur in the highly productive inshore regions of the central Namibian Benguela upwelling system. The surf clam *Donax serra* (Röding, 1798) dominates the intertidal and upper subtidal of large exposed sandy beaches of southern Africa and its recruitment seems to be affected by sulphide events. The reaction of juvenile surf clams to low oxygen concentrations and sulphide occurrence (0.1 mmol l^{-1}) was examined by *in vitro* exposure experiments in a gas-tight continuous flow system. After 2 h of hypoxic- and hypoxic-sulphidic conditions clams moved to the sediment surface, aiding their passive transport to areas with more favourable conditions. The clams showed a high sulphide detoxification capacity by oxidising the penetrating hydrogen sulphide to non-toxic thiosulphate. Moreover, juvenile *D. serra* switched to anaerobic energy production, indicated by the significant accumulation of succinate and, to some extent, alanine. Test animals were not able to reduce their energy requirements enough to withstand long periods of exposure, leading to a median survival time (LT_{50}) of 80 h under hypoxic sulphide incubation. In conclusion, natural "sulphide eruptions", especially those with a large spatial and temporal extension, have to be considered as an important factor for *D. serra* recruitment failures. Hydrogen sulphide is assumed to be a potential community-structuring factor.

Keywords: Benguela upwelling system; *Donax serra*; Hydrogen sulphide; Hypoxia; Succinate; Sulphide oxidation; Thiosulphate

INTRODUCTION

Coastal upwelling regions are frequently exposed to hypoxic conditions (Rosenberg et al., 1983; Arntz et al., 1991; Tarazona et al., 1991; Diaz and Rosenberg, 1995; Gallardo et al., 1998) owing to extremely high primary production and subsequent oxidative degeneration of organic matter (van der Plas, 1999, Fossing et al. 2000), Advancing hypoxic water has the potential to cause mass mortalities of benthos and fish (Brongersma-Sanders, 1957; Hart and Currie, 1960; Arntz, 1981). Further anaerobic degradation of organic matter due to sulphate reducing bacteria results in the production of hydrogen sulphide (e.g. Huxtable, 1986; Widdel, 1988; Jørgensen, 1990). For the Benguela upwelling system permanent hypoxic conditions are recorded in the inshore areas downstream of the major upwelling centres (Bailey, 1991, 1999, Fossing et al., 2000). Especially in summer, a combination of physical and biochemical processes causes sulphidic water to rise sporadically to the surface during "sulphide eruptions" (Bailey, 1991). As a consequence, extremely low oxygen concentrations and high hydrogen sulphide concentrations are occasionally recorded in the intertidal and upper subtidal (Currie, 1999). The impact is perpetual as hydrogen sulphide has a half-life of a few hours even in oxygen-saturated seawater (Morse et al., 1987).

Many benthic organisms live in environments with regular occurrence of hypoxia and hydrogen sulphide. Hydrogen sulphide is highly toxic in nanomolar concentrations to aerobic eukaryotic organisms (Evans, 1967; Nicholls, 1975; National Research Council, 1979). Most intertidal bivalves are capable of sustaining their energy production by the use of anaerobic pathways (reviews: de Zwaan, 1977; Storey and Storey, 1990; Grieshaber et al., 1994). Furthermore, they have evolved a detoxification strategy by oxidising the poisonous hydrogen sulphide to non-toxic compounds, mainly thiosulphate (Jahn and Theede, 1997; Jahn et al., 1997). However, there is extensive evidence for stressful and harmful effects of hypoxia and anoxia from both *in situ* and laboratory experiments (review: Diaz and Rosenberg, 1995). These effects are even more pronounced in the presence of hydrogen sulphide (National Research Council, 1979; Shumway et al., 1983; Diaz and Rosenberg, 1995; Jahn and Theede, 1997).

Ecophysiological studies concerning hypoxia and occurrence of hydrogen sulphide are almost entirely devoted to species from hydrothermal vents or from eutrophicated coastal areas of the Northern Hemisphere. At present, not much is known about the metabolic effects of naturally occurring hydrogen sulphide on macrobenthic species in a coastal upwelling ecosystem such as the Benguela. The surf clam *Donax serra* (Röding, 1798) dominates the intertidal and upper subtidal of large exposed sandy beaches of southern Africa and was affected by sulphide events (Bailey, 1999). Laudien et al. (2001) hypothesised that an observed *D. serra* recruitment failure could have resulted from the occurrence of hypoxia and hydrogen sulphide affecting meroplanktic larvae and early juvenile survivorship. Therefore, the objective of the present

study were (i) to investigate how juvenile *D. serra* deal with severe hypoxia and sulphide exposure (100 μmol l^{-1}) and (ii) to investigate as to what extent juvenile *D. serra* are able to detoxify this poisonous compound?

MATERIAL AND METHODS

Study area and sampling

D. serra was collected during low tide in the intertidal of an exposed Namibian sandy beach (Langstrand: 22°47´S, 14°33´ E) which is impacted by continuous wave action. The area is subjected to subequal semi-diurnal micro- to macrotides with a maximum range of about 2 m (McLachlan, 1986). The study site is described by Laudien et al. (2001) and main features of the beach are summarised in McLachlan (1985).

During a recruitment event in November and December 1999 post-settled *D. serra* of the smallest cohort (2-6 mm anterior-posterior length, approx. 5.5 mg) were collected by gently sieving the sediment (1 mm mesh). Within 1 h after sampling animals were transported to the laboratory (burrowed in wet sand) and transferred into the experimental chambers. Experiments were started after 3 h.

Experiments

Tolerance of severe hypoxia and sulphide

Survivorship of juvenile *D. serra* was investigated *in vitro* under normoxic and hypoxic conditions in (i) the absence and (ii) the presence of hydrogen sulphide using a gas-tight system with continuous flow of seawater from a refillable reservoir (Fig. 1). The water reservoir (R1) contained 70 l of seawater (S = 35) filtered twice (National aquarium filter system and paper filter Whatman 2V). For deoxygenation of the water to a minimum oxygen content, it was warmed to 30-35 °C with an external red light, bubbled with pure nitrogen gas for 4 h and then cooled to room temperature. Deoxygenation was confirmed titrometrically (Grasshoff et al., 1983). Six single 250-ml filter flasks (F) containing a sand layer (100 ml of sand from the natural habitat, sterilized at 700 °C then cooled) were connected to the reservoir with oxygen impermeable Vitontubes. One juvenile *D. serra* was introduced into each filter flask before it was closed gas-tight with a rubber plug. This experiment was repeated four times using always a new individual.

At the beginning of each incubation period all filter flasks were filled completely and kept at 16 ± 0.5 °C. Flow rate through each filter flask was set to 250 ml h^{-1} with variable clamp valves (CV). Due to varying water level in R1 causing changes in hydrostatic pressure, the flow rate was controlled and readjusted when necessary every 2 h.

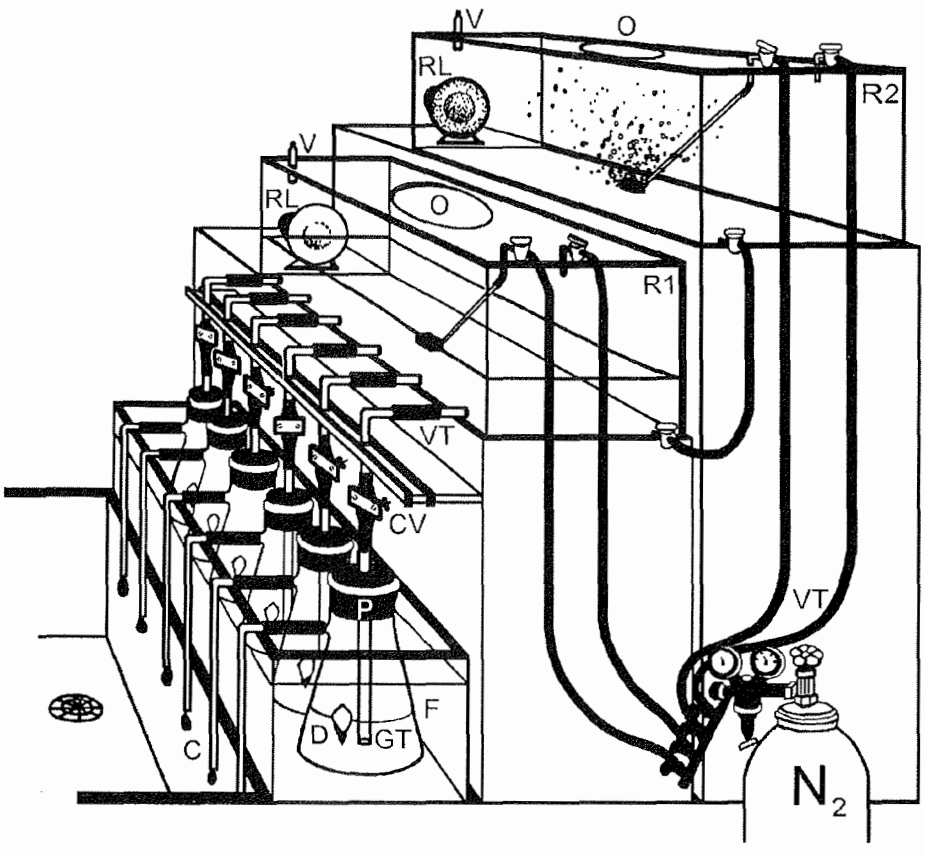

Figure 1: Gas-tight system flow-through system C = glass capillaries, CV = clamp valve, F = filter flask, GT = glass tube, N = nitrogen gas, O = closable opening, P = gas-tight plugs, R1 = water reservoir 1, R2 = water reservoir 2, RL = redlight, V = valve, VT = Viton tube.

The volume of the effluent water of R1 was replaced continuously by overlaying pure nitrogen, and excessive gas could exhaust through a valve (V). Viton tubes (VT) were connected to glass tubes (GT), which were plunged through gas-tight plugs (P) of the filter flasks. The openings of the glass tubes were placed in the sand approx. 5 mm above the flask bottom. Inflowing water replaced the water in the filter flasks, which drained out through glass capillaries (C). A similar reservoir (R2) placed above the first one was set-up in order to be able to prepare new hypoxic water and refill R1. Again, the outflowing volume was replaced by pure nitrogen gas. In addition hypoxic and aerated normoxic controls were set up. In total, 72 clams were tested, 24 under normoxia, 24 under hypoxic conditions and 24 under hypoxic-sulphidic conditions, respectively.

Stock solution of hydrogen sulphide (approx. 10 mmol) was prepared by dissolving (aqua bidest-washed) Na_2S x H_2O crystals (χ = 7-9) in N_2-saturated sea-water taken from R1. Prior to the experiments, the concentration of the stock was determined by

iodometric titration (Poethke, 1973) and sulphide was added to R1 through a closeable opening (O) to a final sulphide concentration of 0.1 mmol l^{-1}. To monitor the sulphide concentration during the course of the incubation, a 50-ml water sample was collected once every 2 h at the outflow of one glass capillary. Sulphide concentrations were determined spectrophotometrically at 660 nm according to the methylene blue method (Fonselius, 1976). Whenever R1 was refilled, at the start as well as at the end of the experiment, oxygen concentration in the water was checked titrometrically (Grasshoff et al., 1983). Sulphide did not change the pH of the incubation medium significantly as confirmed by pH-controls (see also Hahlbeck et al., 2000).

Mortality assessment of test animals was based on failure of the valve closure reflex (Jahn and Theede, 1997) when the gaping surf clams were touched with the glass pipe of the water inlet. The number of surviving bivalves was monitored once every 2 h over a period of 7 days and mortality is given as median survival time (LT_{50}) using the probability relation between percent mortality and time (Litchfield, 1949).

Short term incubations in the presence of sulphide (0.1 mmol l^{-1})

Three replicated groups, with six juvenile *D. serra* each, were introduced into the above-described set-up for seven different time periods. In total, 21 groups of test animals (3 replicates x 7 time periods) were investigated. Incubation was terminated after 0, 1, 3, 6, 12, 24 and 48 h of exposure. Thereafter the tissues of the test animals were rapidly dissected, blotted dry and the pooled sample of each replicate immediately stored in liquid nitrogen until biochemical analysis.

Biochemical analyses

Sulphide oxidation products, e.g. sulphite and thiosulphate were determined in the juvenile surf clams as described in Schiedek et al. (1997): The pooled sample of six shell free juvenile *D. serra* (one replicate; approx. 0.15 g wet mass) were ground to fine powder in a mortar precooled by liquid nitrogen. Concentrations of sulphite (SO_3^{2-}), sulphide (H_2S), cystein ($C_3H_7NO_2HS$), thiosulphate ($S_2O_3^{2-}$) and glutathione ($C_{20}H_{32}N_6O_{12}S_2$) were quantified in the tissue by High-Performance Liquid Chromatography (HPLC) after derivatisation with monobromobimane (e.g. Völkel and Grieshaber, 1994; Jahn et al., 1996; Jahn 1997). The content of succinate and of the free amino acids alanine, aspartate and glutamate were measured as indicators for the onset of anaerobiosis. The free amino acids were analysed from perchloric acid (PCA) extracts. 50-mg portion of the frozen tissue powder was transferred to 0.25 ml 3 mol l^{-1} PCA and homogenised with an Ultra-Turrax for 20 s. After centrifugation (12 min, 12,000 × g), the pH of the supernatant was neutralised by adding 5 mol l^{-1} K_2CO_3, controlled by the colour change of methylorange (1 drop). The precipitate was removed by centrifugation (20 min, 17,000 × g). Amino acids were separated and determined via HPLC (5 µm, Superspher 60, 125-4, RP-8, MERCK) according to Schiedek (1997). Succinate was

analysed via capillary electrophoresis according to a modified method after "Agilent" (Organic Acid Analysis Kit P/N 5063-6510) (T. Hirse and H.-O. Pörtner, unpubl.). Trichloric acetic acid (TCA) extracts were prepared using TCA (15%) and 0.12 g l^{-1} tatrate as an internal standard. Frozen tissue powder (100 mg) was suspended in the 3.5-fold volume of cold TCA and homogenised with an Ultra-Turrax for 10 seconds. After centrifugation (3 min, 16,000 × g, 0 °C) the supernatant was neutralised by the 3-fold volume of 1:4 *n*-Octylamine: 1,1,2-Trichlortrifluorethan (Freon), mixed and centrifuged (1 min, 16,000 × g, 0 °C). The upper phase was stored at -80 °C and diluted 1:4 and filtered through a 0.2-µm injection filter prior to analyses. Succinate was separated with a "eCAP Capillary Tubing" (75 µm, 120 cm, Beckman 338473) at 27 KV and 15 °C and detected (PDA) at 214 nm. The separating buffer consisted of 1:5:20 Brij 35 (Fluka):Acetonitrile (Riedel, chromasolv):Organic Acid Buffer (Agilent).

Statistical analyses

In order to test the reduction of survival time a log-rank test was performed. Thiosulphate, succinate and amino acid concentrations were tested for statistical significance using ANOVA at the 5% level. Prior to the test, data of thiosulphate were square-root transformed, data of succinate were logarithmically transformed in order to achieve normality and induce homogeneity of variances.

RESULTS

Experimental hypoxia

The average oxygen concentration of the incubation water was 0.337 ml l^{-1} with very little variation between the different experimental runs and during each run (± 0.002 ml l^{-1}, n = 17). Sulphide levels were in the range of 100 ± 25 µmol l^{-1}. Normoxic control flasks had a constant average oxygen concentration of 10.491 ± 0.153 ml l^{-1}.

Under normoxic conditions, post-settled *D. serra* were buried in the sediment and the tips of the siphons were visible at the sediment surface. In contrast, under hypoxic conditions, test clams remained buried and the siphons extruded only for the first 2 h regardless of the presence or absence of sulphide. Thereafter, the clams were found lying on the sediment surface and extended their siphons into the water column. Finally, siphons collapsed and the valves gaped. When exposed to hypoxia or hydrogen sulphide, survivorship was affected. Under severe hypoxia the first animals were recorded dead after 78 h with 110 h as a median survival time (LT_{50}) (Fig. 2). The presence of sulphide (100 µmol l^{-1}) significantly reduced the survival time even further (log-rank test, z = - 4.07, $p < 0.01$). The first animal died after 46 h and an LT_{50} of 80 h was calculated. All surf clams kept under normoxic conditions were still alive when the experiment was terminated after 200 h.

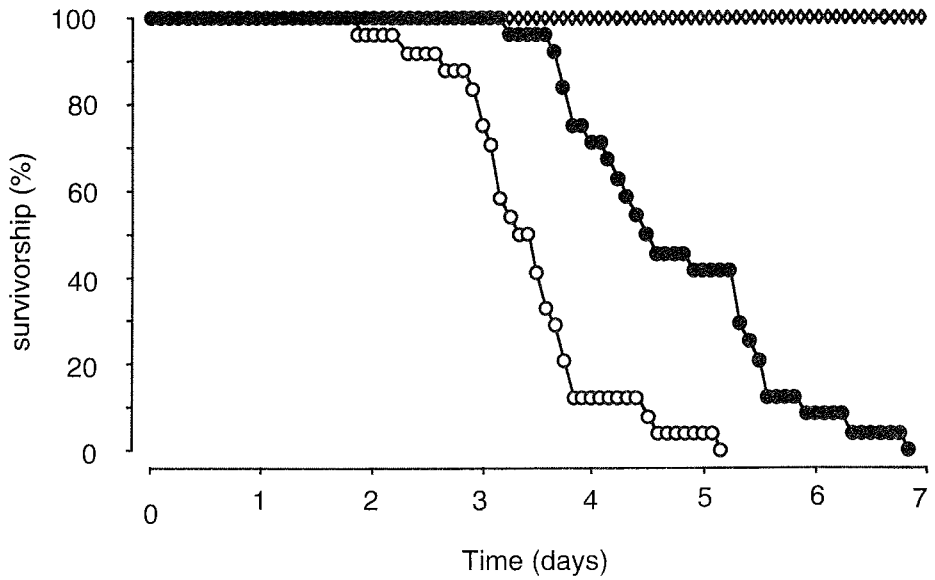

Figure 2: Percentage of surviving post-settled *D. serra* during severe hypoxia in the absence (solid circles) or presence (open circles) of sulphide. Normoxic controls are shown as rhombi. N = 24 per treatment.

Sulphur compounds in the tissue

The time course of sulphide uptake in the soft tissue was followed during 48 h of sulphide exposure (100 µmol l^{-1}). Sulphide itself was not detectable in the soft tissue and sulphite was only found in low concentrations (0.015 µmol g^{-1} wet mass) in some specimens. Thiosulphate was the only sulphur compound that clearly accumulated, reaching a maximum of 0.148 µmol g^{-1} wet mass after 24 h (Fig. 3 a), while cystein was recorded at constant concentrations (0.25 µmol g^{-1} wet mass).

Anaerobic energy production under sulphide exposure

The concentrations of metabolites resulting from anaerobic energy production were followed over the same time course (48 h). Succinate concentration increased 9-fold from the onset of exposure (0.25 µmol g^{-1} wet mass) to 2.23 µmol g^{-1} wet mass after 24 h (Fig. 3 b). The concentration of alanine (17.5 ± 2.5 µmol g^{-1} WM) was significantly elevated after 3 and 24 h (ANOVA, $p < 0.05$), while it decreased significantly between 24 and 48 h (ANOVA, $p < 0.05$). Glutamate levels (6.5 ± 0.8 µmol g^{-1} ww) did not change significantly during the experiment (ANOVA, $p > 0.05$; Fig. 3 d) and aspartate was not detectable.

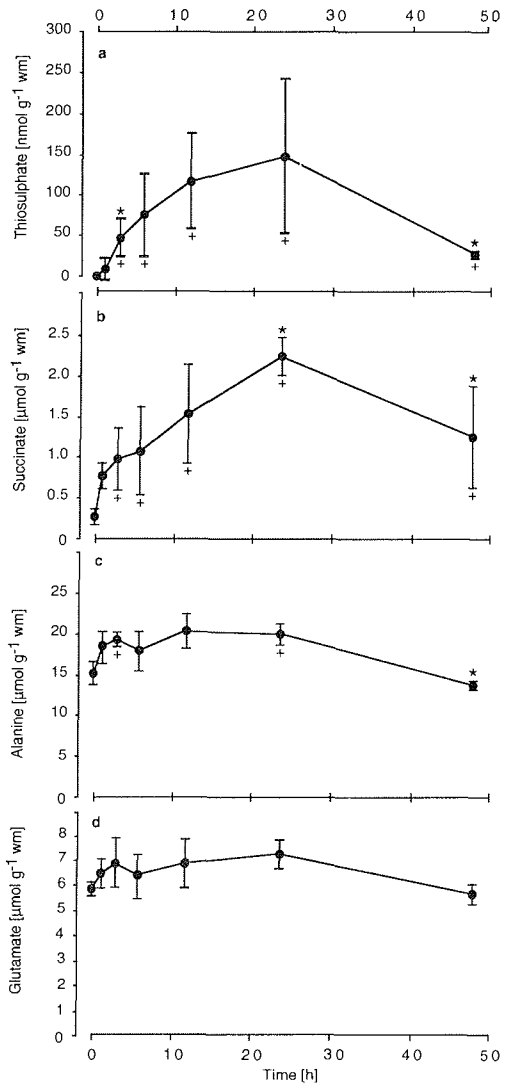

Figure 3: Juvenile *D. serra*: content of thiosulphate (a), succinate (b), alanine (c) and glutamate (d) after hypoxic incubation (0-48 h) in the presence of hydrogen sulphide (100 µmol l^{-1}). Mean ± SE, n = 3, *: significantly different from previous value; +: significantly different from control.

DISCUSSION

Marine invertebrates inhabiting coastal zones with sporadic hydrogen sulphide occurrence such as the central Namibian coastal upwelling system use a variety of behavioural, biochemical and physiological traits to either avoid the contact or to withstand this poisonous substance. The epibenthic blue mussel, *Mytilus edulis*, for instance closes the shells as a passive protection against the poisonous effects of hydrogen sulphide (Jørgensen, 1980). A similar behaviour has been observed for *Perna perna* (Schiedek and Currie, unpublished data). The studied juvenile *D. serra*, however, were observed lying on the sediment surface with siphons extended into the water column when exposed to hypoxia or sulphidic hypoxia. This is in agreement with observations from other infauna bivalves during experimental (*Mulinia lateralis*: Shumway et al., 1983; *Abra alba*: Rosenberg et al., 1991; *Scrobicularia plana*: Oeschger and Pedersen, 1994) and field studies (*Mya arenaria*, *Cerastoderma edule* and *A. alba*: Jørgensen, 1980). Deposit feeding bivalves, such as *D. serra*, use their siphons to take up organic particles by filtering the water. The observed permanent siphon extrusion in the presence of sulphide may reflect a strategy to monitor the water for better conditions and might not be coupled with high ventilation activity. The clam *S. plana*, for instance, reduced ventilatory water flow under exposure to keep the sulphide concentration low in their extrapallial fluid. In addition, a diffusive barrier against sulphide might exist in the siphons (Oeschger and Pedersen, 1994).

The observed migration of *D. serra* to the sediment surface during hypoxic sulphide exposure appears to be an adaptation to the specific conditions prevailing in such kind of coastal systems. Natural "sulphide eruptions" within the Benguela upwelling system often affect restricted surface areas of some 100 m^2 only (Currie, 1999; personal observation). Therefore, moving to the sediment surface favours the transport with currents to other areas where the mobile clams might find better conditions. On the other hand the clams have to cope with dangers like (i) a possible wash up followed by mortality due to overheating in the sun or predation by seagulls (McLachlan et al. 1980, J. Laudien unpublished data); and/or (ii) a constant exposure to pelagic and epibenthic predators, able to enter the toxic water body while feeding (Phil et al., 1992). The advantages of being transported to more favourable conditions seems to compensate for these disadvantages.

As mentioned above, marine invertebrates regularly exposed to hydrogen sulphide have acquired a variety of biochemical adaptations to eliminate this poisonous compound (reviews: Vismann, 1991b; Bagarinao, 1992; Völkel and Grieshaber, 1995; Grieshaber and Völkel, 1998). The detoxification of sulphur compounds to less or non-toxic oxidation products is known to be a common strategy in marine bivalves (Cary et al., 1989; O'Brien and Vetter, 1990) and other invertebrates (Vismann, 1991a; Völkel and Grieshaber, 1995). In *D. serra* thiosulphate appears to be the main product of mitochondrial sulphide oxidation, as it was the only detoxification product found at elevated amounts after experimental sulphide exposure. Thiosulphate accumulated from the onset of exposure to a maximum of 0.148 µmol g^{-1} wet mass (WM) after 24 h. This demonstrates that oxygen stored in body fluids and/or oxygen remains in the incubation water (<0.3 ml l^{-1}, due to the remaining oxygen in the nitrogen gas corresponding to the purity grade noted in the techsheet) seems to be still available for oxidative processes. Thiosulphate is almost non-toxic (Voegtlin et al., 1924; Sörbo, 1972), not inhibiting oxidative metabolism (Vetter et al., 1989) and highly soluble. The detected value is in the same range as found in the Baltic clam *Macoma balthica* (0.177 µmol g^{-1} WM, Jahn and Theede, 1997) but lower than concentrations seen in other marine invertebrates (*Marenzelleria* cf. *wireni*: 1 µmol g^{-1} WM, Schiedek et al., 1997; *Arenicola marina* 30 µmol g^{-1} WM, Grieshaber et al., 1995; *Hediste diversicolor*: 100 µmol g^{-1} DM, Hahlbeck et al., 2000).

The observed decrease of the thiosulphate concentration in the soft tissue of *D. serra* after 24 h may be explained with a decreasing oxygen tension in the tissue. As thiosulphate is highly soluble it can, therefore, not be accumulated to infinite concentrations within tissues. Grieshaber and Völkel (1998) concluded that it is generally not further metabolised but eliminated by outward diffusion. Accordingly, no thiosulphate transport system could be identified in invertebrates (Hauschild et al., 1999).

It is remarkable, that no sulphide could be detected in the clams' soft tissue after exposure to sulphide. This leads to the assumption that sulphide detoxification is very efficient allowing the clams to maintain a very low sulphide level for a period of about 24 h

when exposed to 0.1 mmol l^{-1} hydrogen sulphide. This level represents concentrations measured in the coastal area off Namibia during sulphide eruption events (A. van der Plas, personal communication.). These findings further support that juvenile *D. serra* are able to deal with hydrogen sulphide for a short time period, giving them the opportunity to move to a more suitable location as described above.

Many euryoxic marine bivalves and other invertebrates use alternative pathways of anaerobic energy production for long-term hypoxic survival (e.g., Storey and Storey, 1990; de Zwaan, 1991; Grieshaber et al. 1994). One predominant anaerobic product is succinate, which accumulates in the tissue (e.g., Kluytmans et al., 1977; Widdows et al., 1979; Kluytmans and Zandee, 1983; Demers and Guderley, 1994; Sukhotin and Pörtner, 1999). The present results support these findings: when exposed to sulphidic hypoxia post-settled *D. serra* switched to anaerobiosis — which resulted in a 9-fold increase of succinate levels. Starting from relatively high levels, alanine accumulated to an even higher extent. For intertidal species this might be a regular event, since they must switch to anaerobiosis during low tide when the substratum is exposed to air and the penetration of oxygen into the sediment is not sufficient to support fully aerobic energy production (reviews: Schöttler and Bennet, 1991; Grieshaber et al., 1994). Cockcroft (1990) stresses that anaerobic metabolism includes a significant energy saving strategy and may even explain the obvious success of *D. serra* on southern African coasts.

Relying on anaerobic metabolism, however, does not provide complete protection against "sulphide eruptions". Under severe hypoxia and in the absence of sulphide all of the juvenile *D. serra* survived for more than 4 days (LT_{50} 110 h, Fig. 2). Under hypoxic-sulphidic conditions, survivorship was significantly reduced (LT_{50} 80 h). Non-dissociated hydrogen sulphide diffuses easily through biochemical membranes (Powell, 1989; Julian and Arp, 1992) and reversibly inhibits the last enzymatic reaction of the respiratory chain by forming a stable complex with cytochrome *c* oxidase. In the presence of sulphide, aerobic respiration is therefore impossible (Nicholls, 1975; Nicholls and Kim, 1981, 1982). In the juvenile surf clams succinate was accumulated almost directly after the onset of hypoxic-sulphidic conditions. At the same time, thiosulphate started to increase. As pointed out earlier this suggests that the animals used much of the remaining oxygen to detoxify the penetrating hydrogen sulphide and as a consequence switched to anaerobiosis immediately. A similar reduction in survival and a more pronounced anaerobic metabolism in the presence of hydrogen sulphide has been reported for several other marine invertebrates, e.g. the polychaete worms *Arenicola marina*, *Nephtys hombergii* or *Marenzelleria* cf. *wireni* (Völkel and Grieshaber, 1992; Arndt and Schiedek, 1997; Schiedek et al., 1997). This leads to a faster breakdown of glycogen, which is known to be the major energy resource during long-term anaerobiosis (Schöttler and Bennett, 1991) and may limit survival upon its depletion. Glycogen content was not measured in the juvenile *D. serra* because of limited amount of tissue, but their

stores are likely to be lower than in the adults. A size and age related increase in glycogen content has been shown for instance for juvenile lugworms (Schiedek and Schötttler, 1990).

In terms of survival periods, juvenile *D. serra* are 3-fold more sensitive to hydrogen sulphide than adult Baltic clams *Macoma balthica* (100 µmol l^{-1}, 10 °C) (Jahn and Theede, 1997). However, some of these differences are probably due to the higher temperatures used in our experiments (15 °C). Furthermore, the susceptibility might not only be species related, but seems also to decrease with increasing age and size (e.g. *Theora lubrica*: Imabayashi, 1986, *Mytilus edulis*: Wang and Widdows, 1991; C. Bittkau unpublished data). Jahn et al. (1997) argued that in contrast to larger animals, small individuals with a higher surface-to-volume ratio are unable to detoxify hydrogen sulphide effectively enough but can only survive in sulphidic habitats due to their anaerobic capacity.

This argument leads us to postulate that not only post-settled *D. serra* but especially larvae may be affected even more than juveniles by hydrogen sulphide and that natural "sulphide eruptions" have to be considered as an important factor for *D. serra* recruitment failures.

The Namibian coastline can be separated into two zoogeographical provinces, the cool temperate southwest coast (Namaqua) and the cool temperate northwest coast (Namib) (Emanuel et al., 1992). Various explanations have been given for the differing species numbers and community structures, including the Lüderitz upwelling cell acting as a barrier to northward larval dispersal. However, some species found in the area around Lüderitz are absent in the central Namibian region and reappear further north in a small pocket at Möwe Bay (Currie, 1999). Since the northern province correlates well with the post-upwelling cell characterized by oxygen-deficient bottomwater and opal-rich deposits, Currie (1999) hypothesises that the hypoxia and hydrogen sulphide associated with the Lüderitz upwelling cell could contribute considerably as a community structuring force. Various studies confirmed the structuring effect of hydrogen sulphide (Hiroki, 1977; Diaz and Rosenberg, 1995; Gamenick et al., 1996, 1998; Jahn et al., 1997). However, adult *D. serra* seem to be adapted to sulphidic conditions and can therefore be abundant in the southern and the northern province. Populations seem to withstand moderate sulphide eruptions even after recruitment failures, since the adults reproduce several times during their 5-year life span (Laudien et al., 2001). Further research should include larval stages as well as adult animals from different provinces to confirm a possible structuring effect of naturally occurring hydrogen sulphide in the Benguela Current upwelling system.

CONCLUSION

The results of the present study show that juvenile *D. serra* respond to sulphide exposure conditions by moving to the sediment surface which favours their transport by currents to nearby areas with better conditions. Initially, the clams are well adapted to detoxify hydrogen sulphate to non-toxic thiosulphate and thus keep concentrations of the toxin at very low levels within the valves. The juvenile clams are able to gain energy by switching to anaerobiosis during exposure. However, anaerobiosis and the reduction in energy requirements only supported time-limited survival, leading to a significantly reduced survivorship after 2 days. Therefore we postulate that natural "sulphide eruptions", especially when they have large spatial and temporal extensions, have to be considered as an important factor for recruitment failures of the surf clam *D. serra*.

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PUBLICATION IV:

GENETIC AND MORPHOLOGICAL VARIATION IN FOUR POPULATIONS OF THE SURF CLAM *DONAX SERRA* (RÖDING) FROM SOUTHERN AFRICAN SANDY BEACHES.

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ABSTRACT

The surf clam *Donax serra* (Bivalvia, Donacidae) dominates sandy beach communities of two southern African biogeographical regions, a cold (Benguela current) and warm province (Agulhas current). Morphometric and behavioural differences led to a controversial discussion of whether or not populations from the two provinces belong to the same species. Shell size measurements confirmed morphological differences: clams from the cold province were significantly rounder, flatter and less wedge-shaped than clams from the warm province. In this study a genetic approach was used to relate phenotypic differences to genetic variability of four *D. serra* populations separated by up to 2 500 km of shoreline. Genetic analysis of twenty-two protein-coding loci was carried out by starch-gel electrophoresis. Populations studied are conspecific (genetic distances range from 0.003 to 0.044) and possess genetic variation (alleles per locus: 1.73 - 1.91; mean heterozygosity: 18 - 22%; percentage polymorphism: 45.5 - 59.1%) in the range of most other marine bivalves, which allows for potential adaptation to environmental changes. Wright's fixation indices show little to moderate genetic differentiation among the subpopulations relative to the limiting amount under complete fixation (F_{ST} = 0.016 - 0.089), moderate differentiation of individuals relative to the total population (F_{IS} = 0.265 - 0.452), and comparably high differentiation of individuals relative to the compound population (F_{IT} = 0.300 - 0.473). The effective number of individuals exchanged between populations in each generation is high enough (1.44 - 8.65) to counteract genetic drift. We propose that the observed differences represent phenotypic plasticity enabling this species to inhabit different biogeographic regions. Gene flow, balanced selective pressure and evolutionary inertia are discussed as explanations for similarities of the two outlying populations. The substantial subdivision of the two Namibian populations indicates a potential biotic barrier and requires separate studies of the population dynamics.

Keywords: Allozyme electrophoresis; Biogeography; *Donax serra*; Evolutionary inertia; Morphology; Phenotypic plasticity; Population genetics; Southern Africa

INTRODUCTION

The surf clam *Donax serra* (Röding, 1798) is the largest-sized member of the genus Donacidae and inhabits the intertidal and upper subtidal zone of extended and exposed sandy beaches along the Namibian and South African coast. Biomass reaches 8 500 g shell free dry mass per meter shoreline (Donn, 1987; McLachlan, 1996). Clams are exploited for bait and food and might be a valuable aquaculture species with their unique and delicate taste showing rapid growth and high densities (Donn, 1987; McLachlan, 1996). Based on an economic evaluation Sims-Castley and Hosking (in press) calculated a possible price range between US$ 6.50 - 60.00/kg for export markets. Morphological studies on Donacidae indicated high interspecific (Ansell, 1985; Nelson et al., 1993; McLachlan et al., 1995) and intraspecific (Wade, 1967; Nelson et al., 1993) variability. Regarding *D. serra* populations from the West and the Southeast Coast, it is not clear whether all clams belong to the same species ("population" in this paper refers to all animals inhabiting the geographic location without any implication of reproductive connections). Shell morphology differs significantly (Donn, 1990a; Soares et al., 1998) and colour differences of soft tissue are common between coasts (Donn and Els, 1990). Additionally behavioural dissimilarity was found between adults and juveniles inhabiting different beach zones (e.g. Donn, 1990b; Soares et al., 1998). These differences may indicate that the populations belong to different (sub-) species or stocks. They have been associated with habitat differences (Donn, 1990a) and directional selection with microevolutionary changes maintained by geographical isolation (Soares et al., 1998). However, significant differences in sperm morphology (van der Horst et al., 1986) and growth rates (Schoeman, 1997) were not detected. Further, culturing of larvae from crosses was successful (H. van der Horst pers. comm., *fide* Donn, 1990a) supporting the hypothesis that populations are closely related.

Genetic analysis may not only clarify the phylogenetic relationships, but provides also an estimate to analyse intraspecific larval dispersal between regions and the dependence of recruitment on local stocks. Levinton and Suchanek (1978) postulate that high gene flow occurs in species with planktonic larvae resulting in high levels of genetic variation provided that the populations import reconfigured genes. If populations belong to the same species, if large amounts of variation had occurred in the founder population, and if the recent populations are not isolated, we expect the northern population to be genetically more connected due to larval import by the northerly surface-flow of the Benguela current. Conversely we expect Southeast Coast populations to be most differentiated due to restricted genetic input. Our study examines biometric parameters of *D. serra* populations along the southern African coastline to confirm morphological differences. In order to relate these variations to genetic determination we analysed enzymatic proteins with starch-gel electrophoresis in order to: (i) compare genotypic variation within the populations with expectations for a sexually reproducing species mating randomly under free recombination of genes, (ii) find partition allelic variation among the different populations in order to infer the levels and patterns of gene flow, and (iii) determine if there is an association between allelic and morphological variation, possibly linked to environmental parameters.

MATERIAL AND METHODS

Study areas and sampling

Four exposed sandy beaches of two different biogeographic provinces, two Namibian (Langstrand, 22°47´S, 14°33´E; Meob Bay, 24°38´S, 14°43´E) and two South African beaches (Bloubergstrand, 33°51´S, 18°09´E; Maitlands in St. Frances Bay 34°6´S, 25°13´E) were selected (Fig. 1). For detailed descriptions of the habitats see McLachlan (1986) (Langstrand, I), Holtzhausen (1999) (Meob Bay, II), Soares et al. (1996) (Bloubergstrand, III) and Schoeman (1997) (Maitlands, IV).

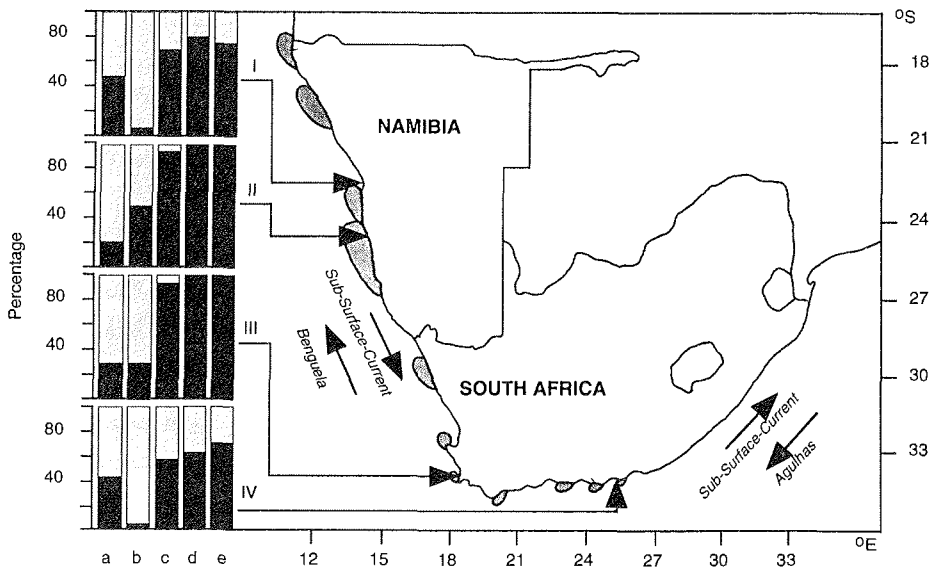

Fig. 1. Study sites along the southern African coast: (I) Langstrand, (II) Meob Bay, (III) Bloubergstrand and (IV) Maitlands. Principal upwelling cells (stippled areas) in the Benguela and Agulhas and the sub-surface-currents are included (after Shannon and Nelson 1996). Allozyme variability is shown on the left side (a: **EST-2**, b: **PEP-B2**, c: **PEP-C2**, d: **PGM-1**, e: **PGM-2**), bars indicate percentage of A (black) and B (grey) allele. Note the related pattern of the two outlying in contrast to the two intermediate populations.

Due to coastal upwelling the water temperature of the Benguela current is 13°C on average (Walker et al., 1984). Sea surface temperature (SST) for Site I decreases to about 12°C in winter and rises to 23°C in summer with seasonal means at Walvis Bay of 16°C (summer/autumn) and 14°C (winter/spring) (Shannon, 1985). The SST at Site II is annually 13.5°C ranging between 10°C and 19.5°C (summer/autumn: 15°C, winter/spring: 12°C) (C. Bartholomae, unpubl. data). Site III is exposed to even lower temperatures with an annual mean of 13°C (8 – 14°C in summer and 11 – 17°C in winter) (Walker et al., 1984). Site I and III are in a high energy intermediate morphodynamic state (Soares et al., 1996; Schoeman, 1997; Laudien et al., 2001), whereas II is in a reflective state with waves breaking almost directly in the intertidal zone (J. Laudien, pers. obs.). All three are, however, open ocean beaches exposed to con-

tinuous wave action and subject to subequal semidiurnal tides with a maximum tide range of about 2 m (springs average 1.4 m, neaps 0.7 m) (McLachlan, 1986).

Site IV (Maitlands) is located at the Southeast Coast and is influenced by the south-eastwards flowing Agulhas current. At the Agulhas Bank it is deflected southwards (Brown and Jarmann, 1978). The mean annual SST is 22°C (15 – 17°C in winter, 26°C in summer) (Ansell and McLachlan, 1980). Sporadically this region may however experience cold water upwelling during strong eastern winds in summer (Goschen and Schumann, 1995) albeit with a much lesser frequency than at the West Coast (Shannon, 1985). The beach is in a high-energy intermediate to dissipative morphodynamic state (McLachlan, 1990; Soares et al., 1998).

Sampling was done during spring low water in March 1999. A total of 32 adult *D. serra* were collected randomly within a 50 m stretch at each sample site. Surf clams were transported to the laboratory alive.

Morphometric and analytic methods

Four morphological variables were measured according to Soares et al. (1998): shell length (antero-posterior), height (ventro-dorsal) and width (left-right) to 0.01 cm with vernier callipers. Additionally wet mass (including shell) was recorded to the nearest 0.01 g. Thereafter clams were frozen at –25°C and extracts were prepared by homogenisation of the body (except stomachs in order to exclude interference from nutrition) in an equal volume of distilled water.

Allozymes were analysed by starch-gel electrophoresis (12% potato starch, Sigma Chemicals Co.), using the electrophoretic procedures, method of interpretation of gel banding patterns and locus nomenclature in van der Bank et al. (1992). Loci were numbered from the anodal end of the gel. Gels were stained for allozymes listed in Table 1. Aspartate aminotransferase (**AAT**, 2.6.1.1), alcohol dehydrogenase (**ADH**, 1.1.1.1), glyceraldehyde-3-phosphate dehydrogenase (**GAP**, 1.2.1.12), glycerol-3-phosphate dehydrogenase (**GPD**, 1.1.1.6), hexokinase (**HK**, 2.7.1.1), L-iditol dehydrogenase (**IDDH**, 1.1.1.14), L-lactate dehydrogenase (**LDH**, 1.1.1.27), malate dehydrogenase (**MDH**, 1.1.1.37), 6-phosphogluconate dehydrogenate (**PGDH**, 1.1.1.44), **AK-2** and **IDH-2** were also stained for, but showed insufficient activity for interpretation.

Table 1

Locus abbreviations, enzyme commission numbers (E.C. No.) and buffers providing the best results (see Material and Methods for abbreviations of buffers), *= monoallelic loci.

Protein	Locus	E.C. No.	Buffer
Adenylate kinase	**AK**	1.1.1.1	TC-EDTA
Creatine kinase	**CK***	2.7.3.2	MF
Esterase	**EST-1, -2**	1.2.1.12	MF
Glucose-6-phosphate isomerase	**GPI**	5.3.1.9	RW
Isocitrate dehydrogenase	**IDH**	1.1.1.27	TC
Malic enzyme	**ME**	1.1.1.38	MF
Mannose-6-phosphate isomerase	**MPI-1*, -2**	5.3.1.8	MF
Peptidase		3.4.-.-	
Substrate: Glycyl-$_{L}$-leucine (GL)	**PEP-A***		MF
$_{L}$-Leucylglycyl-glycine (LGG)	**PEP-B1, -B2**		MF
Leucyl-$_{DL}$alanyl (LA)	**PEP-C1, -C2**		MF
$_{L}$-phenylalanyl-$_{L}$-proline (PHP)	**PEP-D**		MF
$_{L}$-leucine-_-naphthylamide (CAP/LAP)	**PEP-E**	3.4.11.1	LiOH
$_{L}$-leucyl-tyrosine (LT)	**PEP-S**		MF
Phosphoglucomutase	**PGM-1, -2**	5.4.2.2	RW
General protein	**PROT***		RW
Superoxide dismutase	**SOD-1*, -2***	1.15.1.1	LiOH

The following buffers were used to separate the enzymes: (i) **MF** – a continuous Tris, boric acid and EDTA buffer system (pH 8.6) (Markert and Faulhaber, 1965), (ii) **RW** – a discontinuous Tris, citric acid (gel pH 8.7), lithium hydroxide and boric acid (electrode pH 8.0) buffer (Ridgway, et al. 1970), (iii) **TC** – a continuous Tris and citric acid buffer system (pH 6.9) (Whitt, 1970), (iv) **LiOH** – a discontinuous lithium hydroxide, boric acid (gel pH 8.4), additionally Tris and citric acid monohydrate (electrode pH 8.3) buffer (Selander et al., 1971) and (v) **TC-EDTA** – a continuous Tris and EDTA buffer (pH 9.6) (Harris and Hopkinson, 1976) (Table 1).

Statistics

Electrophoretic results were analysed using BIOSYS-1 (Swofford and Selander, 1981). Descriptive statistics were calculated for each population including percentage polymorphic loci (*P*), mean number of alleles per locus (*A*) and average heterozygosity (*H*) according to Nei (1978). To assess statistical significance of allele class deviations from expected numbers of heterozygotes and homozygotes and χ^2 analyses according to Levene (1949) (adapted for small samples) were done. Results were adjusted for the number of tests performed with the Bonferroni technique (Lessios, 1992). For polymorphic loci, allele classes among populations were compared using contingency χ^2 analysis. Allele frequency differences were integrated across loci by calculating genetic dis-

tances for all pairs of populations (Nei, 1978; Wright, 1978) using BIOSYS-1. Different fixation indices were used to analyse genetic differentiation between populations (Wright, 1978) where F_{IT} and F_{IS} are the fixation indices of individuals relative to the total population and its subpopulation respectively, and F_{ST} measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation. To apply *F*-statistics the four populations were considered as sub-samples of a hypothetical compound population resulting from the sum of all analysed specimens. F_{IS} values were tested according to the method of Waples (1987):

$$\chi^2 = \boldsymbol{F}_{IS}{}^2 \times N \ (k-1\ ); \mathrm{DF} = k \times \frac{(k-1)}{2} \tag{1}$$

where N is the total number of individuals and k is the number of alleles at the locus. Significant differences (5% level) of F_{IT} values were tested according to Brown (1970):

$$|\boldsymbol{F}_{IT}|\sqrt{\mathrm{N}} \geq 1.96 \tag{2}$$

F_{ST} values were tested according to Workman and Niswander (1970):

$$\chi^2 = 2N \times \boldsymbol{F}_{ST}\ (\mathrm{k}-1); \mathrm{DF} = (\mathrm{k}-1)(\mathrm{s}-1) \tag{3}$$

where N is the total number of specimens, k is the number of alleles per locus, and s is the number of populations. For an essentially one-dimensional array of sampling sites such as described in this study Wright's (1969) "island model" may be used to express approximate equilibrium levels of gene flow (expressed as the effective number of migrants per generation, N_{EM}) within a structured population. Pairwise N_{EM} values were estimated from F_{ST} according to Takahata (1983):

$$N_{EM} = \frac{\frac{1}{\boldsymbol{F}_{ST}} - 1}{4\alpha} \tag{4}$$

where N_E is the effective population size, M the number of migrants per generation, $\alpha = [n/(n\text{-}1)]^2$ and n = total number of populations. The genetic distances, D (standard; Nei, 1972) and D_{78} (unbiased and adapted for small sample sizes; Nei, 1978) were calculated between populations. Thereafter the distance Wagner procedure was applied, using the coefficient of Prevosti distance (D_1), which measures the distance for a single locus as half the sum of the absolute differences between the allelic frequencies of the populations (Wright, 1978):

$$D_1 = 0.5 \sum^{k} |q_x - q_y| \tag{5}$$

Criterion 3 (see Swofford, 1981) was used to determine addition sequence. An unrooted tree of genetic relationship was computed using D_{78} values and its reliability indicated by values of cophenetic correlation and bootstrapping support values (Efron, 1982; Felsenstein, 1985) via DISPAN (Ota, 1993).

RESULTS

Morphometric comparisons between *D. serra* populations

Morphological comparisons between width *vs.* height (W/H) and height *vs.* length (H/L) revealed a significant difference in shell shape (Kruskal-Wallis, W/H: $p < 0.001$, DF = 3; H/L: $p < 0.001$, DF = 3). West Coast clams were rounder, flatter and less wedge-shaped than Southeast Coast clams. Figure 2 presents W/H plotted against H/L; population means of W/H and H/L (filled symbols) are (I) 0.68 and 0.55, (II) 0.67 and 0.54, (III) 0.68 and 0.54, (IV) 0.63 and 0.59, respectively.

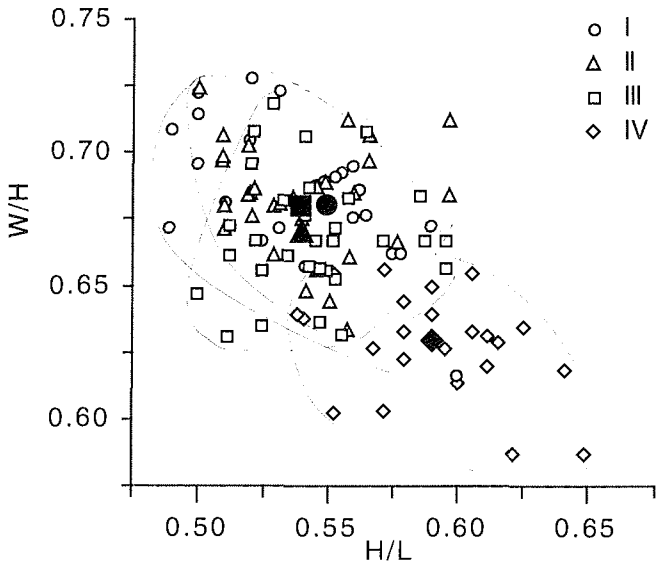

Fig. 2. Shell shape variability of four *D. serra* populations from the West Coast (I-III) and the Southeast Coast (IV) of southern Africa. Width to height ratios (W/H) are plotted against height to length ratios (H/L), means of populations are given as filled symbols.

Allozyme variation

Twenty-two protein-coding loci provided interpretable results. Locus abbreviations, enzyme commission numbers, buffers used giving the most satisfactory results and monoallelic loci are presented in Table 1. Products of all protein-coding loci migrated anodally. Significant (χ^2, $p < 0.05$) deviations of allele distributions occurred between Population I and II at seven (53.8%) loci (**EST-2**, **PEP-B2**, **PEP-C1**, **-2**, **PEP-E**, **PGM-1**, **PGM-2**); between Population I and III at four (31.25%) loci (**PEP-B2**, **PEP-C1**, **-2**, **PGM-1**, **-2**); between Population I and IV at five (33.3%) loci (**MPI-2**, **PEP-C1**, **PEP-E**, **PEP-S**, **PGM-1**); between Population II and III at two loci (15.4%) (**PEP-B2**, **PEP-E**); between Population II and IV at eight (57.1%) loci (**EST-2**, **MPI-2**, **PEP-B2**, **PEP-C1**, **-2**, **PEP-S**, **PGM-1**, **-2**); and between Population III and IV also at eight (57.1%) loci (**MPI-2**, **PEP-B2**, **PEP-C1**, **-2**, **PEP-E**, **PEP-S**, **PGM-1**, **-2**). Only the two intermediate populations did not deviate significantly when all loci were taken into consideration. Table 2 presents the allele frequencies, coefficients of heterozygote deficiency (negative val-

ues) or excess, degrees of freedom, the individual heterozygosity values (*h*) and sample sizes at all polymorphic loci. The *h* values ranged from 0.031 to 0.728. Significant ($p < 0.05$) genotypical deviations from Hardy-Weinberg proportions are indicated in Table 2. Allele classes at **MPI-2** deviated in all populations, **PEP-B2** and **-E** in I and III, **EST-2**, **PEP-S** and **PGM-2** in I and IV, **PEP-C1** in I, II and IV, **PEP-C2** and **PGM-1** only in I, **PEP-B1** in I, II and III and **PEP-D** only in III.

Table 2

Allele frequencies, heterozygote deficiency (indicated with -) or excess from Hardy-Weinberg proportions (*d*), chi-square (χ^2) values (*= significant deviation of allele classes from expected Hardy-Weinberg proportions, $p < 0.05$, adjusted by Bonferroni technique), degrees of freedom (DF) and individual heterozygosities (*h*) for all polymorphic loci and sample size (N) (see Materials and Methods for population designations).

Locus	Population	Allele frequencies				d	χ^2	DF	h	N
		A	B	C	D					
AK	I		1.000							24
	II		1.000							32
	III		0.969	0.031		0	0	1	0.061	16
	IV	0.016	0.984			0	0	1	0.031	16
EST-1	I	0.968	0.032			0.017	0.017	1	0.062	31
	II	0.969	0.031			0.016	0.016	1	0.061	29
	III	1.000								32
	IV	1.000								32
EST-2	I	0.437	0.563			-0.420	4.419*	1	0.492	24
	II	0.200	0.800			0.024	0.019	1	0.320	21
	III	0.286	0.714			-0.089	0.178	1	0.408	21
	IV	0.429	0.571			-0.431	4.096	1	0.490	30
GPI	I	0.984	0.016			0	0	1	0.031	32
	II	0.969	0.031			0.016	0.016	1	0.061	32
	III	1.000								32
	IV	0.969	0.031			0.016	0.016	1	0.061	32
IDH	I	0.984	0.016			0	0	1	0.031	32
	II	1.000								32
	III	1.000								32
	IV	1.000								32
ME	I	1.000								7
	II	1.000								6
	III	0.900	0.100			0.056	0.059	1	0.180	10
	IV	0.917	0.083			0	0	1	0.153	13
MPI-2	I	0.281	0.703	0.016		-0.423	8.578*	3	0.426	32
	II	0.306	0.694			-0.627	12.733*	1	0.425	29
	III	0.354	0.646			-0.554	7.752*	1	0.457	24
	IV	0.069	0.897	0.034		-0.287	57.145*	3	0.190	31

Table 2 continued

Locus	Population	Allele frequencies				d	χ^2	DF	h	N
		A	B	C	D					
PEP-B1	I	0.367	0.367	0.266		-0.454	13.387*	3	0.660	30
	II	0.350	0.367	0.283		-0.357	10.508	3	0.663	30
	III	0.177	0.468	0.355		-0.288	16.978*	3	0.624	31
	IV	0.283	0.434	0.283		-0.195	6.756	3	0.652	30
PEP-B2	I	0.062	0.938			-1.000	42.034*	1	0.117	32
	II	0.500	0.500			-0.158	0.726	1	0.500	32
	III	0.293	0.707			-0.428	5.574*	1	0.414	29
	IV	0.047	0.953			0.033	0.051	1	0.089	28
PEP-C1	I	0.286	0.179	0.393	0.142	-0.704	46.981*	6	0.712	28
	II	0.060	0.240	0.320	0.380	-0.434	25.434*	6	0.692	26
	III	0.154	0.135	0.346	0.365	-0.358	12.019	6	0.705	26
	IV		0.135	0.692	0.173	-0.840	42.279*	3	0.473	25
PEP-C2	I	0.700	0.300			-0.847	11.852*	1	0.420	15
	II	0.944	0.056			0.039	0.061	1	0.105	23
	III	0.938	0.062			0.050	1.105	1	0.117	32
	IV	0.565	0.435			0.385	3.563	1	0.491	27
PEP-D	I		1.000							32
	II	0.096	0.339	0.565		-0.032	4.692	3	0.557	32
	III	0.031	0.969			-0.489	24.691*	3	0.061	32
	IV		1.000							32
PEP-E	I	0.179	0.214	0.554	0.053	-0.256	22.061*	6	0.613	28
	II	0.015	0.266	0.516	0.203	-0.110	5.786	6	0.622	31
	III	0.217	0.250	0.367	0.166	-0.460	21.837*	6	0.728	30
	IV	0.032	0.242	0.516	0.210	-0.144	11.978	6	0.630	32
PEP-S	I	0.250	0.359	0.391		-0.625	27.422*	3	0.656	32
	II		1.000							27
	III	0.156	0.328	0.516		-1.000	63.016	1	0.602	32
	IV	0.018	0.593	0.389		-0.708	16.752*	3	0.497	31
PGM-1	I	0.800	0.200			-0.795	20.428*	1	0.320	30
	II	1.000								32
	III	1.000								32
	IV	0.625	0.375			-0.213	1.498	1	0.469	32
PGM-2	I	0.750	0.250			-0.645	9.899*	1	0.375	22
	II	1.000								32
	III	1.000								16
	IV	0.703	0.297			-0.779	20.306*	1	0.417	17

Within populations genotypic frequencies at five of the polymorphic loci (31.25%) (**AK**, **EST-1**, **GPI**, **IDH**, **ME**) closely approximated Hardy-Weinberg expectations, while the other polymorphic loci showed deficiencies of heterozygotes (Table 2). Between popu-

lations, significant ($p < 0.05$) deviations of allele distributions occurred at nine (56.25%) of the 16 polymorphic loci, namely **EST-2**, **MPI-2**, **PEP-B2**, **-C1**, **-C2**, **-E**, **-S**, **PGM-1** and **-2**. Rare alleles for the **AK** locus occurred in population III (C alleles) and population IV (A alleles) where the other two populations were fixed for B alleles. Rare B alleles were found in I and II at **EST-1** where the other two populations were monoallelic. In contrast, rare B alleles were found at the **ME** locus for III and IV, whereas the two Namibian populations were monomorphic. **GPI** was fixed only in III and **IDH** only polymorphic in I. Three alleles were detected at I and IV, and two alleles in the other two populations at **MPI-2**. The outlying populations were fixed at **PEP-D***B, whereas III had two and II three alleles. Populations II and III were fixed for **PGM-1** and **-2**, whereas II and III were polyallelic. The mean number of alleles per locus (*A*) 1.91 ± 0.21 (I), 1.73 ± 0.21 (II), 1.77 ± 0.21 (III) and 1.86 ± 0.19 (IV) was not significantly different among populations (Kruskal-Wallis Test, n = 22, H = 0.784, $p>0.05$). The mean heterozygosity per locus (*H*), 0.223 ± 0.057 (I), 0.182 ± 0.056 (II), 0.198 ± 0.057 (III) and 0.211 ± 0.052 (IV) was also not significantly different (Kruskal-Wallis Test, n = 22, H = 0.526, $p>0.05$). The percentage of polymorphic loci (*P*) (0.95 criterion) was 45.5% (I), 36.4% (II), 40.9% (III) and 45.5% (IV) respectively.

Genetic differentiation

The genetic distances (*D*) and (D_{78}) are presented in Table 3.

Population	Parameter	II	III	IV
I	D	0.030	0.020	0.016
	D_{78}	0.025	0.015	0.010
	F_{IT}	0.435	0.473	0.442
	F_{IS}	0.402	0.452	0.426
	F_{ST}	0.055	0.037	0.028
	N_{EM}	2.416	3.660	4.882
II	D	-	0.008	0.049
	D_{78}	-	0.003	0.044
	F_{IT}	-	0.300	0.331
	F_{IS}	-	0.289	0.65
	F_{ST}	-	0.016	0.089
	N_{EM}	-	8.648	1.439
III	D		-	0.041
	D_{78}		-	0.036
	F_{IT}		-	0.372
	F_{IS}		-	0.323
	F_{ST}		-	0.073
	N_{EM}		-	1.786

Table 3
Genetic comparison between four *D. serra* populations: *D*: genetic distance according to Nei (1972), D_{78}: genetic distance according to Nei (1978), genetic differentiation according to Wright (1965, 1978), indicating the mean weighted F_{IT}, F_{IS} and F_{ST} statistics values. Additionally the effective number of individuals exchanged between populations in each generation (N_{EM}) using formula 4 (Takahata 1983) is included (see Materials and Methods for population designations).

A genetic distance of 0.030 (D) was computed between I and II. The two intermediate populations (II and III) have the smallest genetic distance of 0.008 (D) and 0.003 (D_{78}), whereas highest values were obtained between Sites II and IV with 0.049 (D) and 0.044 (D_{78}). The calculated fixation indices (Wright, 1965, 1978) also describe genetic differentiation between populations: The F_{IS} statistic indicates the degree of allelic fixation in individuals relative to the sub-population and averaged 0.362 (Table 3). Pair-wise pooling revealed the following values: 0.402 (I+II), 0.452 (I+III), 0.426 (I+IV), 0.289 (II+III), 0.265 (II+IV) and 0.323 (III+IV). These values are moderate since a value of one shows fixation of alleles due to inbreeding and a value close to zero indicate random mating (Nei, 1986). The F_{IT} statistic is also moderate (0.409) and indicates the amount of inbreeding in the population due to population subdivision. Pair-wise comparisons yielded 0.435 (I+II), 0.473 (I+III), 0.442 (I+IV), 0.300 (II+III), 0.331 (II+IV) and 0.372 (III+IV). F_{ST} (Table 3) measures the amount of differentiation among subpopulations relative to the limiting amount under complete fixation and provides a mean measure of the affiliation of individuals within the population (Swofford and Selander, 1981). The average weighted F_{ST} values from pair-wise comparisons revealed the following values: 0.055 (I+II), 0.037 (I+III), 0.028 (I+IV), 0.016 (II+III), 0.089 (II+IV) and 0.073 (III+IV).

According to Wright's (1969) "island model" the N_{EM} values in the present study are high enough to counteract genetic drift and ranged between 1.44 (II and IV) and 8.65 (II and III) (Table 3). The unrooted tree of genetic relationship obtained (distance Wagner procedure, implemented in BIOSYS-1; Farris, 1972), rooted at midpoints of greatest patristic distance is shown in Fig. 3. The graph separates two groups, indicating that the population of Langstrand is closer related to the geographically furthest population of Maitlands than to Meob Bay. The length of the branches indicate differentiation within subpopulations, showing that IV is approximately 2.5 times more differentiated than I. The reliability of the given illustration is indicated by values of cophenetic correlation (100%) and by bootstrapping support values of > 96% (Efron, 1982; Felsenstein, 1985) via DISPAN (Ota, 1993).

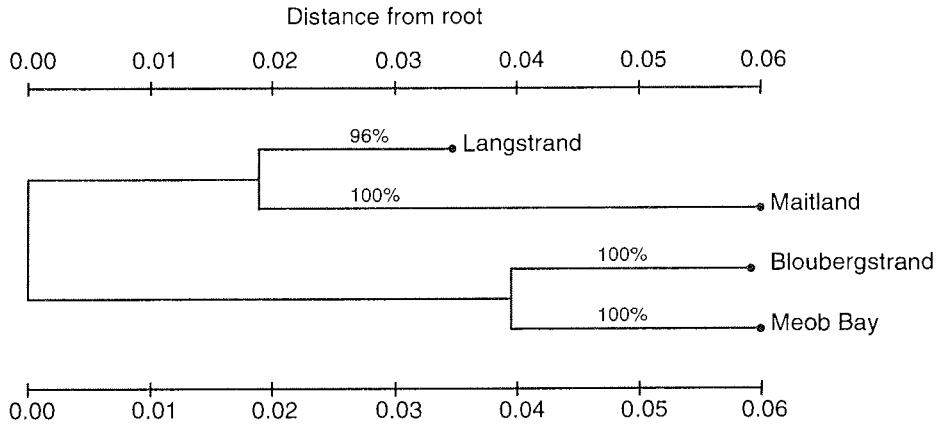

Fig. 3. Wagner tree (using D_{78} values) of four *D. serra* populations by rooting at the midpoint of the longest path (Prager and Wilson 1976), cophenetic correlation = 100%. The values above branches were obtained by bootstrapping (via DISPAN; Ota, 1993) and indicate the reliability of the given illustration.

DISCUSSION

Variability of morphological and behavioural characteristics of many species are believed to be determined by changes in the physical (Grant, 1991; Soares et al., 1999) and/or biological (Levitan, 1988) environment. The phenotypic characteristics may be determined exclusively by the genotype (Rothwell, 1993), or by the interaction between genes and the environment (Pigliucci, 1996; Soares et al., 1999). Selection pressure acting upon a diverse, genetically determined phenotype will trigger evolutionary changes while environmentally determined plasticity may buffer them (Grant, 1991). Therefore, it has been controversially discussed whether different phenotypes of the surf clam *D. serra*, inhabiting two biogeographic provinces along the southern African coast, belong to the same species (Donn, 1990a; Soares et al., 1998).

Morphological variation

Results of our morphological analyses supported significant differences in shell shape between both coasts (Donn, 1990a; Soares et al., 1996). Soares et al. (1998) reported these differences already in juvenile stages. Since smaller and less wedge shaped clams burrow faster than thick shelled ones (Trueman et al., 1966), the authors proposed that the different shell morphologies are the result of directional selection: more wedge shaped, elongate clams would be selected for in the intertidal (Southeast Coast) because they burrow faster and swash-ride more efficiently and thus are less exposed to predators, whereas flat, disc shaped shells are selected for on the West Coast due to increased stability in the subtidal habitats (Donn, 1990b). If the observed low differences in allozymes between Maitlands (IV) and the most northerly located population would mirror differences of the total genomes, it would be unlikely that significantly different shell shape is genetically determined and therefore environmental factors would be likely to cause the different morphologies resulting from phenotypic plasticity. Transformation experiments of marked spat could verify this hypothesis.

Genetic variation

Within populations, *D. serra* showed moderate to high levels of genetic variation: *A*, *H* and *P* values were in the range commonly found in other marine bivalves (Table 4).

Significant heterozygote deficiency relative to Hardy-Weinberg expectations was found for all analysed populations (confirmed by a moderate mean F_{IS} value: 0.362). Results from other bivalves range between 50-67%, whereas Population I is 77% and the other three populations less deficient (Table 5).

Table 4
Average number of alleles per locus (*A*) mean heterozygosity (*H*) and percent polymorphism (*P*) for different freshwater (F) and marine (M) bivalves.

Species	A	H	P	Reference
Aulacomya ater regia (M)	2.31	22 - 29%	-	Blot et al. (1987)
Bathymodiolus spp. (M)	1.27 - 2.09	-	9-36%	Moraga et al. (1994)
Coelatura kunenensis (F)	1.31 - 1.55	-	28.6%	van der Bank (1995)
Donax deltoides (M)	3 - 4			Murray-Jones and Ayre (1997)
D. serra (M)	1.73 - 1.91	18 - 22%	45.5-59.1%	This study
Mytilus edulis (M)	-	9.5%,	30%	Ahmad et al. (1977),
		13 - 23%	-	Grant and Cherry (1985)
M. galloprovincialis (M)	-	18 - 30%	-	Grant and Cherry (1985)
M. desolationis (M)	2.02	23 - 27%	-	Blot et al. (1987)
Ostrea edulis (M)	1.88	8.9%	18.2-40.9%	Saavedra et al. (1993)
Quadrula quadrula (F)	2.06	20 - 27%	50-70%	Berg et al. (1998)
Venus antiqua (M)	1.88	8.8%	38.5-69.2%	Gallardo et al. (1998)

Table 5
Percentage of deficiencies related to Hardy-Weinberg expectations of four freshwater (F) and one marine (M) clam species.

Species	Percentage of deficiency related to Hardy-Weinberg expectations	Reference
Quadrula quadrula (F)	67%	Berg et al. (1998)
European anadontines (F)	64%	Nagel et al. (1996)
Utterbackia imbecillis (F)	65%	Hoeh et al. (1998)
Utterbackia sp. (F)	50%	Hoeh et al. (1998)
Donax serra (M)	population I: 77%, II: 20%, III: 45% and IV 31%	This study

The deficiencies could be expected since ideal Hardy-Weinberg proportions do not occur in nature (Altukhov, 1981) and significant heterozygote differences are common in bivalve species (e.g. Gaffney et al., 1990; Johnson and Joll, 1993; Gallardo et al., 1998). Singh and Green (1984) proposed four possible explanations for such deficiencies: inbreeding, the Wahlund (1928) effect, the presence of null alleles and selection. Population size of *D. serra*, the dispersal via planktonic larvae (Birkett and Cook, 1987) and the fact that only specific loci are affected in the analysed populations provide sufficient evidence to discount inbreeding or the Wahlund effect, resulting from the inclusion of genetically distinct groups into a single population sample (e.g. Gaffney et al., 1990). Nevertheless, different timing of gametocyte release between genotypes throughout the six months spawning period (Laudien et al., in press) may play a significant role for the deficiency (Smith, 1987), which is supported by relatively high F_{IT} values. In addition, spatially or temporally separated cohorts may mimic a Wahlund effect within the population. Null alleles were not detected. Additional factors like non-random mating, self-sorting crossings and mutations are unlikely to be involved since they usually affect all loci.

Five loci (**EST-2, PEP-B2, PEP-C2, PGM-1, PGM-2**) show clinal variation from Sites II and III to the two Sites I and IV respectively (Fig. 1). While population II and III inhabit the cold bases of upwelling cells, periods of wind-relaxation (mid to late summer) result at beach I in a stratification (Shannon and Nelson, 1996) and rapid temperature increase (23 °C, unpubl. data). Therefore Langstrand (I) is only 3°C colder than Maitlands (IV), and the maximal SST may be associated with genetic variability since specific activities of enzymes or additional digestive isozymes may be induced by temperature (Lombard and Grant, 1986). The repeated trend in the parameters *A*, *H*, and *P* appear to be related to temperature. Further the *D*-values are also highest, when a cold and a warm site are paired. This is in agreement with Gartner-Kepkay et al. (1980), who also conclude that environmental factors can explain deficiencies and variation for mussels.

Although the analysed *D. serra* populations show no specific fixed allele differences, the genetic differentiation (D_{78} = 0.003 - 0.044) (Table 3) is in the range for conspecific populations (0 - 0.05; Ferguson, 1980) and compares well with *Mytilus galloprovincialis* (*D*: 0.032 ± 0.001) (Quesada et al., 1995) and *Macoma balthica* (*D*: 0.025 - 0.029) (Meehan, 1985). The present values are also much lower than those of sympatric *D. variabilis* and *D. parvula* populations (Nelson et al., 1993) and also between *Mytilus* species (D_{78}: 0.16 - 0.28 based on 16 - 23 loci) (Skibinski et al., 1980; Grant and Cherry, 1985; Väinölä and Hvilson, 1991). The observed low genetic differentiation of *D. serra* populations is supported by the relatively low *F*-values of differentiation among subpopulations (F_{ST} = 0.016 - 0.089). In essence, 1.6% - 8.9% of the allozyme diversity was explained by differences among samples, and therefore 91.1% - 98.4% by variation within local populations. Values of genetic variation reported for other bivalve molluscs with meroplanktonic larvae cover a wide range (*D. deltoides*, F_{ST} = 0.009 for 1 200 km, Murray-Jones and Ayre, 1997; *Ostrea edulis*, F_{ST} = 0.062, Saavedra et al., 1993; *Pinctada maxima*, F_{ST} = 0.104 over 3 400 km, Johnson and Joll, 1993). High similarities have been related to habitats with current systems (e.g. *Chlamys opercularis*, Macleod et al., 1985, *Choromytilus meridionalis*, Lombard and Grant, 1986, *D. deltoides*, Murray-Jones and Ayre, 1997), evolutionary inertia (i.e. not diverged genetically through evolutionary time, Soares et al., 1999) and a long meroplanktonic time period of widespread bivalve populations (e.g. Varvio et al., 1988). Our study reflects a low state of population separation indicating that larval life of *D. serra* is long enough to allow dispersal. The relatively low genetic differences between populations observed in the present study are in line with results from the black mussel *C. meridionalis* in the Cape Province (Lombard and Grant, 1986) and the sand-whelk *Bullia digitalis* (Grant and Da Silva-Tatley, 1997) and can be explained a) as the result of extensive gene flow between populations, b) due to balancing selective pressure acting upon populations and/or c) evolutionary inertia.

a) Gene flow

N_{EM} values ranged between 1.44 and 8.65, and therefore indicate substantial gene flow as a global value of $N_{EM} > 1$ prevents genetic drift/differentiation (e.g. Slatkin, 1987).

The present data compare well to estimates for *Mytilus edulis* populations (N_{EM}: 8.4; Slatkin, 1985; Quesada et al., 1995), whereas much higher levels (N_{EM} > 23) have been reported for *D. deltoides* separated by up to 1 200 km distance (Murray-Jones and Ayre, 1997). Larvae of *D. serra* from the southern West Coast beaches may be transported equator wards with the Benguela current and thus contribute to populations located further north. This hypothesis was supported as Population I shows higher genetic variability than II or III (Fig. 3). We hypothesise that the back transport of larvae may occur via the sub-surface current (e.g. Gordon et al., 1995), which flows conspicuous pole-wards throughout the year (e.g. Shannon and Nelson, 1996) (Fig. 1). However, hydrographic patterns are unlikely to explain similarities between the two outlying populations. Recruits would be closer related to the bypassed populations (II and III) and larvae would have to stay meroplanktic over a very long time period as the Agulhas current leaves the shelf at the southern termination of the Agulhas Bank and eddies move commonly westwards (Shannon and Nelson, 1996). Only an analogous trend for the outlying populations in contrast to the intermittent beaches could explain their low genetic distance with both getting recruits from beaches mainly located against the surface currents while the counter-currents may back-transport some larvae. However, the intrapopulational differentiation is much higher at IV than at the other beaches (Fig. 3) and therefore gene import at IV much lower. Grouping of the two outlying populations is therefore unlikely to be related to hydrography.

There is a possibility of gene exchange by human interference, which could explain the lower genetic distance of I and IV as transfer of *D. serra* has happened at some beaches of the eastern Cape and at Cape Cross (J. Laudien, pers. comm. from local fishermen, nature conservationists and staff of the Namibian Ministry of Fisheries and Marine Resources).

b) Balancing selective pressure

Balanced selection related to SST is much more likely to explain similarities between Site I and IV. Karl and Avise (1992) also suggested selection to explain the uniformity of their genetic analyses. Allozymes may have effects on overall fitness and growth rates (Koehn and Gaffney, 1984; Singh and Green, 1984) and therefore selection for the most beneficial enzyme could be the cause for heterogeneity of the allozyme frequencies. This is supported by results from Gartner-Kepkay et al. (1980), who found indications in *M. edulis* that variable environmental parameters are mirrored in populations with variable genomes and thus reflect a functional aspect of adaptability. In *D. serra* several loci are affected differently, thus we postulate that there is a different sensitivity of the enzyme systems to various environmental selective forces. Analysed clams show allelic diversity particularly at the peptidase (**PEP**) locus. Variations at the cytosol aminopeptidase (**PEP-E**, formerly **CAP** or **LAP**) locus, are in agreement with findings for *M. edulis* and *Geukensia demissa* (Young et al., 1979; Garthwaite, 1986). Different **PEP-E** genotypes provide differences in growth rate and tissue weight (Koehn and Gaffney, 1984; Garthwaite, 1989) and therefore this could be a useful locus for

selective breeding and aquaculture. As a digestive enzyme **PEP** may be subject to the availability of certain food compounds. Thus varying nutrition between sites may contribute, although not significantly (e.g. McMillan 1993), to genetic distances.

c) Evolutionary inertia

Sandy beach organisms are generalists (Brown and McLachlan, 1990) and a co-adapted genome supporting high plasticity allows them to withstand and rapidly adapt to changes of the dynamic environment. Kreitman and Akashi (1995) assume that strong selection pressure for phenotypic plasticity may override stochastic processes, such as random genetic drift and mutation, which promote divergence of populations. If different phenotypes are fit for different environmental conditions (Sultan, 1995), populations can theoretically survive long geological periods without the necessity for genetic changes. This also may explain the genetic similarity of *D. serra* populations, in line with explanations for other sandy-beach molluscs (Soares et al., 1999).

Genetic isolation

For species with planktonic larvae, direct determination of the subdivision of populations is virtually impossible and genetic comparison provides an indirect but powerful tool. Our results show a substantial geographic separation of the two Namibian populations. Several studies indicated that the intense upwelling cell at Lüderitz (e.g. Shannon, 1989) (Fig. 1) could cause a biological discontinuity (e.g. van der Bank and Holtzhausen, 1998/99), initiated near 24°30'S in the vicinity of Meob Bay (Agenbag and Shannon, 1988). This effective biotic barrier could be an expanded cold water filament generated in the Lüderitz upwelling cell, a deflection of the poleward under-current located in the vicinity of Meob Bay (*cf.* Monteiro, 1999) or the combination of changes in circulation, turbulence and stratification (Agenbag and Shannon, 1988). The present genetic analysis resembles the picture observed for other marine bivalves separated by biogeographic borders (e.g. Saavedra et al., 1993; Sanjuan et al., 1994; Quesada et al., 1995) and supports the hypothesis that environmental forces may be the major causal elements determining genetic differentiation. We emphasise that the observed genetic differences between populations could also reflect historical patterns of gene flow such as large-scale climatic changes, which occurred in the Atlantic over the Pleistocene (e.g. Thunell and Belyea, 1982) and could have initiated the gene divergence among neighbouring populations (Endler, 1977). Therefore population dynamics for stocks should be analysed separately. If these parameters are not similar, proper management of future commercial *D. serra* harvests must be adjusted respectively.

CONCLUSIONS

This preliminary genetic study on *D. serra* reveals that populations are conspecific and possess genetic variation in the range of most other marine bivalves that potentially allows for adaptation to environmental changes. Our results reveal a discrepancy between morphological differences and genetic similarity. Therefore it is likely that different shell shapes are resulting from phenotypic plasticity caused by environmental factors. The allozymes described show a good basis for further studies on genetic distribution patterns and enzyme polymorphism. Within-area and additional population sampling is required to substantiate the present results. Further confirmation of the strength of larval connections may involve surveying genetic variation in newly settled larvae and juveniles in particular. The substantial subdivision of the two Namibian populations requires separate analyses of population dynamics.

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8 LIST OF SELECTED ABBREVIATIONS

Abbreviation	Explanation (unit in parentheses)
I	Langstrand
IA	Paaltjies IV
II	Meob Bay
III	Bloubergstrand
IV	Maitlands in St. Frances Bay
A	Mean number of alleles per locus
AFDM	Ash-free dry mass (g)
B	Biomass (g AFDM)
BIOSYS	Comprehensive analysis of electrophoretic data in population genetics and systematics
Borax	Natriumtetraborat-10-hydrat
c	Index of cohort
CI	Condition index
D	Standard genetic distance
D_1	Prevosti distance
D_{78}	Unbiased genetic distance
DISPAN	Genetic Distance and Phylogenetic Analysis
DM	Dry mass (g)
EDTA	Ethylenediaminetetra-aceticacid
F_{IS}	Fixation index of individuals relative to its subpopulations
F_{IT}	Fixation index of individuals relative to the total population
F_{ST}	Amount of differentiation among subpopulations relative to the limiting amount under complete fixation
H	Average heterozygosity (%)
h	Hour(s)
HPLC	High-Performance Liquid Chromatography
K	Growth constant of VBGF (y^{-1})
k	Number of alleles at the locus
L_1	Length at time of release (mm)
L_2	Length at time of recapture (mm)
L_∞	Asymptotic length (mm)
L_j	Midlength of size class j (mm)
L_t	Length at age t (mm)
LFD	Length-frequency distribution
LT_{50}	Median survival time (a^{-1})
M	Number of migrants per generation
M_t	Total wet mass (g)
M_v	Wet mass of visceral mass (g)
N	Number of individuals

n	Number of populations
N_E	Effective population size
N_{EM}	Effective number of migrants per generation
N_j	Number of specimens of size class j
OGP	Overall growth performance
P	Percentage of polymorphic loci (%)
P	Production (g AFDM)
PCA	Perchloric acid
PDA	Photo Diode Array
s	Number of populations
SST	Sea surface temperature (°C)
t	Age (y)
t_0	Age at zero length (a^{-1})
TCA	Trichloric acetic acid
VBGF	Von Bertalanffy growth function
WM	Wet mass (g)
Z	Total instantaneous mortality rate

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