Growth, condition, survival and feeding rate of the Pacific oyster (*Crassostrea gigas* Thunberg) cultured in three distinct South African environments

by

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Declaration

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Chapter 1

General introduction

Aquaculture is a growing sector of agriculture due to declining natural fish stocks and a growing global population's need for food security. The term "mariculture" is used for the culture of marine species and oysters are a high-value mariculture product. South African commercial oyster culture dates back to 1948, with cultured indigenous Cape rock oysters (*Striostrea margaritacea*) in Knysna (Haupt *et al.* 2010a). Since 1973, the introduced Pacific oyster (*Crassostrea gigas*) has been the only commercially cultured species due to its fast growth rates, a high feeding efficiency and a tolerance to a wide range of environmental conditions (Quayle 1980; Hecht and Britz 1992; Almeida *et al*. 1997; Bayne *et al.* 1999; Bayne 2002; Haupt *et al*. 2010a).

Among all the indigenous oysters which include; *S. margaritacea*, the Natal rock oyster (*Saccostrea cucullata*), the red oyster (*Ostrea atherstonei*) and the Cape weed oyster (*Ostrea algoensis*), only *S. margaritacea* has the required combination of a larger adult size and a suitable cup-shape for mariculture purposes (de Keyser 1987; Haupt *et al*. 2010a). Although relatively slow growth for *S. margaritacea* compared to *Crassostrea gigas* has been established, previous growth trials were only performed in the Knysna estuary (de Keyser 1987; Haupt *et al*. 2010a). Growth trials with *S. margaritacea* within other environments might yield different results, and it is important to monitor environmental variables at potential and existing oyster culture sites to explain growth for future trials using this indigenous oyster.

Until 2001, Pacific oyster culture in South Africa was most prominent in the Knysna estuary, but since then has moved to Algoa Bay (Eastern Cape) and Saldanha Bay (Western Cape) due to floods (Haupt *et al.* 2010a). South African oyster culture is limited by the small number of protected bays and permanently open estuaries (Haupt *et al.* 2010a). Algoa Bay and Saldanha Bay are relatively protected from wave-action, but only Saldanha Bay is situated within the nutrient-rich Benguela upwelling system (Pitcher and Calder 1998;

Monteiro *et al*. 1998; Monteiro and Largier 1999). South African oyster culture is therefore dominated by operations in Saldanha Bay (Olivier *et al.* 2013), which is a favourable culture environment due to high phytoplankton biomass combined with moderate temperatures (Korringa 1956; Pitcher and Calder 1998).

Saldanha Bay also supports all of the country's mussel farms (Pitcher and Calder 1998), and its carrying capacity for bivalve culture is influenced by nitrogen flux from the upwelling system into the bay, phytoplankton production and carbon flow through the farming area ecosystem (Grant *et al*. 1998; Monteiro *et al*. 1998; Pitcher and Calder 1998). Currently, Saldanha Bay is theoretically capable of producing 10.6 to 28.3 times more bivalves than is currently produced. Oyster production has increased by 42% from 2005 to 2008 and the current total for oysters produced in Saldanha Bay is 176 tons per year (Britz *et al.* 2009; Olivier *et al.* 2013).

The annual production of *C. gigas* in South Africa in 2010 was 276 tons, compared to 95 000 tons for France, 29 169 tons for USA, and 200 298 tons for Japan (FAO 2010). Since Saldanha Bay is not utilized to its full potential and because production is relatively low compared to other countries, local oyster culture is not yet fully developed, but this is not only due to the limited number of sheltered culture areas. In addition to challenges regarding funding of aquaculture practices, government has, until recently, limited oyster culture (and aquaculture in general) through the lack of financial investment, and through issues related to regulation (Britz *et al*. 2009; Haupt *et al.* 2010a; Olivier *et al*. 2013). Once these obstacles are overcome, South Africa displays a high potential for export of oysters, because oysters are in good condition in the winter (May – July) when oysters in the Northern hemisphere experiences summer mortality (Cheney *et al.* 2000; Olivier *et al.* 2013).

Fisheries on the South African West Coast have decreased, thus oyster and mussel culture operations can provide employment to unskilled local workers previously employed

by the fisheries sector (Olivier *et al.* 2013). For government to provide more financial investment foroyster culture, more knowledge is needed on viable oyster farming practices, particularly with regard to suitable culture environments in South African waters. Despite studies on oyster growth globally, none are published on parameters affecting oyster growth and other commercially beneficial traits in South Africa. Published works on oysters in South Africa have covered treatments to reduce *Polydora* spp. on *C. gigas* (Nel *et al.* 1996), the distribution of naturalized *C. gigas* along the coast (Robinson *et al.* 2005), the history and status of oyster culture and exploitation (Haupt *et al.* 2010a), alien species introduced through oyster culture (Haupt *et al.*2010b), and an early survey of local oyster culture sites (Korringa 1956). Only an unpublished M.Sc. thesis (de Keyser 1987) focused on oyster culture.

In South Africa, long-line suspended culture predominates, where oyster cages are tied to lines (spaced approximately 2 m apart) which are suspended from a horizontal longline. The longline is kept afloat by buoys, and suspended cages hang above the bottom sediment. Suspended culture is advantageous because oysters are permanently immersed and therefore their time for filter-feeding is maximized, and higher growth rates are achieved than those for intertidal-grown oysters (Wisely *et al*. 1979). Because oyster cages are fixed at a specific level below the sea-surface (typically 1 – 1.5 m), they can be fastened where light-intensity and water movement, which both contribute to food distribution, are optimal. Suspended culture can be based offshore (Pogoda *et al.* 2011), in semi-enclosed bays (Brown and Hartwick 1988; Kobayashi *et al.* 1997; Hyun *et al.* 2001) and in fully enclosed ponds ("pond culture"; King 1977; Almeida *et al*. 1997). With pond-culture seawater is usually pumped from the sea (King 1977).

Oyster spat, typically 0.3 – 3 g, are imported from the U.S.A., Namibia and Chilé for "grow-out" at local oyster farms. "Grow-out'' refers to the period between the planting of oyster spat in the farm and harvest for market. Oysters are nursed from larvae in a protected

environment until they have formed shells and are largeenough to be planted for grow-out. For South African oyster culture, spat are imported because currently there is no local hatchery. Once oyster spat have been planted for grow-out, they are usually "graded" every 6 – 8 weeks (Haupt *et al*. 2010a) during removal of epibionts, and sorted into different size groups to avoid re-planting of slow- and fast-growing oysters within the same compartments. When "fast-growers" are placed within the same immediate vicinity, they out-compete smaller oysters for space and food. This competition becomes more pronounced at high stocking densities at which growth rates are impaired (Mann and Ryther 1977; Héral 1993). Oysters are therefore re-planted within optimal stocking densities after each grading session.

The purpose of this study was to compare growth rate, feeding rate, condition and survival of *C. gigas* among three environmentally distinct grow-out localities, spanning the range of oyster farms along the South African coastline. These localities included: two sea-based oyster farms located 1 – 2 km from the shore at Algoa Bay (Eastern Cape) and Saldanha Bay (Western Cape), and a land-based farm at Kleinzee (Northern Cape). Oyster spat of similar initial sizes were planted within long-line cages for grow-out. Environmental parameters monitored included temperature, phytoplankton abundance (measured as chlorophyll *a* concentrations; Chapter 2) and fatty acid composition (Chapter 3). These were related to growth rate, condition and survival (Chapters 2 and 3), and feeding rate and morphology (Chapter 4). Since South African oyster culture shows great potential for expansion within Saldanha Bay (Olivier *et al.* 2013), this study aimed to establish the potential of oyster culture expansion within Algoa Bay and within pond-culture systems, and to compare oyster performance and environmental suitability to existing oyster farms in South Africa.

Within the first year of the study, a growth trial on three cohorts of *Crassostrea gigas* was conducted from May 2010 – March 2011 (Chapter 2) in these three environments, with

continuous monitoring of temperature and chlorophyll *a*. A second growth trial for a comparison of two different cohorts commenced in July 2011 and lasted until June 2012 (Chapter 3). Temperature and chlorophyll *a* were logged again and the collection of seawater samples for fatty acid analysis was included as an additional environmental variable to measure the nutritional value of each environment. Two feeding trials (Chapter 4) and subsequent measurements of feeding organ size were conducted after the second growth trial for comparison between oysters from Algoa Bay and Saldanha Bay.

In Chapter 2, oyster growth rate, condition, survival, temperature and chlorophyll *a* are compared among the three sites for the first growth trial ("Study 1"). Very little is known about how different South African environments affect oyster performance. Grow-out sites fall within distinct sea-temperature ranges, with Saldanha Bay being situated in the cool Benguela current system and Algoa Bay in the warmer Agulhas current system. Depending on the pond system, pond farms are known to have unique temperature dynamics. The viable range of commercial oyster grow-out environments are established in this Chapter. Growth rates are compared to those found for *C. gigas* in other countries to place the feasibility of local oyster culture practices into perspective.

The second growth trial ("Study 2", Chapter 3) permitted inter-annual comparisons of temperature and chlorophyll *a* for each site. This chapter focuses on a comparison between two *C. gigas* cohorts of different origin, grown within each environment. These cohorts were imported from a Namibian and a Chilean hatchery, selected within the same size-range, and grown-out at Algoa Bay, Saldanha Bay and Kleinzee. Information gained on the performance of different cohorts at each environment, can be used to identify the relative influences of environment and cohort on performance of *C. gigas* under normal culture practices. This Chapter provides an indication of the benefits that can be gained from building a local hatchery, where trials on the effect of environment and cohort on oyster

growth and survival can be performed. As an additional environmental parameter, fatty acid composition, which reflects the nutritional value of phytoplankton and seston in the seawater, was also compared between the two sea-based farms (Chapter 3). Differences in fatty acid composition and their proportions between Algoa Bay and Saldanha Bay, could reflect both a difference in nutritional value and, possibly, phytoplankton species composition between sites for any given time (Langdon and Waldock, 1981; Parrish *et al*. 2000; Rico-Villa *et al.* 2006; Kharlamenko *et al*. 2008; Burnell and Allan 2009).

To explain growth differences between the two sea-based farms further, feeding efficiency between oysters from Algoa Bay and Saldanha Bay are compared, since growth rate is related to feeding rate (Bayne *et al*. 1999). For this, clearance rate (the number of particles removed from the water per unit time) was compared, simultaneously for oysters from each grow-out site within water from both Algoa Bay and Saldanha Bay separately. Clearance rates were also compared within a laboratory setup; within a flow-through system with an artificially supplied diet. Feeding rate, in turn, is largely dependent on morphological adaptations in the size of feeding organs which can display considerable plasticity (Barillé *et al.* 2000; Honkoop *et al*. 2003; Bayne 2004; Benninger *et al*. 2008). Therefore the relative sizes of gills and labial palps, both involved in feeding, are compared between oysters from both Algoa Bay and Saldanha Bay (Chapter 4). Finally, within a single cohort, differences in growth can be explained by specific responses to food availability, food quality and temperature at the different sites.

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Chapter 2

Growth and condition of the Pacific oyster (*Crassostrea gigas*) at three environmentally distinct South African oyster farms

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ABSTRACT

The Pacific oyster *Crassostrea gigas* is cultured at eight commercial farms in South Africa. Worldwide, environmental-specific intensive selection on the species optimizes commercially beneficial traits, but its performance has not been studied in South Africa. From May 2010 – March 2011, we compared two-monthly measurements of growth rate, condition and survival of three cohorts of different origin in long-line culture at three different South African environments: two sea-based farms located in Saldanha Bay (Western Cape) and Algoa Bay (Eastern Cape); and a land-based farm at Kleinzee (Northern Cape). Overall, Saldanha Bay was cooler (mean sea surface temperature of 16.0°C; C.V. = 16.2%) than the other two localities, which did not differ significantly from one another (Kleinzee 18.6°C; 20.4%; Algoa Bay 17.8°C; 8.9%). The high variability at Kleinzee reflected stronger summer warming than at the other two farms. Saldanha Bay had higher phytoplankton biomass (mean 14.3 mg Chl *a* m^{-3}, C.V. 54.2%, May 2010 – March 2011) than did Algoa Bay (mean 5.3 mg Chl *a* m^{-3}, 81.0%, September 2010 – March 2011). The three cohorts showed similar trends in growth and condition. Growth rates, expressed as live or dry mass gains, were two to ten times higher than those reported elsewhere in the world, and dry weight condition indices (DWCI) were also high. High rates in Algoa Bay (measured as live mass) despite its relatively low phytoplankton biomass seem to reflect a similar phenomenon to that reported in other relatively phytoplankton-poor grow-out environments, such as the Mediterranean Thau Lagoon in France. Gain in dry meat mass and condition were highest for oysters in Saldanha Bay, with high food availability offsetting the thermal advantages of the warmer Algoa Bay site. Oysters in the bottom layers of the cages grew significantly faster than those in the top layers, particularly in Saldanha Bay, possibly reflecting fine-scale vertical differences in phytoplankton biomass and seston. Saldanha Bay proved to be the best of the three locations for producing market-ready oysters. Algoa Bay yields faster growth,

but leaner oysters and is a good nursery location, as is Kleinzee which yields overall slow growth, but good shell quality in winter and early spring.

1. Introduction

Worldwide, the Pacific oyster (*Crassostrea gigas*) has been cultured for centuries, with recently intensified selection for commercially beneficial traits such as growth rate (Hedgecock *et al.* 1995; Dégremont *et al.* 2005; Taris *et al.* 2007), survival (Ward *et al*. 2000; Langdon *et al*. 2003; Evans and Langdon 2006), disease resistance (David *et al*. 2007), shell shape (Ward *et al*. 2000), feeding efficiency (Bayne *et al.*1999), and meat quality (Langdon *et al*. 2003). Oyster farms in Australia, New Zealand, the U.S.A., France, and the UK produce their own larvae for culture and export. Although South Africa currently has eight commercial oyster farms, it lacks a hatchery, and thus has no locality-specific breeding programs. Oyster spat and seed are imported from Namibia, Chilé and the U.S.A. for grow-out, a practice that carries substantial environmental risks of importing both invasive alien species as epibionts, and bivalve pathogens.

Oyster growth and survival are affected by genotype and environmental parameters such as temperature, salinity, pH, particulate organic matter (POM), particulate inorganic matter (PIM), dissolved oxygen (DO), and phytoplankton productivity (Langdon *et al*. 2003; Dégremont *et al*. 2005; Evans and Langdon 2006; Swan *et al*. 2007). Phytoplankton serves as the main food source for filter-feeding oysters, and can be measured as the concentration of chlorophyll *a* in the water of the grow-out environment (Gangnery *et al*. 2003). Among all these environmental parameters, temperature, PIM, POM and chlorophyll *a* are the most important determinants of oyster growth rate (Brown 1988; Brown and Hartwick 1988a, b; Bougrier *et al*. 1995; Barillé *et al.* 1997; Toro *et al.* 1999; Gangnery *et al.* 2003; Flores-Vergara *et al.* 2004).

Growth of the Pacific oyster in culture has been well-studied worldwide, such as in Malta (Agius *et al*. 1978), France (Gangnery *et al*. 2003), Canada (Brown & Hartwick 1988a & b), México (Chávez-Villalba *et al.* 2007), Australia (Li *et al*. 2009), and New Zealand

(Handley 2002). Some sheltered South African bays are suitable for culture of this species: for example, moderate temperatures combine with high phytoplankton biomass to make Saldanha Bay a particularly promising environment (Korringa 1956). South Africa has a 20-year history of Pacific oyster culture, with annual production ranging from 1.6 million oysters in 1985 to a maximum of 8 million in 1991 (Haupt *et al.* 2010). This promise notwithstanding, there have been no published studies comparing growth of *C. gigas* at different South African sites – information valuable to the industry. An unpublished M.Sc. thesis on growth of this species in Algoa Bay (de Keyser 1987) constitutes the only available information on this topic. To address this shortfall, the aim of this Chapter was to compare growth rate and condition of different cohorts between three different localities that spanned the range of culture conditions in the country. These variables were related to sea temperatures and phytoplankton biomass on the farms.

2. Materials and methods

2.1. Study sites

From May 2010 until March 2011, the growth trials were conducted on two sea-based farms located 1 – 2 km from the shore in water of 12 – 14 m: one in Saldanha Bay (Western Cape, 33.0°S, 18.0°E) and the other in Algoa Bay (Eastern Cape, 33.95°S, 25.6°E). The third site was a land-based farm at Kleinzee (29.7°S, 17.1°E) in the Northern Cape. Saldanha Bay is a semi-enclosed embayment that, because of its links to the highly productive Benguela upwelling system off the West Coast of South Africa, has high subsurface nitrate input and productivity (chlorophyll levels) for most of the year (Pitcher and Calder 1998; Monteiro *et al.* 1998; Monteiro and Largier 1999). Phytoplankton biomass is dominated by diatoms in spring and early summer, and by dinoflagellates in late summer and autumn (Pitcher and Calder 1998). The Eastern Cape site is an open sea farm situated adjacent to the harbour

within Algoa Bay in the Agulhas current system, but lacks the strong summer upwelling of the Benguela. Phytoplankton in both Algoa Bay and Saldanha Bay comprises mostly larger (> 5 µm in diameter) diatoms and dinoflagellates (B. Hubbart, G. Pitcher, and S. Jackson, unpublished data), but abundance in Algoa Bay is much lower and proportionally more phytoplankton are < 5 µm compared to Saldanha Bay. The nursery ponds at Kleinzee are pump-ashore systems approximately 200 m from the ocean, and were included because they are an important nursery site in South Africa; however, the slow water turnover (for which daily volumes are unavailable) results in a less productive environment.

2.2. Temperature and chlorophyll a

Sea temperature was logged at each study site at 30 min intervals in the top and bottom layers of two cages, using Thermochron iButton recorders in waterproof plastic bottles. Hourly estimates of chlorophyll *a* were obtained through deployment of a Turner Designs Submersible Fluorometer (SCUFA®) in Saldanha Bay and a WET Labs ECO Fluorometer in Algoa Bay. *In situ* fluorescence readings were calibrated through comparison with extracted chlorophyll concentrations (Parsons *et al*. 1984). These instruments were secured to the suspension rope of one of the cages, 20 cm above the cage (approximately one and a half meters below the sea surface). These measurements were conducted throughout the study in Saldanha Bay, but commenced only in September 2010 in Algoa Bay. Phytoplankton biomass in saltwater ponds is generally low (see Discussion and references therein), and therefore chlorophyll *a* was only measured and compared for the sea-based localities.

2.3. Oyster stocks and husbandry

Three cohorts of the Pacific oyster, *Crassostrea gigas* were imported*:* one from Coast Seafoods in Washington State, U.S.A., with an initial mass of 0.34 g ("US" cohort, two

months old), and two from Cultivos Marinos in Bahía de Tongoy, Chilé with initial mean masses of 4 g ("CS", "Chilé Small", four months old) and 19 g ("CL", "Chilé Large", six months old). Each cohort was equally divided to give a starting sample of 3 000 oysters per cohort per farm, and planted for grow-out at the end of May 2010. Live mass and condition index measurements of oysters were taken every two months (see below), and so the study consisted of five two-month grow-out periods between May 2010 and March 2011.

Oysters were planted in cylindrical five-layer plastic *Ostriga*® cages with a diameter of 600 mm, total cage height of 750 mm, compartment height of 150 mm, and an outer wall minimum mesh diameter of 9.4 mm. Each cage layer was divided into four identical quadrants or compartments, each containing two bags of oysters for the first two months, and one bag thereafter. Oysters in one compartment were used throughout for individual masses for growth estimation, and those in the other three to assess mortality. Fine mesh tulle bags were used for the first two months on the sea farms and for four months at Kleinzee, then replaced with mesh *Netlon*® bags of appropriate sizes (maximum mesh diameters 10 and 26 mm; lengths 650 – 750 mm). Bags were individually numbered and color-coded so that growth and mortality could be related to position within the cages, and track each bag throughout the study.

Cages were suspended from long lines one and a half to two meters beneath the sea surface. Stocking densities within cages conformed to commercial husbandry practices, and were adjusted by discarding oysters once every two months to keep the total biomass per compartment at approximately 650 g, while maintaining a standard number of oysters per bag for any given grow-out period on each farm. Initial stocking density was two bags per compartment, each containing 62 or 63 oysters for a total of 125 oysters per compartment, yielding 500 oysters per layer. Final density for all cohorts ranged between 3 – 15 oysters per bag (compartment) at the sea-based farms. The slowest-growing oysters were discarded after

every two months, to "grade" oysters as would be done on a commercial farm. This form of selection means that reported growth rates are optimal, and competition within bags did not impede growth of slower-growing oysters further. This was done to retain relevance to the industry, because avoidance of such selection would have resulted in stocking of oysters of disparate sizes, yielding growth rates not comparable to those obtained under standard husbandry practices.

At the two sea farms, oysters used for determination of condition indices were selected at random at the end of each grow-out period, whereas at Kleinzee we selected oysters that were large enough for shucking without loss of tissue.

2.4. Measurements of growth: live and dry mass gain

Every two months, oysters were cleaned, weighed, counted, and dead animals were removed and counted. Before re-bagging, oyster numbers were adjusted as described above. Individually-weighed oysters from each cage layer (hereafter "individual" oysters) were used for analyses on growth rate. For each of the remaining three bags in the layer, total combined masses for all oysters (hereafter "batch" masses) were used for two comparisons only: seasonal mortality, and effects of depth within cage on oyster mass gain. In addition to increasing sample sizes for these analyses, batch masses were included to keep stocking densities within each cage layer comparable to those in commercial farming practice.

Individual oysters and smaller batches were weighed with a Denver MAXX 120 g scale accurate to 0.01 g. Larger batches were weighed with a bigger, splash-proof Masskot15 kg scale accurate to 1 g.

For comparison with published growth rates of *Crassostrea gigas*, growth rates were estimated from the linear regression of live mass with time as an independent variable, using least-squares linear regressions on individuals only. Growth rate is derived from the slope of

these linear equations, in g.oyster^{-1}.day^{-1}. Also using individual live masses, growth was compared between farms and between the top and bottom layers of cages by fitting polynomial curves to individual oyster masses as a function of time (e.g. Brown and Hartwick 1988a). For each growth curve, the best-fit polynomial was ascertained using the extra sum-of-squares F-test, which compares the difference between residual sums-of-squares for the two models with the difference expected by chance. The result is expressed as an F ratio, from which a p-value is calculated (Haddon 2001).

Once the best-fit models for live mass data for each cohort at each farm were established, the three growth curves for each cohort between farms, also using the extra sums-of-squares F-test were compared.

To further compare allocation of resources to different body components (meat and shell) between grow-out sites, dry meat mass gain was estimated as a function of time for each cohort on each farm. For this, the least-squares linear equation expressing oyster dry meat mass as a function of whole live mass was used, these variables were measured for each cohort in the 40 oysters sub-sampled for condition index (Section 2.5 below). The same procedure as that of above was used to ascertain which polynomials fit best, and to compare the three cohorts' growth curves within each farm. For polynomial-based analyses, GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego California, USA) was used.

2.5. Oyster condition index

From July 2010 onwards, 40 oysters were taken from the biggest cohort (CL) at each farm for assessment of various measures of condition using wet and dry meat and shell masses (Crosby and Gale 1990). From September 2010, once oysters from all cohorts were

large enough, samples of 40 oysters each were likewise taken from the other two cohorts at each farm.

These oysters were weighed whole, shucked, and their meats and shells weighed separately. Shell and meat samples were dried at ± 50°C for four days and then re-weighed. Because it is independent of variability in intervalval fluid volume (Pogoda *et al.* 2011), Dry Weight Condition Index (DWCI *sensu* Handley 2002) calculated as the proportion of dry meat mass (g) to dry shell mass (g): (dry meat mass x 1000) / (dry shell mass) (Walne and Mann 1975) was used as a measure of condition. To assess density, shell dry weight was also expressed as a percentage of shell wet mass (Robert *et al*. 1993).

DWCI was compared for each cohort between farms, and with other published studies. Then, to avoid problems associated with ratio-based analyses that fail to account for departures of scaling exponents from unity, shell wet and dry mass between farms within each cohort were compared using separate slopes-model Generalized Linear Model Analyses of Covariance (GLZ ANCOVAs) with a log-link function. Fresh and dry shell masses were used as dependent variables, with fresh or dry meat mass respectively as covariates (continuous predictors or independent variables).

2.6. Mortality and fouling

Using batches, the number of dead oysters at the end of each grow-out period werre counted and expressed as a percentage of the original number of oysters for each grow-out period in each batch. This was compared between grow-out periods and farms for each cohort using Kruskal-Wallis ANOVA.

Fouling (epibiotic) organisms were identified in the field using a photographic guide (Branch *et al.* 2010). Through summer and autumn (Nov 2010 – Mar 2011) all fouling on the oysters were removed once every two months before weighing, by hand-cleaning, and if

necessary scraping with a shucking knife or paint scraper. Using both batches and individuals, all fouling organisms were weighed together for each bag. The total mass from fouling from batches was then divided by the number of oysters in that bag and expressed as an average fraction of individual oyster mass.

For each two-month grow-out period, temperature, chlorophyll *a,* mortality, DWCI, and shell density were compared between farms using Kruskal-Wallis ANOVA followed by post-hoc pairwise tests. Statistica 10.0 (Statsoft, Tulsa, Oklahoma, U.S.A.) was used for all the above analyses. All data were tested for normality with Shapiro-Wilks tests to differentiate between the use of parametric or non-parametric tests.

3. Results

Where not specified, all differences mentioned in this section are statistically significant ($p < 0.05$).

3.1. Environmental variables

Over the entire study period, daily mean sea temperature did not differ significantly between Kleinzee and Algoa Bay, but was cooler for Saldanha Bay ($p < 0.000001$, Fig. 1). Kleinzee exhibited the greatest temporal variation (mean of all daily sea temperatures = 18.6°C, coefficient of variation (C.V) = 20.4%). Corresponding values for Algoa Bay and Saldanha were 17.8°C (8.9%) and 16.0°C (16.2%). Temperature differences between farms emerged seasonally: in autumn to spring (12 May – 30 Sep 2010), Algoa Bay (mean of daily means = 16.7 ± 0.9 (1 S.D.)) was warmer than Kleinzee (14.6 ± 1.5) which was in turn warmer than Saldanha Bay (13.6 ± 0.8) ($p < 0.00001$ in all cases). In spring to autumn (1 Oct 2010 – 18 Mar 2011), Kleinzee (21.5 ± 1.8) was warmer than Algoa Bay (18.7 ± 1.4), with Saldanha Bay (18.1 ± 1.6) again the coolest ($p < 0.00001$ in all cases).

In waters cooler than 19°C, *Crassostrea gigas* allocates proportionally more of its metabolisable energy intake (Bayne 2004) to growth, and less to reproduction. Above this temperature, reproduction is prioritized (Chávez-Villalba *et al*. 2002, 2007) and total energy available for growth decreases (Bougrier *et al*. 1995). Consequently, the percentage of sea temperature records above this threshold for each farm for periods refered to as late autumn to early spring (May 2010 – Sep 2010) and late spring to early autumn (Oct 2010 – Mar 2011) were determined. In Saldanha Bay, 0% of late autumn to early spring and 33.2% of late spring to early autumn temperatures were above 19°C; in Algoa Bay these percentages increased to 0.4% and 48.0 % respectively. In Kleinzee 0%, of late autumn through to early spring and 91.8% of late spring through to early autumn temperatures exceeded 19°C.

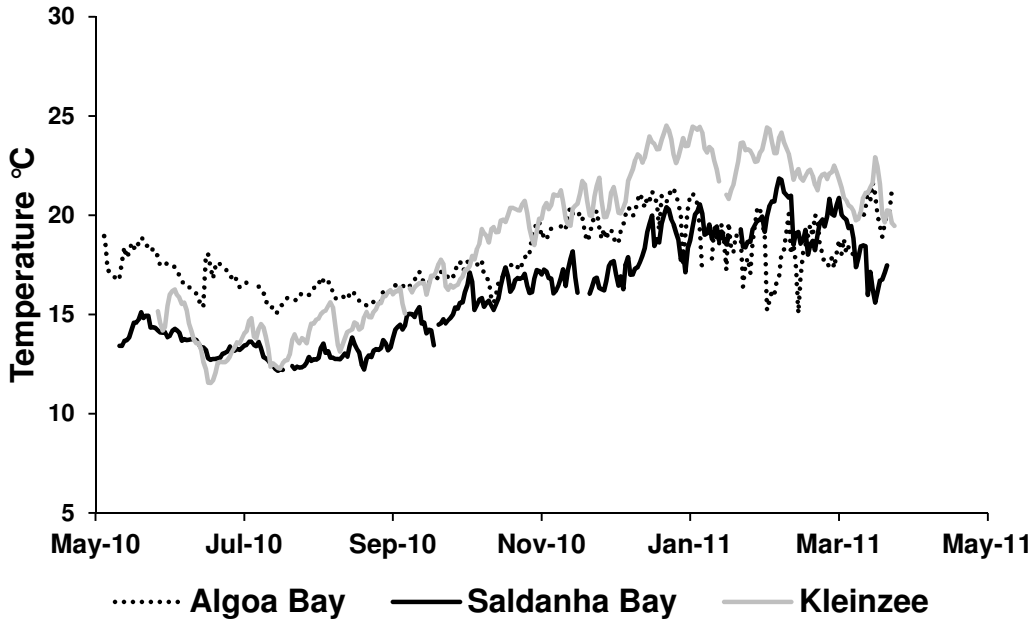

Figure 1: In autumn to spring (12 May – 30 Sep 2010), Algoa Bay was warmer than Kleinzee, with Saldanha Bay the coolest (Kruskal-Wallis $H_{2,409} = 245$, $z = 9.83$, 15.41, and 5.12, $p < 0.00001$ for all pairwise comparisons), but in spring to autumn (1 Oct 2010 – 18 Mar 2011), Kleinzee was warmer than Algoa Bay, with Saldanha Bay again the coolest ($H_{2,499} = 223$, $z = 10.90$, 3.12 and 14.26, $p < 0.00001$ in all cases).

Chlorophyll *a* measured in Saldanha Bay was generally > 5 mgm^{-3}, demonstrating high and variable phytoplankton productivity (Fig. 2). The daily mean chlorophyll *a* for May 2010 – Mar 2011 at Saldanha Bay was 14.3 mgm^{-3} (54.2% C.V.), ranging from 3 to 41.9 mgm^{-3}. At Algoa Bay the daily mean was 5.3 mgm^{-3} (81.0% C.V.; 1.5 – 28.8 mgm^{-3}) for Sep 2010 – Mar 2011. Chlorophyll *a* values were relatively high at both farms from Feb – Apr 2011.

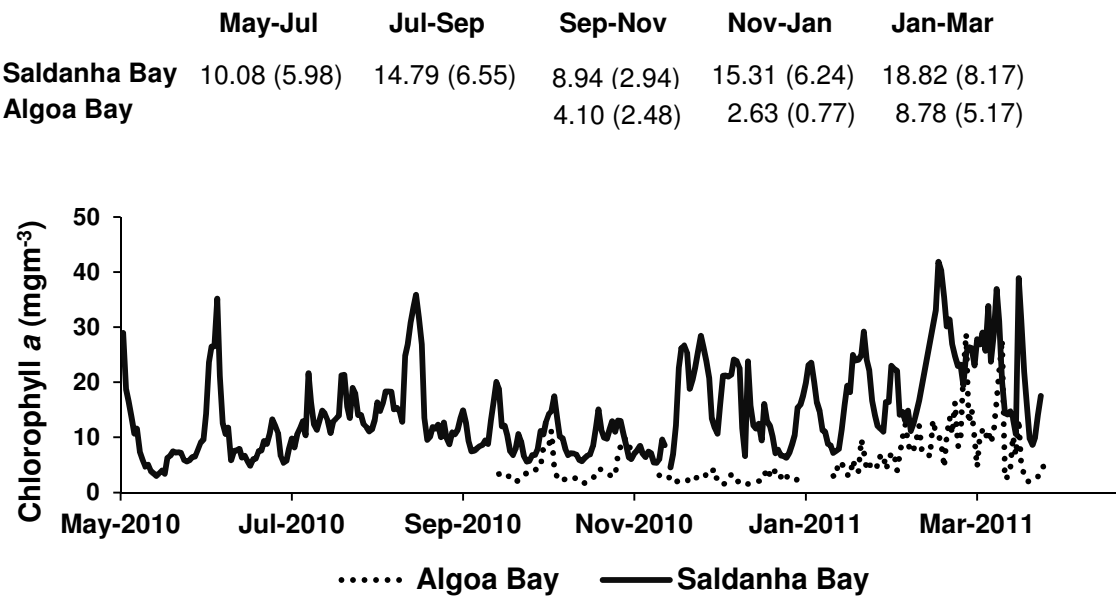

Figure 2: Daily mean chlorophyll *a* ($mg.m^{-3}$) values showed that Saldanha Bay primary productivity was higher than that for Algoa Bay from Sep 2010 to Mar 2011 (Mann-Whitney $U_{329, 117} = 6\ 408$; $z = 14.48$, $p < 0.001$). Means for each grow-out period and both farms are shown above the graph (± 1 S.D.).

3.2. Growth: live mass gain was fastest in Algoa Bay

Using individually-weighed oysters, total live mass gain was most rapid in Algoa Bay, followed by Saldanha Bay, then Kleinzee (Fig. 3). Oyster ages were as follows: US started at 2 and ended at 12 months old, CS started at 4 and ended at 14 months, and CL started at 6 and ended at 16 months old. For comparison with previously published values, least-squares linear regressions of mass gain as a function of time yielded estimates of growth rates (slopes, g live mass; gain.oyster^{-1}.day^{-1}) for the entire study period for all cohorts (Table 1).

Oyster growth is, however, not linear over time (Brown and Hartwick 1988a), and third-order polynomials provided the best-fit growth curves to our data (Fig. 3, Appendix 1). Moreover, total live mass includes water in the tissues, and shell mass, neither of which are of direct value to oyster farmers or their customers. To further compare allocation of resources to meat growth between grow-out localities, without the confounding effects of shell mass and meat water content, dry meat mass growth as a function of time was estimated by using equations obtained from the live masses and dry meat masses of oysters sampled for condition index for the entire study period (Fig. 4). For the CL cohort only, individual live masses measured throughout the study period (Fig. 3) were substituted into equations obtained using least-squares linear regressions of dry meat mass as a function of live mass for each grow-out period (Appendix 2). The ranking of oyster growth changed in this analysis, with Saldanha Bay oysters gaining more meat mass than did those at Algoa Bay, and Kleinzee remaining the site that yielded the slowest growth.

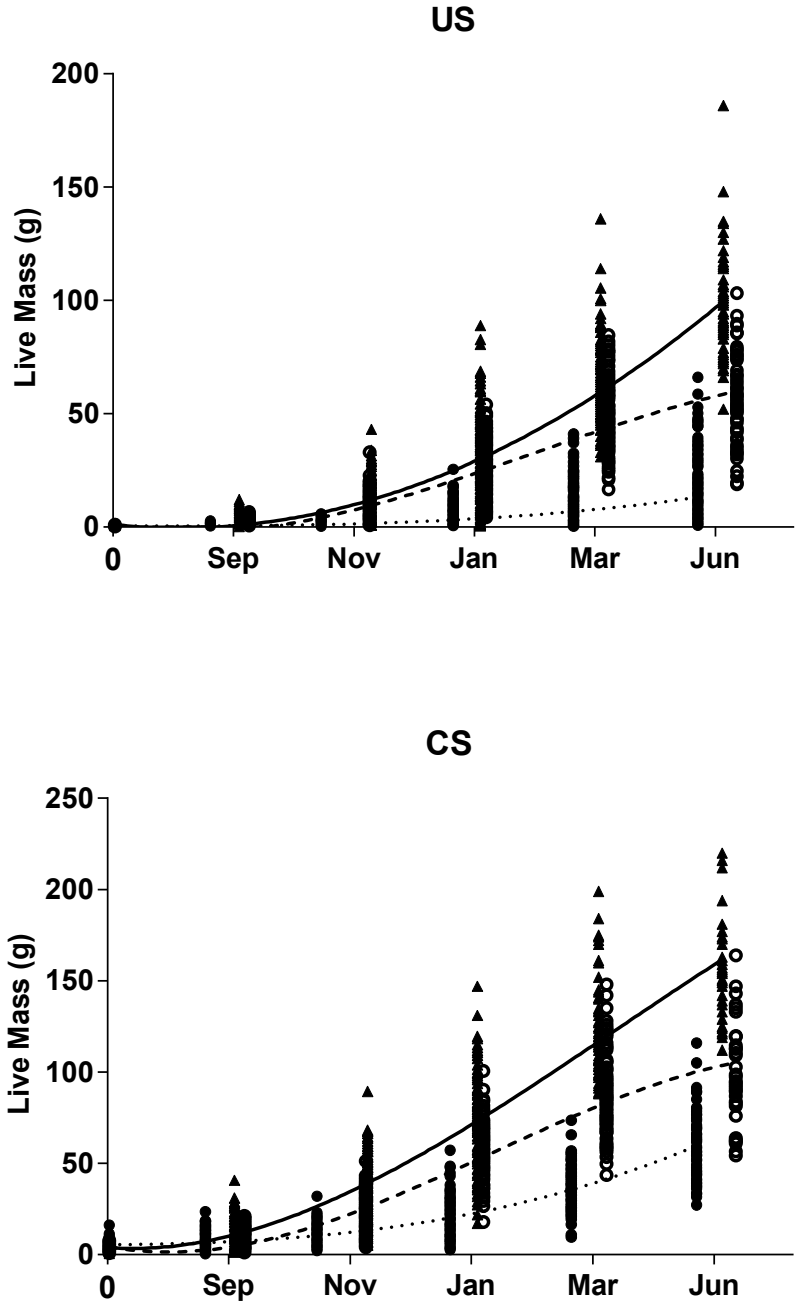

Figure 3: Whole live mass (g) for individual oysters as a function of time. For each cohort, mass gain was fastest at Algoa Bay (triangles ▲ and solid lines), followed by Saldanha Bay (hollow circles ○ and dashed lines), and then Kleinzee (solid circles ● and dotted lines) ($F_{8,4764}$ = 1308 with $p < 0.0001$ and $F_{8,4266}$ = 1000 $p < 0.0001$ for US and CS respectively). Sample sizes for US, CS and CL respectively at Algoa Bay ranged from 375 (start) to 61 (end), 365 – 36 and 250 – 30; for Saldanha Bay were 376 – 61, 370 – 41 and 250 – 49; and for Kleinzee 371 – 209, 371 – 88 and 225 – 91.

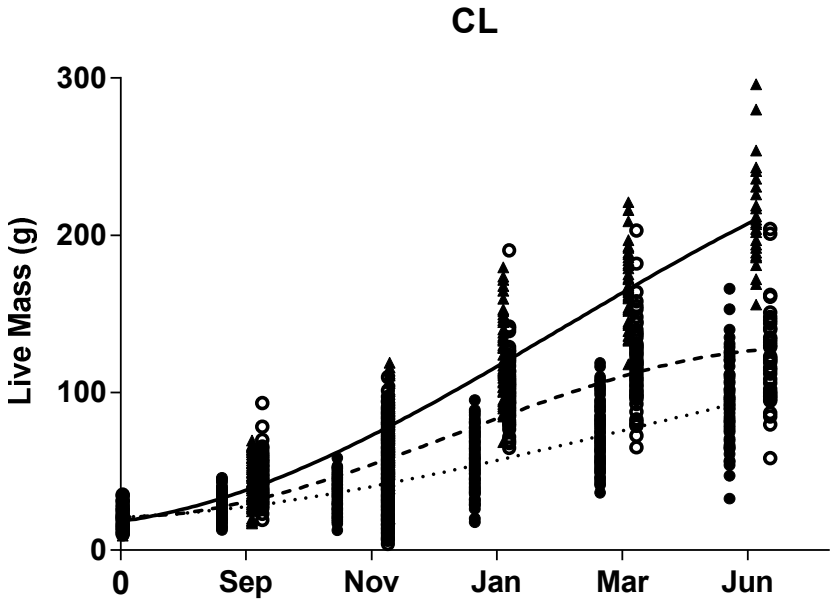

Figure 3 continued: Whole live mass (g) for individual oysters as a function of time for the CL cohort. Mass gain was fastest at Algoa Bay (triangles ▲ and solid lines), followed by Saldanha Bay (hollow circles ○ and dashed lines), then Kleinzee (solid circles ● and dotted lines) ($F_{8,2982} = 418.6$ $p < 0.0001$).

Table 1: Measures of growth in the present study, variously expressed in units comparable to those in the literature, and grouped accordingly.

Location	Growth rate / mass gain	Units	Culture method	Comments	Source
				U.S.A. cohort	
Algoa Bay, South Africa	0.246 ± 0.004 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	Slopes of linear least-squares regression, ± 1 S.D. (R^2 = 0.69, n = 1473)	This study
Saldanha Bay, South Africa	0.173 ± 0.003 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.71, n = 1493)	This study
Kleinzee, South Africa	0.037 ± 0.001 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.27, n = 1810)	This study
				Chilé Small cohort	
Algoa Bay, South Africa	0.439 ± 0.006 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.81, n = 1251)	This study
Saldanha Bay, South Africa	0.298 ± 0.004 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.77, n = 1385)	This study
Kleinzee, South Africa	0.144 ± 0.003 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.65, n = 1642)	This study
				Chilé Large cohort	
Algoa Bay, South Africa	0.580± 0.007 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.89, n = 857)	This study
Saldanha Bay, South Africa	0.351 ± 0.008 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.67, 1069)	This study
Kleinzee, South Africa	0.233 ± 0.004 $g.day^{-1}$	Live mass gain ($g.oyster^{-1}.day^{-1}$)	Long-line	(R^2 = 0.74, n = 1068)	This study

Atlantic coast, France	0.047 – 0.175 g.day^{-1}	Live mass gain (g.oyster^{-1}.day^{-1})	Long-line culture	Oysters 3 – 10 months old, thus comparable to US cohort in our study†	Boudry *et al.* (2003)
Portugal	0.098 ± 0.031 g.day^{-1}	Live mass gain (g.oyster^{-1}.day^{-1})	Intertidal table culture	Oysters ~7 months old at start, ~1 g live mass	Batista *et al.* (2007)
Baie des Veys, North Coast of France	0.178 g.day^{-1}	Live mass gain (g.oyster^{-1}.day^{-1})	Intertidal table culture	Highest growth rates in study	Dégremont *et al.* (2005)
Algoa Bay, South Africa	1.64 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	US cohort (± 0.71 S.D., n = 5)	This study
Saldanha Bay, South Africa	1.5 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	US cohort (± 0.63 S.D., n = 5)	This study
Kleinzee	1.18 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	US cohort (± 0.26 S.D., n = 5)	This study
Algoa Bay, South Africa	1.26 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Small cohort (± 0.46 S.D., n = 5)	This study
Saldanha Bay, South Africa	1.12 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Small cohort (± 0.39 S.D., n = 5)	This study
Kleinzee	1.0 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Small cohort (± 0.15 S.D., n = 5)	This study
Algoa Bay, South Africa	0.96 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Large cohort (± 0.23 S.D., n = 5)	This study

Saldanha Bay, South Africa	0.82 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Large cohort (± 0.36 S.D., n = 5)	This study
Kleinzee	0.82 %.day^{-1}	Instantaneous growth rate (%.oyster^{-1}.day^{-1})	Long-line culture	Chilé Large cohort (± 0.12 S.D., n = 5)	This study
Algoa Bay, South Africa	23 mg.day^{-1}	Dry mass gain (mg.oyster^{-1}.day^{-1})	Long-line culture	US cohort, started at shell heights of 10-20 mm*	This study
Saldanha Bay, South Africa	23.7 mg.day^{-1}	Dry mass gain (mg.oyster^{-1}.day^{-1})	Long-line culture	US cohort, started at shell heights of 10-20 mm	This study
North coast of Sicily	0.06 – 0.12 mg.day^{-1}	Dry mass gain (mg.oyster^{-1}.day^{-1})	Long-line culture	Grown 7 and 13 m below surface	Sarà and Mazzola (1997, Figs 4 & 5)
German Bight, North Sea	0.86 – 2.33 mg.day^{-1}	Dry mass gain (mg.oyster^{-1}.day^{-1})	Suspended culture from buoys	Started at shell heights of 10-20 mm	Pogoda *et al.* (2011, Table 3)
Arcachon Bay, South of France	3 to 40 g	Start to end live mass (g)	Intertidal Stanway cylinders	13 months of growth	Robert *et al.* (1993, Fig. 4)
Algoa Bay, South Africa;	3.0 to 150 g	Start to end live mass (g)	Long-line culture	10 months of growth Chile Small cohort	This study
Saldanha Bay, South Africa;	3.4 to 105 g	Start to end live mass (g)	Long-line culture	10 months of growth Chile Small cohort	This study
Kamakman Bay, South Korea	~2 to ~13 g	Start to end live mass (g)	Long-line culture	10 months of growth	Hyun *et al.* (2001, Fig. 4)
Algoa Bay, South Africa;	0.4 to 8.9 g	Start to end dry mass (g)	Long-line culture	10 months of growth Chile Large cohort	This study
Saldanha Bay, South Africa;	0.2 to 9.2 g	Start to end dry mass (g)	Long-line culture	10 months of growth Chile Large cohort	This study
Kamakman Bay, South Korea	0.3 to 3.2 g	Start to end dry mass (g)	Long-line culture	10 months of growth	Hyun *et al.* (2001, Fig. 4)

Seto Inland Sea, South Honshu, Japan	0.3 to 2.8 g	Start to end dry mass (g)	Long-line culture	7 months of growth	Kobayashi *et al.* (1997, Fig. 3)
Thau Lagoon, South of France	~ 0.15 to 2.7 g	Start to end dry mass (g)	Intertidal table culture	10 months of growth	Gangnery *et al*. (2003) (Fig. 6)
Gulf St Vincent, South Australia,	~1g to 2.2 g	Start to end dry mass (g)	Intertidal culture	13 months of growth	Li *et al.* (2009, Fig. 3)

Note: figure numbers are only given in source references for which values were read from figures.
†: US oysters in our study started at approximately two months old, attaining 12 months at the end of the study.
*: calculated and used for comparative purposes here only: not presented in Fig. 4 (see caption).

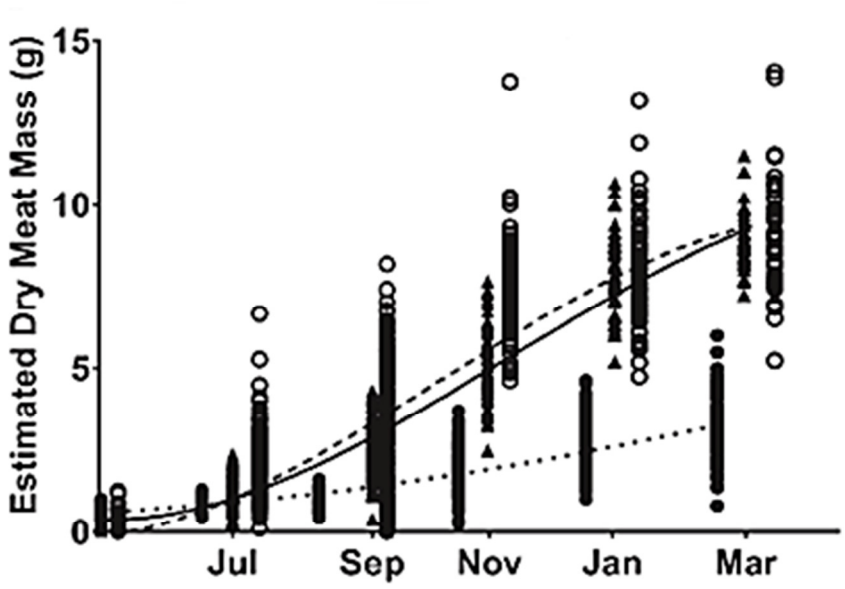

Figure 4: Estimates of dry meat mass (g) for individual oysters of the CL cohort as a function of time (Algoa Bay: triangles ▲ and solid line; Saldanha Bay: hollow circles ○ and dashed line; and Kleinzee: solid circles ● and a dotted line). Best fit polynomial parameters and statistics given in Appendix 3. Within each grow-out period, n = 225 – 250 at the start of the study and 30 – 91 at the end.

3.3. Position within cage influenced growth

Over the entire study period at the two sea farms, individually-weighed oysters in the bottom layers grew significantly faster than did those in the top. Comparisons between top and bottom live mass growth curves showed the following: at Algoa Bay for US and CL cohorts respectively, $F_{3,959} = 11.74$ and $F_{4,335} = 10.82$; at Saldanha Bay, for US, CS and CL respectively, $F_{4,965} = 22.93$, $F_{4,910}$ 86.73, and $F_{4,353}$ =21.83 (in all cases, $p < 0.0001$). For the US cohort in Algoa Bay, the best-fit curves were second-order (quadratic) polynomials; best-fit polynomials for the other four comparisons were third-order (cubic). For brevity, only actual growth curves for the US cohort are shown for this comparison (Appendix 4). These analyses showed no effect of depth within cage at Kleinzee for any cohort.

Comparisons between batch live masses confirmed that the bottom cage layer is usually a more favourable growth environment than the top. The seasonal effect of position within cages on oyster growth was assessed using % mass gains over each grow-out period, calculated from the start and end masses of each batch. Across all three farms, the effect of position within cage on growth was most marked in Saldanha Bay, with seven of a possible 15 comparisons showing significance, followed by Algoa Bay (five of 15) then Kleinzee (three of 15) (Appendix 5).

These within-season comparisons confirmed that the bottom layer of each cage was usually a more favourable micro-environment for growth than was the top: in winter in Kleinzee, and particularly but not exclusively in summer for the two sea farms. In Saldanha in mid-summer (Nov 2010 – Jan 2011), bottom layer oysters for all three cohorts consistently gained more mass than did their top layer counterparts. Within Algoa Bay, depth within cage apparently had no effect on growth for the US (smallest initial size) cohort, but both other cohorts grew faster in the bottom layer.

3.4. Oyster condition at different localities: shell mass relative to body mass and DWCI

Log_{10}-log_{10} fits explained the variance in oyster shell mass as a function of meat mass better than did simple linear fits (Fig. 5). Over the whole study period and for all three cohorts, separate-slopes GLZs with a log link function followed by comparison of 95% confidence limits of Least Squares Means showed that shell dry mass (g) was higher relative to body mass for Algoa Bay and Kleinzee oysters than for Saldanha Bay oysters (CL: χ^2 = 71.7; $p < 0.00001$; CS: $\chi^2 = 30.8$, $p < 0.00001$; US: $\chi^2 = 537.73$, $p < 0.0001$). Saldanha Bay had the lowest slope: $y = 0.69x + 1.13$ ($R^2 = 0.86$, $n = 199$); whereas Algoa Bay ($y = 0.80x + 1.27$, $R^2 = 0.94$, $n = 193$) and Kleinzee have statistically indistinguishable slopes ($y = 0.80x + 1.27$, $R^2 = 0.88$, $n = 187$). As a consequence of their relatively lighter shells, all cohorts of Saldanha Bay oysters showed higher DWCI than those from the other two farms (Fig. 6, $p < 0.000001$ in all cases), and DWCI for all cohorts at Algoa Bay exceeded those from Kleinzee for most grow-out periods ($p < 0.002$ in all cases; statistics given in Appendix 6).

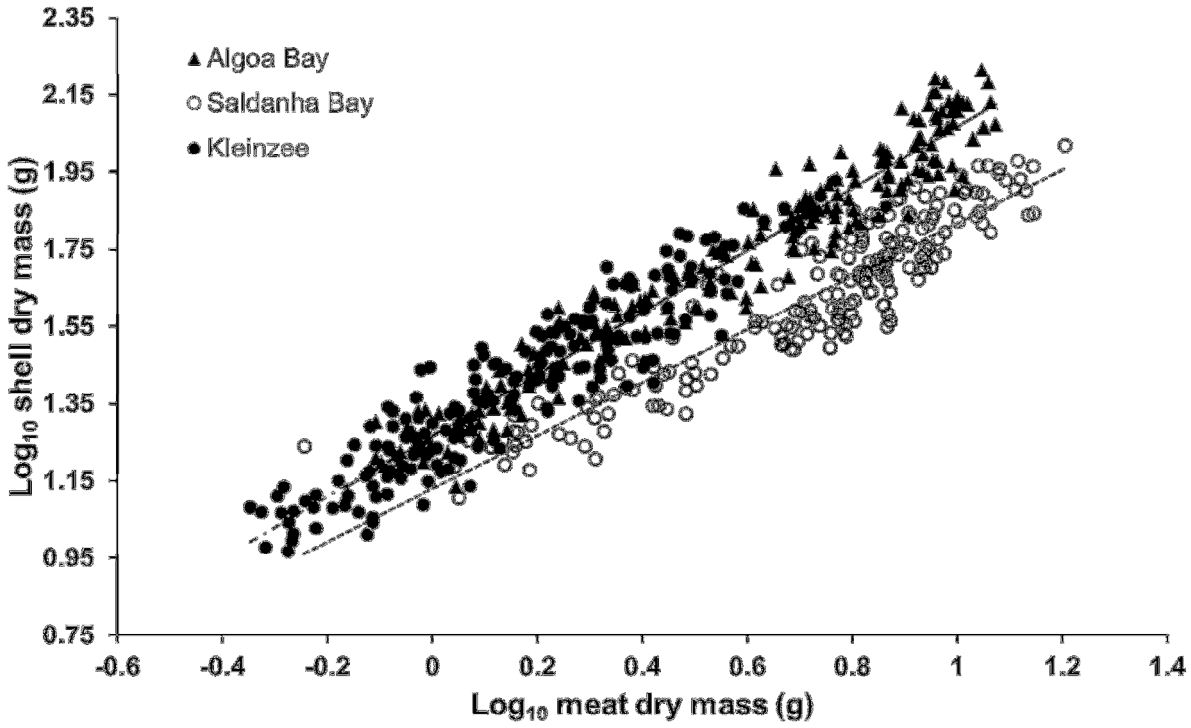

Figure 5: Dry shell mass (g) for individual oysters as a function of dry meat mass for the largest (CL) cohort only. Algoa Bay: triangles ▲ and solid line; Saldanha Bay: hollow circles ○ and dashed line; and Kleinzee: solid circles ● and dashed line.

3.5. Seasonal patterns in DWCI

For the US cohort at Kleinzee, DWCI was lower in spring and early summer (Sep – Nov 2010) than in summer and autumn (Nov 2010 – Mar 2011, all statistics in Appendix 7). The reverse was true at Saldanha Bay, where DWCI was higher in spring and early summer than in summer through autumn. For the CS cohort at Kleinzee, DWCI was lower in the first half of summer (Nov 2010 – Jan 2011) than later in summer through early autumn (Jan – Mar 2011). For the CL cohort (Fig. 6), for which data exists for the entire study period, seasonal trends in DWCI were most evident in Saldanha Bay, with significant dips in late autumn and winter (May – Jul 2010) and late summer to early autumn (Jan – Mar 2011). In Algoa Bay, DWCI was lower in autumn through to early spring (May – Sep 2010) than in late spring right through to autumn (Sep 2010 – Mar 2011). At Kleinzee, DWCI in winter to early spring (Jul – Sep 2010) was significantly lower than all other grow-out periods. For the US cohort at Kleinzee, DWCI was lower in spring (Sep – Nov 2010) than in summer (both Nov 2010 – Jan 2011 and Jan – Mar 2011; Kruskal-Wallis $H_{2, 119} = 49.55$; $z = 6.64$ and 5.38, $p = 0.000001$ in both cases). The reverse was true at Saldanha Bay: DWCI was higher in spring (Sep – Nov 2010) than in summer (both Nov 2010 – Jan 2011 and Jan – Mar 2011; $H_{2, 119} = 28.71$; $z = 4.02$ and 5.07 respectively, $p \leq 0.0002$ in both cases). For the CS cohort at Kleinzee, DWCI was lower in the first half of summer than in the second (Nov 2010 – Jan 2011 < Jan – Mar 2011, $H_{2, 120} = 7.112$; $p = 0.03$, $z = 2.65$, $p = 0.02$).

Shell density (dry mass as a percentage of wet mass) was highest for Kleinzee oysters of the CL cohort during autumn to spring (May – Sep 2010), and for CS during spring to early summer (Sep – Nov 2010; Table 2). However, this trend was reversed in late spring to autumn (Sep 2010 – Mar 2011) in CL, summer to autumn (Nov 2010 – Mar 2011) in CS and during summer (Jan – Mar 2011) in US, when shell density was higher for oysters grown in Saldanha Bay than at the other two locations. For the smaller cohort, shell density at

Kleinzee was lower than both other farms through a summer period; for the US cohort in spring to summer (Sep 2010 – Jan 2011), and for the CS cohort in summer to autumn (Jan – Mar 2011) (statistics for all comparisons in Appendix 8).

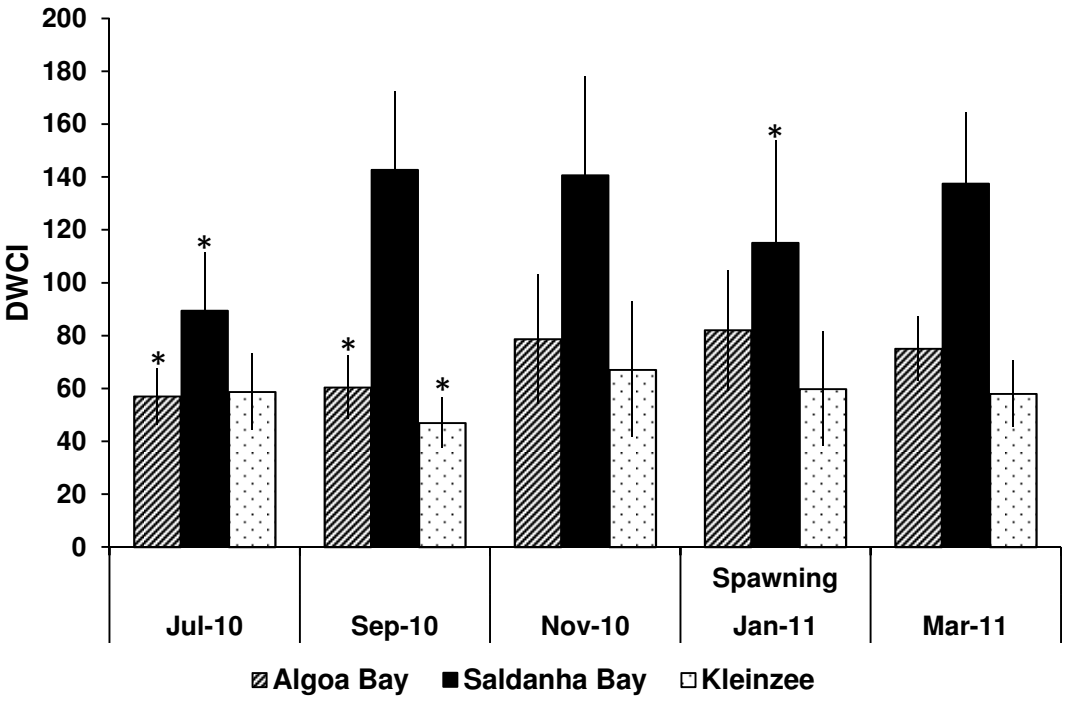

Figure 6: Dry Weight Condition Index for the CL cohort was consistently higher for oysters grown at Saldanha Bay than at the other two locations. For Jul – Sep, Nov – Jan, and Jan – Mar, DWCI for Algoa Bay oysters was in turn higher than for those grown at Kleinzee (Appendix 6). Medians for each grow-out period and farm are displayed (error bars represent the quartile range), where * denotes periods that were significantly different from all others, within each farm: statistics for these Kruskal-Wallis ANOVAs given in Appendix 7. Only the last month of each grow-out period is shown on the x-axis. N = 40 for each farm and each grow-out period.

Table 2: Mean shell densities (shell dry mass as a percentage of shell wet mass), with ± 1 S.D. in parentheses. Within each grow-out period, values in bold are significantly different from other farms. Statistics for between-farm comparisons are given in Appendix 8. Sample sizes are 39 – 41 for each farm and cohort.

U.S.A.	**Algoa Bay**	**Saldanha Bay**	**Kleinzee**
Sep – Nov 2010	75.93 (± 12.41)	81.32 (± 12.24)	**74.7 (± 6.19)**
Nov 2010 – Jan 2011	82.28(± 8.96)	83.67 (± 4.4)	**77.41 (± 4.24)**
Jan – Mar 2011	83.16 (± 4.15)	**92.10 (± 8.41)**	80.81 (± 4.44)
Chilé Small			
Sep – Nov 2010	77.03 (± 8.68)	76.2 (± 6.49)	**79.94 (± 5.61)**
Nov 2010 – Jan 2011	80.63 (± 4.21)	**87.4 (± 4.75)**	81.49 (± 3.71)
Jan –Mar 2011	**84.69 (± 4.1)**	**91.9 (± 6.57)**	**80.41 (± 8.09)**
Chilé Large			
May – Jul 2010	80.70 (± 6.00)	79.66 (± 5.48)	**85.73 (± 3.21)**
Jul – Sep 2010	82.83 (± 5.25)	82.5 (± 4.47)	**87.1 (± 3.49)**
Sep –Nov 2010	79.51 (± 3.72)	**87.21 (± 2.86)**	80.2 (± 3.45)
Nov 2010 – Jan 2011	79.7(± 4.45)	**88.57 (± 3.29)**	83.95 (± 5.86)
Jan – Mar 2011	84.24 (± 3.58)	**92.63 (± 1.85)**	85.73 (± 3.46)

3.6. Mortality and fouling

For the US cohort with the highest mortalities, total mortality at each farm did not exceed 30%, and within each two-month grow-out cycle averaged lower than 10% (Fig. 7). Within each locality, seasonal mortality patterns differed. At Kleinzee, summer mortality predominated, in Algoa Bay winter mortality was higher, and Saldanha Bay showed one peak in winter and another at the end of summer.

Between localities, within the US cohort (Fig. 7), late autumn and winter (May – Jul 2010) mortalities were higher in Algoa and Saldanha bays than at Kleinzee, but as spring approached (Jul – Sep 2010), only Algoa Bay had higher mortalities than Kleinzee. From spring to early summer (Sep – Nov 2010), both Kleinzee and Algoa Bay had higher mortalities than did Saldanha Bay. Within CS, Algoa Bay had higher mortalities than both other farms in late autumn to spring (May – Sep 2010; 2 and 8% for the mortality median, $p < 0.00006$), but from spring to early summer (Sep – Nov 2010) Kleinzee (2%, $p = 0.002$) had higher mortalities than Saldanha Bay.

In mid-summer (Nov 2010 – Jan 2011), Kleinzee mortalities (4% and 1% for CS and CL respectively; $p < 0.008$) exceeded those at the sea farms for all three cohorts. In summer to autumn (Jan – Mar 2011) both Saldanha Bay and Kleinzee showed higher mortalities than Algoa Bay within US and CS (0.2% each; $p < 0.1$), but within CL only Kleinzee (0.05%; $p < 0.03$) again had higher mortalities than both other farms. Oysters at Saldanha Bay were probably affected by stressful fouling removal (see Discussion).

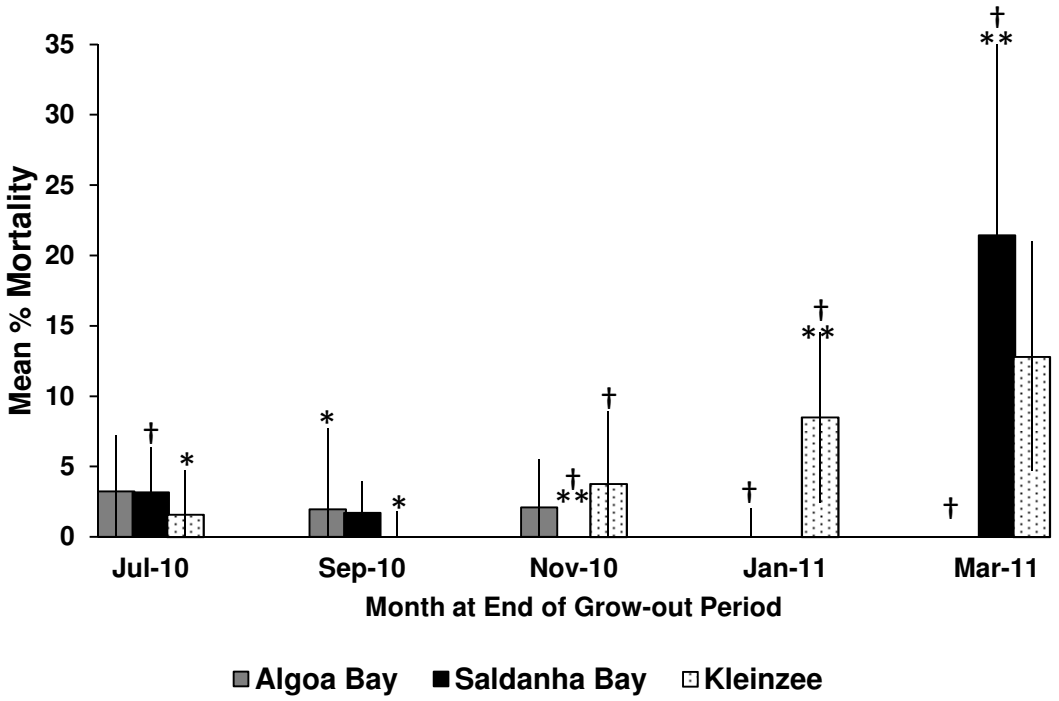

Figure 7: Mortality for the US cohort, expressed as the median for mortality as percentages of the total initial numbers of oysters per bag (error bars represent the quartile range), and calculated separately for each two-month grow out period. Comparisons between farms within each grow-out period: marked values are significantly different from all others; * $p < 0.01$, ** $p < 0.001$. Within each farm, grow-out periods that differed significantly from all others are marked with † ($p < 0.01$ in all cases).

The fouling: oyster mass ratio was higher in Saldanha Bay than at the other localities in spring to summer (Sep 2010 – Jan 2011; Fig. 8). For the CS cohort (Fig. 8), Saldanha Bay oysters had a higher fouling ratio than those of Algoa Bay (Mann-Whitney $U_{1,5} = 0.0$, $z = -2.54$, $p = 0.0079$). Ratios for US and CL were also higher in Saldanha than in Algoa Bay: For CL from Sep – Nov 2010, medians for Saldanha and Algoa bays respectively were 0.059 ($25\text{-}75^{th}$ percentiles = 0.049 – 0.084) and 0.0075 (0.0075 – 0.0127); Mann-Whitney $U_{1,5} = 0.0$, $z = -2.51$, $p = 0.0079$. For US from Nov 2010 – Jan 2011, medians were 0.41 (0.39 – 0.45) and 0.065 (0.061 – 0.098) for Saldanha and Algoa bays; Mann-Whitney $U_{1,5} = 0.0$, $z = -$

2.54, p = 0.0079). Fouling epibionts manifested on the oyster bags and cages and could have obstructed water movement through their mesh to the oysters. Water movement rate directly influences *C. gigas* growth (Walne 1972) and therefore the difference between oyster growth at Saldanha Bay and Algoa Bay could also be related to water flow rate restrictions caused by fouling on the cages. At Kleinzee, we identified the following suspension-feeding epibionts on the oysters themselves: barnacles (*Notomegabalanus algicola)*, black mussels (*Choromytilus meridionalis*) and ascidians (*Ciona intestinalis* and *Ascidia candata*). Sponges (*Leucosolenia* spp.) and ascidians were found at Kleinzee, the latter particularly common on oysters grown in the bottom levels of the cages. Barnacle and mussel fouling at Kleinzee was not as high as that on the sea-based farms. Polychaete worms (*Diopatra neopolitana*, *Marphysa* spp. and *Timarete capensis*) were found in both Saldanha Bay and Algoa Bay, particularly in the latter. In Saldanha Bay, competitive suspension-feeders (the barnacle *Notomegabalanus algicola* and mussel *Mytilus galloprovincialis*) were abundant, as were amphipods (*Jassa spp*.). In Algoa Bay, competitive suspension-feeders included introduced barnacles (*Amphibalanus amphitrite*) and to a lesser degree indigenous oysters (*Striostrea margaritacea*), and bryophytes (*Jellyella tuberculata* and *Bugula neritina*). Fouling was highest in Saldanha Bay during summer, but Saldanha Bay oysters nonetheless achieved a high DWCI and relatively fast dry meat growth.

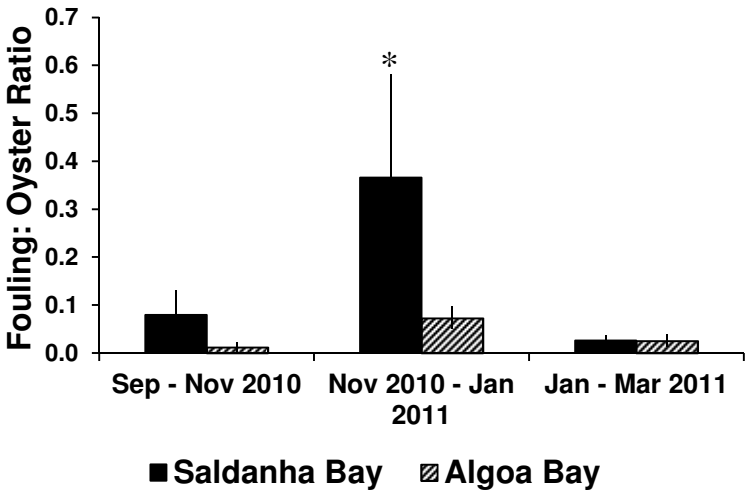

Figure 8: Median fouling: oyster mass ratios for the CS cohort, with the quartile range represented by the error bars. * denotes a significant difference between farms.

4. Discussion

Regardless of the units used for comparison, growth rates of Pacific oysters in long-line culture in South Africa are among the fastest reported in the world (Table 1). Reported values are a minimum of one third and a maximum of six times those in intertidal table culture in Europe (Gangnery *et al*. 2003; Dégremont *et al.* 2005; Batista *et al.* 2007) and in South Australia (Li *et al.* 2009). Mass gains in our study were up to four times those obtained with intertidal culture in Stanway cylinders in the South of France (Robert *et al.* 1993).

Comparison of our data with other suspended culture studies is more appropriate. Long-line culture maximizes growth rate because it avoids emersion of oysters during tidal cycles, hence loss of feeding and growing time. In the relatively warm waters of our study sites, oysters of comparable initial sizes (mass or shell height) grew up to three to ten times faster than those in suspended culture on the Atlantic coast of France (Boudry *et al.* 2003), in the German Bight of the North Sea (Pogoda *et al*. 2011), the northern coast of Sicily (Sarà and Mazzola 1997), South Korea (Hyun *et al.* 2001), and in Japan's Seto Inland Sea (Kobayashi *et al.* 1997). Oyster cultivation fully offshore (e.g. Pogoda *et al.* 2011) has not been explored in South Africa, but in the Benguela Large Marine Ecosystem would be challenged by wave heights often exceeding 3 m and occasionally 6 m, imposing logistical constraints for regular harvest and husbandry. Until offshore oyster culture in South Africa proves viable, Saldanha Bay seems to be the only grow-out site that can produce both fast-growing and marketable oysters.

Chlorophyll *a* concentrations, which are two to six times lower in Algoa than in Saldanha Bay, partially explain the lower dry mass growth rates in Algoa Bay oysters. The difference in the dry mass gain of oysters in Saldanha Bay and Algoa Bay is, however, less than expected given the differences in phytoplankton biomass between these two sites.

During prolonged periods of nutrient shortage, *C. gigas* allocates proportionally more of its total body mass to shell (Brown and Hartwick 1988a). The relatively fast live mass gain for all cohorts in Algoa Bay is largely due to proportionally greater shell growth at this nutritionally-poorer site.

Oyster feeding efficiency shows considerable selectivity of phytoplankton particles based on their size, chemical cues and specific nutrient proportions in a particular environment (Cognie *et al.* 2001; Espinosa *et al.* 2007; Bayne 2009). Therefore oysters at both Algoa Bay and Saldanha Bay would have maximized their selectivity of the most nutritious particles in their environment and it is possible that oysters at different environments are conditioned to select for different particle size ranges. Pacific oysters in the Thau Lagoon have up-regulated their extraction efficiency of seston in response to low food availability (phytoplankton concentrations of < 2 mg.m^{-3}) yielding high clearance rates and relatively high growth rates compared to other oyster farms in France (Dupuy *et al*. 2000). A similar feeding response may explain the paradoxically high growth rates that we report for Algoa Bay despite its relatively low productivity. Assessment of phytoplankton size and species composition at the three sites, and of the nutritional value of phytoplankton mixtures at each site, will give further insights into optimizing commercial oyster culture in South Africa.

Better growth in bottom relative to top cage layers in Saldanha Bay may be a consequence of subsurface phytoplankton biomass maxima (Pitcher and Calder, 1998) that influence lower cage layers. However, the small distance (0.75 m) separating cage top and bottom layers in our study means that this explanation is less likely than is differences in epifaunal settlement, possibly in response to light intensity. Interestingly, Ngo *et al.* (2009) reported the opposite: better growth in top layer oysters in long-line culture, with greater distances separating levels (1 to 3 m). In addition to assessment of epifaunal settlement on

cages at different depths, it is recommended that thorough exploration of oyster growth at different levels in the water column for both Algoa and Saldanha bays is undertaken.

Slow growth at Kleinzee, coupled with proportionally high shell masses, was probably caused by very low phytoplankton concentrations in this relatively shallow pond system (B. Hubbart, G. Pitcher, and S. Jackson, unpublished data). This effect would be exacerbated by poor food distribution through cages (Wilson-Ormond *et al*. 1997) in the absence of wave action and tidal cycles. In summer, low body condition (DWCI) and shell density, and high mortality at this locality are likely due to the combination of rising temperatures and low food availability. This is particularly detrimental to Pacific oysters (Malouf and Breese 1977), as the demands of metabolism driven upwards by seasonal warming cannot be met by a poor food supply (Barillé *et al*. 2003). Recall that for the entire study period, 91.8% of all summer temperatures at Kleinzee were above 19°C. However, good shell growth and high shell density for spat at this locality in winter and early spring confirm that its use as a nursery is appropriate in this season.

Oyster condition (DWCI) was lowest at Algoa and Saldanha bays in winter, with an additional dip at Saldanha Bay in summer that may have reflected spawning (Chávez-Villalba *et al*. 2007): food availability and temperature were both high during this period at Saldanha Bay. For all cohorts at all farms, oysters would have reached sexual maturity by Sep – Nov 2010, since oysters with a dry meat mass of only 0.25 g may be ready to spawn (Pouvreau *et al*. 2006; Normand *et al*. 2009). Since dry meat mass and chlorophyll *a* was not measured for Kleinzee for Jul – Sep 2010, a dip in DWCI here can not acurately be related to spawning with an increase in water temperature from winter to spring. Mean DWCI of Saldanha oysters of all three cohorts was much higher (127.5, 158.6, and 128.4), than that for *C. gigas* in longline culture in the Gulf of California, México (24 – 44, with a maximum of 96; Chávez-Villalba *et al.* 2007), and the same is true for Algoa Bay (73.5, 86.6 and 72.4) and

Kleinzee (60.5, 59.6 and 59.6). Oysters cultured on intertidal racks in New Zealand have corresponding values of 37 – 47 (estimated from Fig. 3c in Handley 2002), and in suspended culture in Tunisia 23.1 – 49.6 (values from Dridi *et al.* 2007, multiplied by 10 for comparison with our study).

The pronounced summer mortality peak at Saldanha Bay (Jan – Mar 2011) was propbably an artifact of husbandry: heavy fouling necessitated extensive cleaning of these oysters in Jan 2011, causing evident stress as oysters gaped after cleaning. This did not occur for any other farm or grow-out period.

5. Summary

South African waters, particularly the upwelling-influenced, cool system in Saldanha Bay, clearly supply optimal food and temperatures to produce exceptionally fast-growing, high quality market-ready oysters in long-line culture. Algoa Bay is a good nursery location: oyster spat with initial live masses of 3 g gained mass extremely fast in this relatively unprotected bay. High summer temperatures at Kleinzee ponds inhibit growth and cause mortality. Fine scale within-cage differences in growth rates likely arise from settlement patterns of fouling organisms, and vertical zonation of phytoplankton, both of which require further investigation at Algoa and Saldanha bays. In this study, environment influenced growth rate to a much greater extent than did oyster origin, but more work is needed comparing oysters of different stocks at the same initial masses and environments in South Africa.

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Appendix 1: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of individual oysters presented in Fig. 3. F-ratios are for comparisons of curves between farms within each cohort (Haddon, 2001). Sample sizes within each grow-out period are given in the caption for Fig. 3; total sample sizes for each polynomial regression below may be inferred from the degrees of freedom. SS: Sum of Squares.

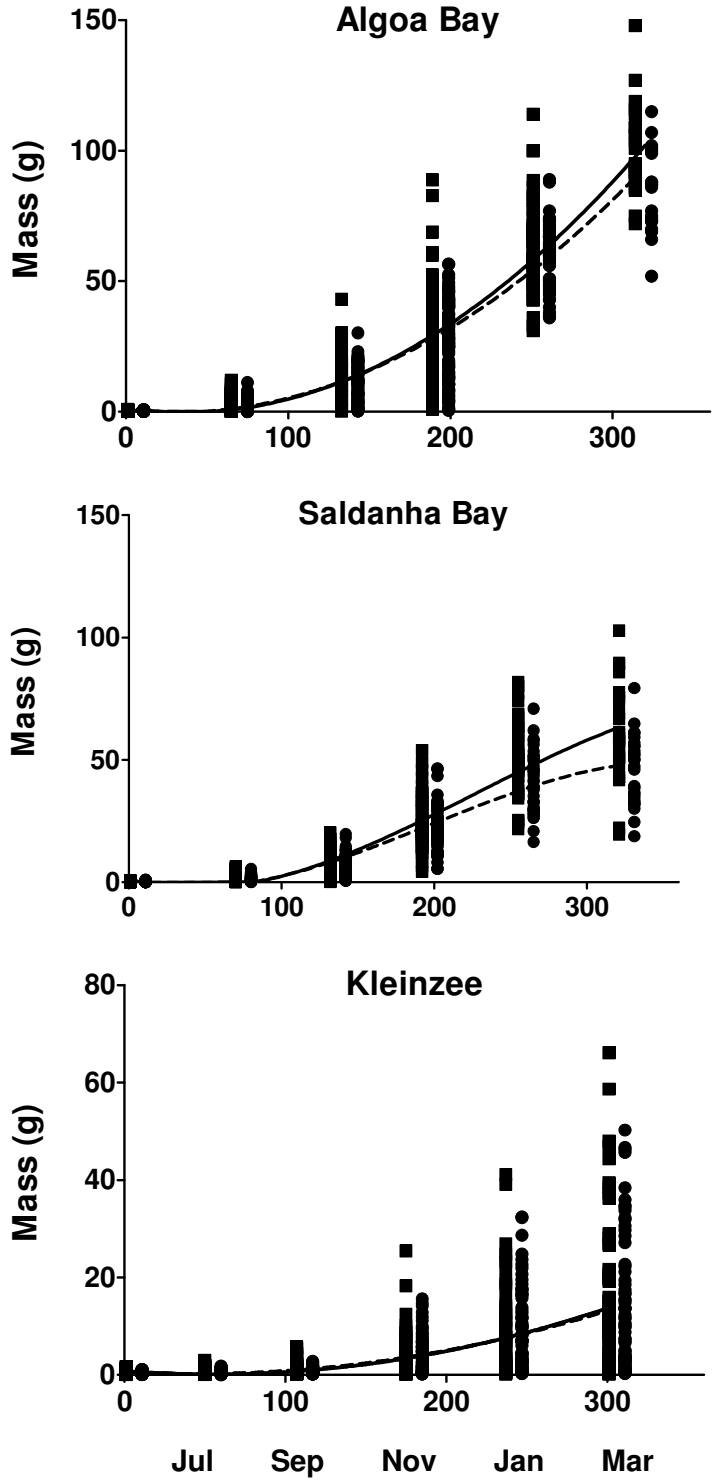
Cohort	Algoa Bay	Saldanha Bay	Kleinzee
US			
$F_{8,4764} = 1308$			
$P < 0.0001$			
B0	0.8332 ± 0.5749	1.1430 ± 0.4051	0.5380 ± 0.3009
B1	-0.0656 ± 0.0204	-0.1628 ± 0.0137	-0.0042 ± 0.0104
B2	$1.065 \pm 0.177 \times 10^{-3}$	$2.134 \pm 0.118 \times 10^{-3}$	$4.27 \pm 8.78 \times 10^{-5}$
B3	$4.92 \pm 3.99 \times 10^{-7}$	$-3.29 \pm 2.59 \times 10^{-6}$	$3.65 \pm 1.96 \times 10^{-7}$
df	1 469	1 489	1 806
r^2	0.8379	0.8312	0.3314
Absolute SS	178 606	90 076	61 566
$S_{y.x}$	11.03	7.78	5.84
CS			
$F_{8,4266} = 1000$			
$P < 0.0001$			
B0	3.8180 ± 0.7322	4.5230 ± 0.5352	5.4210 ± 0.3738
B1	-0.0739 ± 0.0275	-0.2036 ± 0.0187	0.0069 ± 0.0134
B2	$3.088 \pm 0.254 \times 10^{-3}$	$3.548 \pm 0.170 \times 10^{-3}$	$0.256 \pm 0.118 \times 10^{-3}$
B3	$-3.99 \pm 0.593 \times 10^{-6}$	$-6.03 \pm 0.389 \times 10^{-6}$	$1.060 \pm 0.273 \times 10^{-6}$
df	1 247	1 381	1 638
r^2	0.8889	0.8726	0.7838
Absolute SS	238 405	143 212	85 505
$S_{y.x}$	13.83	10.18	7.23
CL			
$F_{8,2982} = 418.6$			
$P < 0.0001$			
B0	18.35 ± 0.95	20.96 ± 1.3	20.77 ± 0.817
B1	0.1639 ± 0.0353	-0.00211 ± 0.0440	0.0535 ± 0.0286
B2	$2.739 \pm 0.325 \times 10^{-3}$	$2.89 \pm 0.396 \times 10^{-3}$	$0.9946 \pm 0.2459 \times 10^{-3}$
B3	$-4.20 \pm 0.754 \times 10^{-6}$	$-5.76 \pm 0.888 \times 10^{-6}$	$-1.28 \pm 0.554 \times 10^{-6}$
df	853	1 065	1 064
r^2	0.9179	0.6924	0.7652
Absolute SS	187 894	443 091	161 707
Sy.x	14.84	20.40	12.33

Appendix 2: Equations from linear regression analyses of meat dry mass as a function of whole live mass (g), for each farm and grow-out period for the CL strain only (n = 40 for each regression). These equations were used to determine estimated dry meat mass from live masses for each farm and grow-out period (Appendix 3). In all cases $p \leq 0.00002$.

Location	**Grow-out period**	Slope	Intercept	r^2
Algoa Bay	May – Jul 2010	0.0346x	-0.2444	0.7323
	Jul – Sep 2010	0.0396x	- 0.4111	0.8447
	Sep – Nov 2010	0.0467x	- 0.7221	0.5323
	Nov 2010 – Jan 2011	0.0529x	- 1.0357	0.5567
	Jan – Mar 2011	0.0303x	+ 2.5212	0.3820
Saldanha Bay	May – Jul 2010	0.0495x	- 0.3166	0.6176
	Jul – Sep 2010	0.0933x	- 2.0474	0.8553
	Sep – Nov 2010	0.0726x	- 0.0772	0.5445
	Nov 2010 – Jan 2011	0.0613x	+ 0.7289	0.6691
	Jan – Mar 2011	0.0606x	+ 1.6991	0.7048
Kleinzee	May – Jul 2010	0.0334x	- 0.0189	0.7345
	Jul – Sep 2010	0.0252x	+ 01191	0.6377
	Sep – Nov 2010	0.0443x	- 0.542	0.6641
	Nov 2010 – Jan 2011	0.0437x	- 0.5758	0.7693
	Jan – Mar 2011	0.0391x	- 0.4889	0.8625

Appendix 3: Best-fit polynomial parameter estimates (± 1 S.E.) for dry meat mass growth curves for CL cohort presented in Fig. 4. F-ratios are for comparisons of curves between farms within each cohort (Haddon, 2001).

	Algoa Bay	**Saldanha Bay**	**Kleinzee**
$F_{8,2982}$ = 440 **P < 0.0001**			
B0	0.4013 ± 0.0423	0.3479 ± 0.1118	0.5368 ± 0.0299
B1	$7.289 \pm 1.568 \times 10^{-3}$	$-0.180 \pm 3.784 \times 10^{-3}$	$1.961 \pm 1.048 \times 10^{-3}$
B2	$1.219 \pm 0.145 \times 10^{-4}$	$2.49 \pm 0.34 \times 10^{-4}$	$0.364 \pm 0.090 \times 10^{-4}$
B3	$-18.7 \pm 3.35 \times 10^{-8}$	$-49.5 \pm 7.64 \times 10^{-8}$	$-4.67 \pm 2.03 \times 10^{-8}$
df	853	1 065	1 064
R^2	0.9179	0.6924	0.7651
Absolute SS	372	3 277	216.7
$S_{y.x}$	0.6604	1.754	0.4513



Appendix 4: Live mass growth curves from individual oysters of the US cohort, compared between the top and bottom cage layers. Dashed lines and solid circles: top cage layer; solid lines and solid squares: bottom cage layers.

Appendix 5: Comparisons of live mass gain for batches between cage top and bottom layers. For each batch, total mass gain over each two-month period was expressed as a percentage of that batch's mean start mass. Only significant comparisons are shown. For all but three (typed in italics), bottom oysters gained more mass than did those in the top layer.

Location	**Cohort**	**Grow-out period**	**Mann-Whitney U**	**z**	***P***
Saldanha Bay	US	May – Jul 2010	$U_{12,16} = 19$	-3.55	0.0004
		Nov 2010 – Jan 2011	$U_{8,8} = 5$	-2.78	0.003
	CS	Jul – Sep 2010	$U_{8,8} = 11$	-2.15	0.03
		Nov 2010 – Jan 2011	$U_{8,8} = 10$	-2.26	0.02
	CL	*May – Jul 2010*	*$U_{16,16} = 31$*	*3.64*	*0.0001*
		Sep – Nov 2010	$U_{8,8} = 9$	-2.36	0.015
		Nov 2010 – Jan 2011	$U_{7,7} = 1$	-2.94	0.0012
Algoa Bay	CS	May – Jul 2010	$U_{16,15} = 70$	-1.96	0.05
		Nov 2010 – Jan 2011	$U_{8,8} = 10$	-2.26	0.02
		Jan – Mar 2011	$U_{8,8} = 3$	-2.99	0.001
	CL	*Nov 2010 – Jan 2011*	*$U_{8,8} = 11$*	*2.15*	*0.03*
		Jan – Mar 2011	$U_{8,4} = 0$	-2.63	0.004
Kleinzee	CL	May – Jul 2010	$U_{16,8} = 9$	-3.34	0.0003
		Jul – Sep 2010	$U_{8,8} = 0$	-3.31	0.0002
		Sep – Nov 2010	*$U_{8,8} = 2$*	*3.10*	*0.001*

Appendix 6: Comparison of DWCI (Fig. 6) between farms, which was consistently higher at Saldanha Bay than at the other two environments and also, higher at Algoa Bay than at Kleinzee. Statistics are for Kruskal-Wallis ANOVAs, followed by z-values for post hoc pairwise tests for which $p < 0.001$ unless otherwise stated. NS: non-significant. N = 39 – 41 oysters for each cohort at each locality.

Cohort	**Location**	**Saldanha Bay**	**Algoa Bay**
US			
Sep – Nov 2010 $\mathbf{H_{2, 119} = 48.89; P < 0.00001}$	Kleinzee	4.82	NS
	Algoa Bay	6.79	
Nov 2010 – Jan 2011 $\mathbf{H_{2, 120} = 93.96; P < 0.0001}$	Kleinzee	9.68	4.48
	Algoa Bay	5.20	
Jan – Mar 2011 $\mathbf{H_{2, 118} = 85.53; P < 0.0001}$	Kleinzee	9.22	3.89
	Algoa Bay	5.29	
CS		**Saldanha Bay**	**Algoa Bay**
Sep – Nov 2010 $\mathbf{H_{2, 120} = 93.97; P < 0.0001}$	Kleinzee	9.66	4.15
	Algoa Bay	5.52	
Nov 2010 – Jan 2011 $\mathbf{H_{2,117} = 84.0595; P < 0.00001}$	Kleinzee	9.09	4.42 ($p < 0.002$)
	Algoa Bay	5.57	
Jan – March 2011 $\mathbf{H_{2, 120} = 95.53; P < 0.0001}$	Kleinzee	9.76	4.40
	Algoa Bay	5.36	
CL		**Saldanha Bay**	**Algoa Bay**
May – Jul 2010 $\mathbf{H_{2, 120} = 69.74; P < 0.00001}$	Kleinzee	6.95	NS
	Algoa Bay	7.49	
Jul – Sep 2010 $\mathbf{H_{2, 119} = 92.59; P < 0.0001}$	Kleinzee	9.56	3.81
	Algoa Bay	5.69	
Sep – Nov 2010 $\mathbf{H_{2, 119} = 83.04; P < 0.00001}$	Kleinzee	8.75 (0.0000001)	NS
	Algoa Bay	6.55	

Appendix 6 continued:

CL		Saldanha Bay	Algoa Bay
Nov 2010 – Jan 2011 **$H_{2, 119}$ =70.37; *P* < 0.00001**	Kleinzee	8.39	4.04
	Algoa Bay	4.38	
Jan – Mar 2011 **$H_{2, 120}$ = 94.44; *P* < 0.0001**	Kleinzee	9.66	3.90
	Algoa Bay	5.76	

Appendix 7: Seasonal trends in Dry Weight Condition Index (DWCI; actual values given in Fig. 6) for the CL cohort. Statistics are for Kruskal-Wallis ANOVAs, followed by z-values for post hoc pairwise tests for which $p < 0.0001$ unless otherwise stated. N = 39 – 41 oysters for each cohort at each locality.

CL cohort	**May – Jul 2010**	**Jul – Sep 2010**	**Sep – Nov 2010**	**Nov 2010 – Jan 2011**
Algoa Bay: $H_{4, 198} = 87.35$; $P = 0.000001$				
Jul – Sep 2010	NS			
Sep – Nov 2010	6.50	5.39		
Nov 2010 – Jan 2011	7.23	6.10	NS	
Jan – Mar 2011	5.65	4.54	NS	NS
Saldanha Bay: $H_{4, 199} = 86.610$; $P = 0.000001$				
Jul – Sep 2010	7.01			
Sep – Nov 2010	7.60	NS		
Nov 2010 – Jan 2011	3.54 (0.004)	3.42 (0.006)	4.01 (0.0006)	
Jan – Mar 2011	7.36	NS	NS	3.78 (0.002)
Kleinzee: $H_{4, 200} = 52.25$; $P = 0.000001$				
Jul – Sep 2010	5.06			
Sep – Nov 2010	NS	6.88		
Nov 2010 – Jan 2011	NS	4.83	NS	
Jan – Mar 2011	NS	4.67	NS	NS

Appendix 8: Shell density (shell dry mass as a % of wet mass) compared between localities using Kruskal-Wallis ANOVAs followed by post hoc pairwise tests (z-values, followed by p-values in parentheses). For the cohort with the smallest initial mass (US), shell density was consistently lower at Kleinzee than at the two sea-based farms. Where differences existed between Saldanha and Algoa bay oysters, those at Saldanha Bay had higher shell densities. This was also true for the CS cohort from Nov 2010 to Mar 2011. Interestingly, a reversal of this trend was apparent in winter and spring for the two Chilean cohorts: CS oysters at Kleinzee had denser shells than those at either of the two sea farms (Sep to Nov 2010), and the same was true for CL oysters (May to Sep 2010).

US		**Saldanha Bay**	**Algoa Bay**
Sep – Nov 2010: $H_{2, 119} = 17.01$, $P = 0.0002$	Algoa Bay	NS	
	Kleinzee	4.08 (0.0002)	2.59 (0.03)
Nov 2010 – Jan 2011: $H_{2, 120} = 30.81$, $P < 0.00001$	Algoa Bay	NS	
	Kleinzee	5.445 (0.00001)	3.66 (0.001)
Jan – Mar 2011: $H_{2, 118} = 66.36$, $P < 0.00001$	Algoa Bay	5.91 (0.00001)	
	Kleinzee	7.82 (0.00001)	NS
CS		**Saldanha Bay**	**Algoa Bay**
Sep – Nov 2010: $H_{2, 120} = 9.81$, $P = 0.007$	Algoa Bay	NS	
	Kleinzee	2.78 (0.02)	2.64 (0.03)
Nov 2010 – Jan 2011: $H_{2, 117} = 40.69$, $P < 0.00001$	Algoa Bay	5.82 (0.00001)	
	Kleinzee	5.17 (0.000001)	NS
Jan – Mar 2011: $H_{2, 120} = 65.42$, $P <$ 0.00001	Algoa Bay	5.05 (0.000001)	
	Kleinzee	7.997 (0.000001)	2.95 (0.01)

Appendix 8 continued:

CL		**Saldanha Bay**	**Algoa Bay**
May – Jul 2010: $H_{2, 120} = 29.44$, $P < 0.00001$	Algoa Bay	NS	
	Kleinzee	5.14 (0.000001)	4.08 (0.0001)
Jul – Sep 2010: $H_{2, 119} = 23.198$, $P < 0.00001$	Algoa Bay	1.0	
	Kleinzee	4.42 (0.00003)	3.86 (0.0004)
Sep – Nov 2010: $H_{2, 119} = 85.71$, $P < 0.0001$	Algoa Bay	7.00 (0.000001)	
	Kleinzee	6.52 (0.000001)	NS
Nov 2010 – Jan 2011: $H_{2, 114} = 31.96$, $P < 0.00001$	Algoa Bay	5.18 (0.000001)	
	Kleinzee	4.51 (0.00002)	NS
Jan – Mar 2011: $H_{2, 120} = 75.54$, $P < 0.00001$	Algoa Bay	8.07 (0.000001)	
	Kleinzee	6.82 (0.000001)	NS

Chapter 3

The effect of different South African environments on growth, condition and survival of Namibian and Chilean cohorts of the Pacific oyster (*Crassostrea gigas*) in South Africa

ABSTRACT

Worldwide, Pacific oyster (*Crassostrea gigas*) spat for grow-out are bred for performance within specific climates and environments. In South Africa, local farmers need to find hatcheries which supply spat of a continuously high quality, and spat may need to be imported from a similar geographical area, since oyster genotypes interact with their environment (Evans and Langdon 2006). To test this spat from Walvis Bay, Namibia, within the same current system as oyster farms on the West Coast were imported, along with spat from Bahía de Tongoy, Chilé, a climatically distinct environment. All oysters within each cohort were half-sibs, and were grown in suspended culture from July 2011 – June 2012 at sea-based farms within Algoa Bay (Eastern Cape) and Saldanha Bay (Western Cape), and at a pond-culture farm in Kleinzee (Northern Cape). Growth and condition were compared between cohorts within each grow-out environment, and related to environmental variables. Daily mean temperatures above the oyster thermal optimum of 19°C were 50.5%, 14.3% and 63.1% of all the daily mean temperatures over the whole study for Algoa Bay, Saldanha Bay and Kleinzee respectively. Saldanha Bay (7.8 $mg.m^{-3}$) had a higher chlorophyll *a* daily mean concentration than Algoa Bay (3.9 $mg.m^{-3}$). Fatty acid (FA) composition differed significantly between the two sea-based farms, both overall, and with regard to the essential FA which characterize diatoms (eicosapentaenoic acid) and dinoflagellates (docosahexaenoic acid). In Algoa Bay there was proportionally more docosahexaenoic acid (12.7% of all the FA found in the sample) than in Saldanha Bay, while Saldanha Bay had proportionally more eicosapentaenoic acid (10.8% of all FA). In addition to indicating periodical differences of phytoplankton groups at the two sites, essential fatty acids and their ratios are important for oyster growth and health and indicate potential differences in nutritional value between sites. Previous relatively high growth rates at Algoa Bay might have been coupled with a high nutritional value of phytoplankton species, and FA sampling has revealed relatively high

proportions of EPA, DHA and their precursors at Algoa Bay. Relative to the Chilean cohort, the Namibian cohort displayed lower mortalities during stressfully high summer temperatures at Algoa Bay and Kleinzee. The Namibian cohort had higher shell densities at all sites, compared to the Chilean cohort, while the Chilean cohort had a higher DWCI than the Namibian cohort within Saldanha Bay. The Namibian cohort proved better suited to grow-out environments with high temperature ranges than did the Chilean cohort.

1. Introduction

Between the genotypic and environmental factors that influence both live mass (as a function of time) and survival in Pacific oysters, mass is influenced more by environment, whereas survival is influenced more by genotype (Degremont *et al*. 2005; Evans and Langdon 2006). It is possible to encounter a genotype x environment interaction where the same oyster cohort might yield differential performances in either oyster mass, survival or both, depending on the environment where it is raised (Langdon *et al*. 2003). These interactions have occurred for *Crassostrea gigas* in other studies (Langdon *et al*. 2003; Degremont *et al*. 2005; Evans and Langdon 2006) and can occur within cohorts of different origin imported for grow-out in South Africa at Algoa Bay, Saldanha Bay and Kleinzee. High-performing cohorts from a family bred for favourable genetic traits such as growth, survival, metabolic efficiency and protein deposition will generally show high performance within a wide range of favourable environments (Langdon *et al*. 2003).

The genotype of an oyster is particularly important in dealing with stress (Zhang *et al*. 2012). The sequencing of the oyster genome has established that genes involved in stress adaptation are highly expressed in *C. gigas*, even in inbred oyster cohorts which are often found among hatchery-reared oyster families used for commercial purposes (Zhang *et al*. 2012). Depending on genotype, some oyster cohorts are hardier and are predisposed to higher survival rates than other cohorts. Genes coding for protection against heat stress are particularly enhanced along with a complex array of genes involved in shell formation (Zhang *et al*. 2012). Oysters depend on shell growth for protection against predators and shell-boring polychaetes. Metabolic functions associated with shell formation are also accelerated with increased temperatures, as increased shell growth often occurs with increasing temperature (Walne and Mann 1975; Shpigel and Baylock 1991).

The oyster culture potential of different South African coastal environments is depends primarily on temperature range, and phytoplankton composition and abundance patterns. Pond-culture oyster farms generally have lower phytoplankton abundance (Chapter 2), coupled with a lower distribution of food through limited water movement. Because of low food availability, pond culture in South Africa is chiefly intended for the nursing of oyster juveniles and spat, before they are transported to sea-based farms for grow-out. The relative performance in shell and meat growth, shell density and survival of different oyster cohorts in a nursing environment, could indicate which oyster cohorts are among the highest quality for grow-out in sea-based farms.

It is important to compare the phytoplankton composition within different oyster grow-out sites, because phytoplankton species differ in nutritional value, and smaller species (< 4 µm) are retained less efficiently by oyster gills (Haven and Morales-Alamo 1970; Ropert and Goulletquer 1999; Ward and Shumway 2004). Of particular importance in this regard is the fatty acid (FA) composition within different phytoplankton species. Although the composition of FA and other nutrients within phytoplankton species varies in response to light intensity and nutrient composition in the water (Harrison *et al*. 1990; Thompson *et al*. 1996; Holland *et al*. 2004) , some FA are characteristically prominent in particular species (Langdon and Waldock 1981; Wikfors and Ohno 2001; Løfstedt 2010).

These FA include polyunsaturated fatty acids (PUFAs) which are essential for oysters such as; docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3), which are known to be vital for oyster growth and survival (Langdon and Waldock 1981; Løfstedt 2010). Arachidonic acid (ARA; 20:4ω6) is also an essential PUFA in adult oysters and a precursor of EPA, and is involved in reproduction and immune responses (Sargent *et al*. 1997; Hurtado *et al.* 2009). Stearidonic acid (SDA; 18:4ω3) and a-linolenic acid (ALA; 18:3ω3) are precursors of EPA and DHA, and linoleic acid (LA; 18:2ω6) is a precursor of

ARA. The relative proportions of these essential PUFAs are also important, since they play a role in reproduction, and the ratio of EPA: PUFAs above a certain threshold inhibits growth (Thompson *et al*. 1996). Phytoplankton essential PUFA ratios and their success in meeting oysters' nutritional requirements vary between phytoplankton species. The phytoplankton strain, *Chaetoceros calcitrans*, has a high nutritional value for larval bivalves, with a (EPA + DHA): ARA ratio which is optimal for larval requirements (Rico-Villa *et al.* 2006). High values of this ratio improve larval settlement rate in *Mytilus galloprovincialis* (Pettersen *et al.* 2010).

Fatty acid biomarkers in seawater samples can be used to identify the dominant phytoplankton in a marine environment, whereby a higher proportion of diatoms is reflected by increased levels of 16:4ω1, 16:1ω7 and EPA (Parrish *et al*. 2000; Kharlamenko *et al*. 2008). Dinoflagellates and other flagellates contain large proportions of DHA (Parrish *et al*. 2000; Kharlamenko *et al*. 2008), in addition to SDA and oleic acid (OA; 18:1ω9) (Kharlamenko *et al*. 2008). Branched and odd-chain number fatty acids such as 15:0 and 17:0 are produced by bacteria and their relative proportions are markers to estimate the presence of bacteria in the environment (Gillian and Hogg 1984; Parrish *et al*. 2000; Budge *et al*. 2006).

Site-specific FA ratios could explain variable effects of grow-out environments on oyster performance. Because oyster growth at Algoa Bay is fast despite low chlorophyll *a* values, phytoplankton species at Algoa Bay might have a high nutritional value for oysters, and FA ratios within these species might suite oyster growth requirements. To compare FA ratios among sites, water samples for FA analysis were collected at Algoa Bay and Saldanha Bay during a summer period.

Within each South African environment, it can be expected that genetically distinct oyster cohorts imported from different hatcheries will differ in performance with regards to

either growth, survival or both. To test this, two oyster cohorts were imported, one from Namibia and one from Chilé, for grow-out at Algoa Bay, Saldanha Bay and Kleinzee. Growth, condition and survival were compared between cohorts at each environment. Differences between cohorts were related to specific responses to temperature, chlorophyll *a* concentration and FA ratios. This was also done to determine whether high performance in one cohort relative to the other would be constant across different grow-out environments, as this would indicate the relative importance of influences of genotype and environment on oyster growth and condition.

2. Materials and methods

2.1. Study sites

For this study, grow-out environments (farms) included the same sea-based farm in the Big Bay of Saldanha Bay and the land-based farm at Kleinzee (Chapter 2). For the Algoa Bay grow-out environment, the study started on the same farm as in the previous study (Chapter 2), but in September 2011 oyster cages were moved to another farm in the same bay, a few hundred meters away (33.9°S, 25.6°E) from the original farm.

2.2. Temperature and chlorophyll a

Sea temperature and chlorophyll *a* estimates were continuously logged with the same individual instruments as those used for the study reported in Chapter 2. Two oyster cages each contained two Thermochron iButtons in the top and bottom cage layers for half-hourly temperature recordings. Hourly chlorophyll *a* estimates were recorded from July 2011 – June 2012 at both farms using a Turner Designs Submersible Fluorometer (SCUFA®) and a WET Labs ECO Fluorometer at Saldanha and Algoa bays respectively. Both fluorometers were secured to the suspension rope of one of the cages, directly above the cage, one and a half to

~three meters below the sea surface, depending on the cages' position on the suspension rope. Chlorophyll *a* was not measured at Kleinzee. Temperature and chlorophyll *a* daily means were calculated from the average of all measurements within a single day, and was compared between farms using Kruskal-Wallis ANOVAs followed by post-hoc pairwise tests with Statistica 10.0 (Statsoft, Tulsa, Oklahoma, U.S.A.).

2.3. Water samples for fatty acid analysis

Water samples were collected at Saldanha and Algoa Bay three times: within November 2011 and January 2012, and then early April and May 2012 for Saldanha and Algoa Bay respectively, with three replicates each. Only a limited number of water samples for fatty acid analysis could be collected during the course of this study due to the logistical and time constraints associated with fatty acid analysis of samples including two geographically distinct sites. One of the Saldanha Bay sample replicates for the month of November was contaminated during sample preparation and was excluded from the analysis.

For each sample replicate, 3 l of seawater were concentrated onto pre-combusted Advantec™ glass microfibre filters (GF/F) with a 0.5 µm mesh diameter. Seawater for filtering was collected within the vicinity of the oyster cages and kept on ice during travelling and filtered within two hours after collection on average. Filters were then immediately stored at -80 ± 2°C. For analysis of FA content, frozen samples were taken to the Fatty Acid Facility at Rhodes University in Grahamstown, where filter samples were analysed with gas chromatography and mass spectrometry (GC-MS). At this facility, samples were freeze-dried (VirTis, benchtop "K"Manifold freeze dryer) for five hours at -40 to -60°C and all equipment for analysis were lipid-cleaned: glassware (pipettes, tubes, vials and vial-inserts) were ashed in a muffle furnace for 5 hours at 450°C, and all caps and septa with were rinsed with chloroform ($CHCl_3$) and methanol.

Fatty acids were extracted by means of a one-step method which combines extraction and trans-esterification with a sulphuric acid catalyst (H_2SO_4), adapted from Indarti *et al*. (2005) as follows. Filters were transferred to 15 ml glass tubes to which 2 ml of $CHCl_3$ and an anti-oxidant (0.01% butylated hydroxytoluene) were added. An internal non-naturally occurring FA standard namely 19:0 (0.05 ml aliquots) was added to each sample to aid in GC peak quantification. A solution of $H_2^-SO_4$ and anhydrous methanol (0.3: 1.7) was added to each sample to remove fatty acids from their glycerol backbones and to methylate the free fatty acids to produce fatty acid methyl esters (FAMEs). FAME samples were then topped with nitrogen gas, sealed, and heated at 100°C for 30 minutes.

Samples were cooled and Milli-Q water was added to create a soluble medium for hydrophilic compounds so that lipid compounds could be adequately separated. These were then vortexed for ten seconds and centrifuged at 3000 rpm for three minutes, after which the upper aqueous layer was removed using a pipette. The new samples were dried with Na_2SO_4 and the extracts (without filter residues) were separated and transferred to 2 ml vials through a column of Na_2SO_4. Extracts were evaporated to dryness and topped with 0.5 ml hexane. For the more dilute samples, glass inserts were placed into the 2 ml vials to reduce the volume of solvents used.

For analysis, an Agilent GCQQQ Gas Chromatography-Mass Spectrometer (GC-MS) instrument was used (Agilent Technologies 6890, USA; Supplier: Chemetrix Pty Ltd). FAMEs were injected at 260^{o}C, and the oven temperature set at 70°C (the first minute) ramped at 40°C/min until 170°C was reached, and then ramped again at 2.5°C/min until 250°C. Depending on sample concentration, both splitless and 2 to 1 split injections were run to obtain clear chromatogram peaks. Each sample ran for forty minutes. FAMEs were carried by a stream of helium at 1.08 ml/min through a Zebron™ Capillary GC column with

a 30 m length, 0.32 mm inner diameter and a 0.25 µm film thickness. The instrument was linked to a computer with online software.

Individual FAMEs retained on the column for different times (depending on the chain length and number of double bonds) were detected with a flame ionization detector (FID). Gas chromatograms were analyzed with Agilent Chemstation 2.01, and mass spectrometer chromatograms were analyzed with Agilent Mass Hunter software through comparisons of peak molecular masses to a National Institute of Standards and Technology (NIST) library of compounds. Chromatogram peaks were identified from a subset of the samples and three standards (37 Component FAME Mix, Marine Pufa no.1, Bacterial Acid Methyl Esters Mix; Supelco, U.S.A.) analyzed with MS and identified with the NIST library. For each sample, the areas under individual FA peaks were expressed as percentages of the total area under all FA peaks. These proportions were qualitatively compared between samples collected at Algoa Bay and Saldanha Bay with PAST© statistical software, and a Bray-Curtis measure of distance was used for non-metric multi-dimensional scaling (nMDS), ANOSIM and SIMPER analysis (Clarke and Warwick 1994).

2.4. Oyster stocks and husbandry

Two cohorts of two-month-old *Crassostrea gigas* spat were imported from two distinct hatcheries: one from Beira Aquaculture in Walvis Bay, Namibia, and one from Cultivos Marinos in Bahía de Tongoy, Chilé. All oysters from both cohorts were from matings with a single male and two females, and were therefore half-sibs. Although comparisons of genetic vs. environmental impacts elsewhere in the world were done on full-sib families (Langdon *et al*. 2003; Degremont *et al*. 2005; Evans and Langdon 2006), the design for this study is modeled on standard South African spat import protocols. Oysters were selected from both cohorts in order to size-match Namibian and Chilean oysters to have the same initial masses

at each farm (approximately 0.5 g). A starting sample of 3000 oysters per cohort and farm (1500 oysters per cage) were planted for grow-out in July 2011 in two three-layer suspended cages. From July – November 2011 three high density polyethylene (HDPE) cylindrical Cesto® cage layers (415 mm diameter, 80 mm height) were tied to a rope and separated 500 mm with rigid piping. A total of four "cage-assembles" (two per cohort) were hung from longlines with the top cage layer one and a half to three meters from the sea surface at each farm, depending on the cages' position on the longline and its proximity to floating buoys.

A weight was tied below the bottom layer to provide structure within the cage. Cage layers had an outer mesh diameter of 10 mm square blocks, and were divided into four identical compartments each containing a small-mesh (4 x 4 mm) basket with a lid (mesh 2 x 2 mm). Each cage layer was sealed with an empty layer fixed above it using cable ties. A specific compartment from each cage layer was used throughout for measurements of individual oyster masses and shell heights, while oysters from the other three compartments were used to determine mortality.

In July 2011, 125 oyster spat were placed in each of the four cage layer compartments. Live mass, shell height and condition index measurements of oysters were measured every two months (see below) and therefore the study consisted of four two-month grow-out periods until March 2012. Individually-measured oysters (Section 2.5) at the two sea-based farms were grown-out until June 2012, and therefore these oysters have measurements for five grow-out periods. At the end of September 2011 after two months of grow-out, stocking density per compartment was reduced to 50 oysters to maintain a constant biomass per compartment to conform to commercial husbandry practices. For the sample size reduction to 25% of the original number of oysters, oysters from both cohorts were selected to center around the mean masses, while the smallest oysters were also discarded as is practice in

industry (Chapter 2). There was no difference between cohorts at the start of the Sep – Nov 2011 grow-out period for the two sea-based farms.

At the end of November 2011, all oysters were transferred to flat HDPE mesh envelope bags (mesh size is 25 mm), which were suspended vertically in stacks of five, with each bag separated by 200 mm of rope. Envelope bags were kept open by gravity and maintained in a rectangular shape by means of a rigid square frame of HDPE tubing. For grow-out in these cages oysters from the specific marked compartments were moved to specifically marked *Netlon*® (mesh diameter 28 mm long and can stretch to 28 mm across) bags (lengths 650 – 750 mm). Oyster batches from the previous four compartments were placed into four different compartment-specific Netlon bags within the same envelope in order to maintain these oysters within the same position in the cage. The top-middle and bottom-middle layers (the second and the fourth layer of the five-layer cage) of the new cages were filled with the discarded oysters to weigh down the cage.

For these new cages, stocking density at the two sea-based farms was reduced from 50 to 40 oysters per bag, when oysters from selected compartments were marked individually (Section 2.5.). Selection for this reduction was random, but oysters too small for marking with a number were discarded from compartments meant for marked individuals. At the end of the next grow-out period (end Jan 2012), stocking density at the two sea-based farms was reduced for the final time to 30 oysters per bag through random selection as the number of slow-growing oysters for that were discarded decreased. At Kleinzee, oyster numbers were reduced from 50 to 40 oysters per bag within the bags for batch measurements for the last grow-out period (Jan – Mar 2012). This was also done through random selection, which aided in avoiding the effect a size bias would have on the number of mortalities during a high-temperature period. Kleinzee oysters for individual measurements were not reduced at that stage, although the slow growing oysters were kept separate from the fast-growers, but

within the same cage layer. Discard or separation of slow-growing oysters from fast-growing oysters optimizes oyster growth rates and survival, which makes them comparable to those obtained under standard husbandry practices (Chapter 2).

2.5. Measurements of growth: live and dry mass gain

Oyster shell height and mass measurements were taken every two months at the end of each grow-out period, individually for one of the bags in each cage layer (hereafter "individual" oysters), while oysters in the remaining three bags were counted and weighed as a batch (hereafter "batches"). For both of these measurements oysters were cleaned of excessive debris and epibionts by means of rinsing and scraping with a paint scraper. At Kleinzee, ascidians and soft epibionts were removed by hand. The counted live and dead oysters from each batch were used to determine the percentage mortality (see below). Dead oysters in each bag were removed and counted at the end of each grow-out period.

Individual oysters from each cohort and cage layer were used for growth rate analyses. At the two sea-based farms within November 2011, oysters from the individually measured compartments within the top and bottom layer (~1.4 m apart) were marked individually with numbers. For this small numbers were printed on plastic embossed Dymo® tape, which were cut out individually and stuck to the flat right valve of the oyster shell by submerging it in a drop of non-toxic epoxy. To prepare the shell surface for number attachment a dremel-tool was used to smooth and clean an area between 0.5 and 0.75 cm^2 on the anterior part of the oyster shell next to the umbo. After smoothing, the surface was dried with acetone to remove shell dust. Individual oysters and smaller batches were weighed with a Denver MAXX 120 g scale accurate to 0.01 g and measured with digital calipers (accurate to 0.01 mm) for the longest shell height (from umbo to the posterior edge; Galtsoff 1964; Brown and Hartwick

1988). Larger batches were weighed with a bigger, splash-proof Masskot 15 kg scale accurate to 1 g.

To ascertain the most important determinants of growth rate in the sub-sample of marked individuals, best-fit multivariate regression models were compared using generalized least squares (GLS) and maximum likelihood estimates with R© 2.15.2 statistical software (R Development Core Team 2012) and the "nlme" package (Pinheiro *et al.* 2012). This analysis was chosen to correct for differences in the range of scatter and a relationship of residuals between successive grow-out periods. For these analyses, percentage body mass gain per day (instantaneous growth rate) for each grow-out period was the dependent variable and independent variables included categorical and continuous predictors. The instantaneous growth rate was calculated as: (((end mass - start mass) x100)/(start mass))/(number of days). The combination of independent variables which had the biggest influence on oyster growth was identified through comparison of the Akaike's information criterion (AICc) values of fitted models with different combinations of these variables (factors) by means of the "MuMIn" package (Bartoń 2010). The AICc together with a one-way ANOVA F-test on the difference between models with stepwise exclusions of individual factors from the full model indicated which variables should be included in the model on oyster growth. The slope coefficients of factors in the best-fit model indicated whether their influence on growth was significant, and either positive or negative.

Whole live mass and dry mass as a function of time for all individuals (not only the marked ones) were compared between farms, cohort and cage layer (top vs. bottom) by fitting polynomial curves (Chapter 2, see also Brown and Hartwick 1988). Best-fit growth curves were compared using the extra sum-of-squares F-test (Haddon 2001). Dry meat mass gain for all individual oysters from Algoa Bay and Saldanha Bay was estimated using cohort-specific data for each grow-out period from the sub-sample (N = 40) of oysters used for

condition indices. Comparison of dry meat mass growth was performed only on oysters from the sea-based farms as this chapter is focused on inter-strain oyster comparison, and because the suitability of grow-out environments for optimal growth is less obvious between the two sea-based farms.

For these oysters, dry meat mass was regressed on whole live mass separately for each cohort and farm. These least-squares linear equations were used to estimate dry meat masses from the whole live mass of each individual oyster (marked and unmarked). The estimated dry meat masses were also fitted to best-fit polynomials and compared with the extra sum-of-squares F-test. Between-farm comparisons of dry meat mass gain coupled with those of whole live mass gain provide insight into the combined water and shell contents of oysters grown in different environments. A combination of low food and high temperatures favors investment of energy into shell growth (Walne and Mann 1975; Brown and Hartwick 1988; Shpigel and Baylock 1991). For all polynomial-based analyses we used GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego California, USA).

2.6. Oyster condition index

From the end of the second grow-out period (November 2011), 38 – 40 oysters were taken from both cohorts at each farm, and shucked in the laboratory. Shell and meat samples were weighed separately on the balance accurate to 0.01 g (Section 2.5), dried at ± 50°C for four to six days and re-weighed. Dry meat mass was expressed as a fraction of dry shell mass for DWCI (Chapter 2 for equation), and shell dry mass as a percentage of shell wet mass was used to express shell density. For each two-month grow-out period, DWCI and shell density was compared between farms and cohorts using Kruskal-Wallis ANOVAs followed by post-hoc pairwise tests with Statistica 10.0.

2.7. Mortality and fouling

Using batches, we counted the number of dead oysters at the end of each grow-out period and expressed this as a percentage of the original number of oysters for each grow-out period in each batch. This was compared between grow-out periods and farms for each cohort using a Kruskal-Wallis ANOVA. To reflect the fact that mortalities for most grow-out periods were low, and medians for all grow-out periods seldom reached 10% mortality and did not exceed 35% (Appendix 11), the total percentage mortality was calculated. For each cohort and farm, all the mortalities per bag were added for all cage layers and grow-out periods to obtain the total mortality over the whole study. The total mortality was then expressed as a percentage of the initial number of oysters per bag (the number of oysters at the beginning of each grow-out period prior to mortalities) added for all cage layers and grow-out periods to obtain the total percentage mortality (Table 2). To calculate the final percentage mortality, the total mortality was instead expressed as a percentage of the initial number of oysters per cohort and farm, without the oysters which had been discarded over the course of the study. For each two-month grow-out period percentage mortality was compared between cohorts and farms using Kruskal-Wallis ANOVAs followed by post-hoc pairwise tests with Statistica 10.0.

3. Results

Because environmental parameters are very variable in nature, temperature and chlorophyll *a* data from the previous growth trial (data set) in Chapter 2 from May 2010 – March 2011 (hereafter referred to as "Study 1") was included for comparison with the data set from this growth trial from July 2011 – June 2012 (hereafter referred to as "Study 2"). This was done to explore inter-annual variations. Parametric or non-parametric tests were used based on Shapiro-Wilks normailty tests on all data, and $p < 0.05$ was used as a threshold

for significance. Environmental parameters and consequent oyster responses are described separately for a winter and a summer period. The winter period constitutes the period of mid to late July 2011 until late September 2011 as well as the period of late March 2012 to early June 2012, and is characterized by moderate temperatures and relatively high chlorophyll *a* values because of phytoplankton blooms. Medians for daily mean temperatures within winter were 14.4°C (quartile range of 13.3 – 15.6°C), 17.6°C (16.5 – 18.9°C) and 17°C (14.7 – 19.5°C), for Saldanha Bay, Algoa Bay and Kleinzee respectively. Summer (late November 2011 – late March 2012) was characterized by relatively low chlorophyll *a* values due to the lack of phytoplankton blooms, and high water temperatures at all farms, with sharp high temperature peaks at Algoa Bay and Kleinzee (Fig. 1). Medians for daily mean temperatures within summer were 18.6°C (17.8 – 19.3°C), 21.2°C (19.9 – 22.6°C) and 21.2°C (20.4 – 22.5°C) for Saldanha Bay, Algoa Bay and Kleinzee respectively.

3.1. Temperature and chlorophyll a

3.1.1. Inter-annual variation in temperature

From Study 1 (May 2010 – March 2011) to Study 2 (July 2011 – June 2012) at the two sea-based farms, the median of daily mean sea temperatures changed from 15.1°C (quartile range of 13.7 – 17.5°C) to 16.2°C (14.1 – 18.4°C) for Saldanha Bay, and from 17.5°C (16.5 – 19.2°C) to 19°C (17.4 – 20.8°C) for Algoa Bay (Fig. 1) and differed significantly between studies at each farm (Mann-Whitney $U_{307, 392} = 52857$, $z = -2.76$, $p = 0.0058$; Mann-Whitney $U_{297, 391} = 38871$, $z = -7.4$, $p < 0.0000001$ and Mann-Whitney $U_{263, 380} = 42124$, $z = -3.9$, $p = 0.0007$ for Saldanha Bay, Algoa Bay and Kleinzee respectively). Although Study 1 did not include the month of April, all the other months and all seasons were covered by both studies, and therefore a definite inter-annual difference in temperatures occurred between the two studies.

3.1.2. Temperature comparison between farms for Study 2

Within Study 2, daily mean sea temperatures at both Algoa Bay (mean with quartile range: 19°C, 17.4 – 20.8°C, N = 297) and Kleinzee (20°C, 16.9 – 21.3°C, N = 263) were higher than those of Saldanha Bay (16.2°C, 14.1 – 18.4°C, N = 307) (Kruskal-Wallis ANOVA: $H_{867} = 200$, $p < 0.0001$), with no difference between Algoa Bay and Kleinzee. Among these 50.5%, 14.3% and 63.1% of all the daily mean sea temperatures at Algoa Bay, Saldanha Bay and Kleinzee respectively were above the oyster thermal optimum of 19°C. Within winter, Algoa Bay (N = 174) had higher daily mean sea temperatures than Kleinzee (Kruskal-Wallis ANOVA; N = 134, z = 2.9, p = 0.01), with no difference in summer.

3.1.3. Inter-annual variation in chlorophyll a

Daily mean concentrations of chlorophyll *a* (Fig. 2) for Study 2 were lower than those for Study 1 at both farms (Mann-Whitney $U_{299, 174} = 22353$, z = 2.6, p = 0.01 and Mann-Whitney $U_{316, 247} = 20773$, z = 9.53, $p < 0.0000001$ for Algoa Bay and Saldanha Bay respectively).

3.1.4. Comparison of chlorophyll a between farms for Study 2

For both the winter and the summer period, Saldanha Bay had higher chlorophyll *a* daily mean concentrations than Algoa Bay (Mann-Whitney $U_{124, 183} = 1988$, z = -12.3, $p < 0.001$; and Mann-Whitney $U_{123, 116} = 3002$, z = -7.73, $p < 0.0000001$ for winter and summer respectively). The overall median for Algoa Bay daily mean chlorophyll *a* concentrations (N = 299) was 3.9 $mg.m^{-3}$ (with quartile ranges of 2.3 – 5.2 $mg.m^{-3}$), while the seasonal medians were 2.84 $mg.m^{-3}$ (1.92 – 2.63 $mg.m^{-3}$) and 4.63 $mg.m^{-3}$ (3.12 – 5.9 $mg.m^{-3}$) for winter and summer respectively. For Saldanha Bay, the overall median for daily mean chlorophyll *a* concentrations (N = 247) was 7.8 $mg.m^{-3}$ (5.4 – 10.96 $mg.m^{-3}$), with 9.8 $mg.m^{-3}$ (5.9 – 13.4 $mg.m^{-3}$) and 6.8 $mg.m^{-3}$ (4.9 – 8.7 $mg.m^{-3}$) for winter and summer respectively. Within

Saldanha Bay, chlorophyll *a* concentrations in the winter were higher than those of summer (Mann-Whitney $U_{124, 123} = 5135$, $z = 4.4$, $p = 0.000009$) due to phytoplankton blooms in early spring and late autumn, but within Algoa Bay the reverse was true with higher chlorophyll *a* concentrations in summer (Mann-Whitney $U_{183, 116} = 6290$, $z = -5.93$, $p < 0.0000001$).

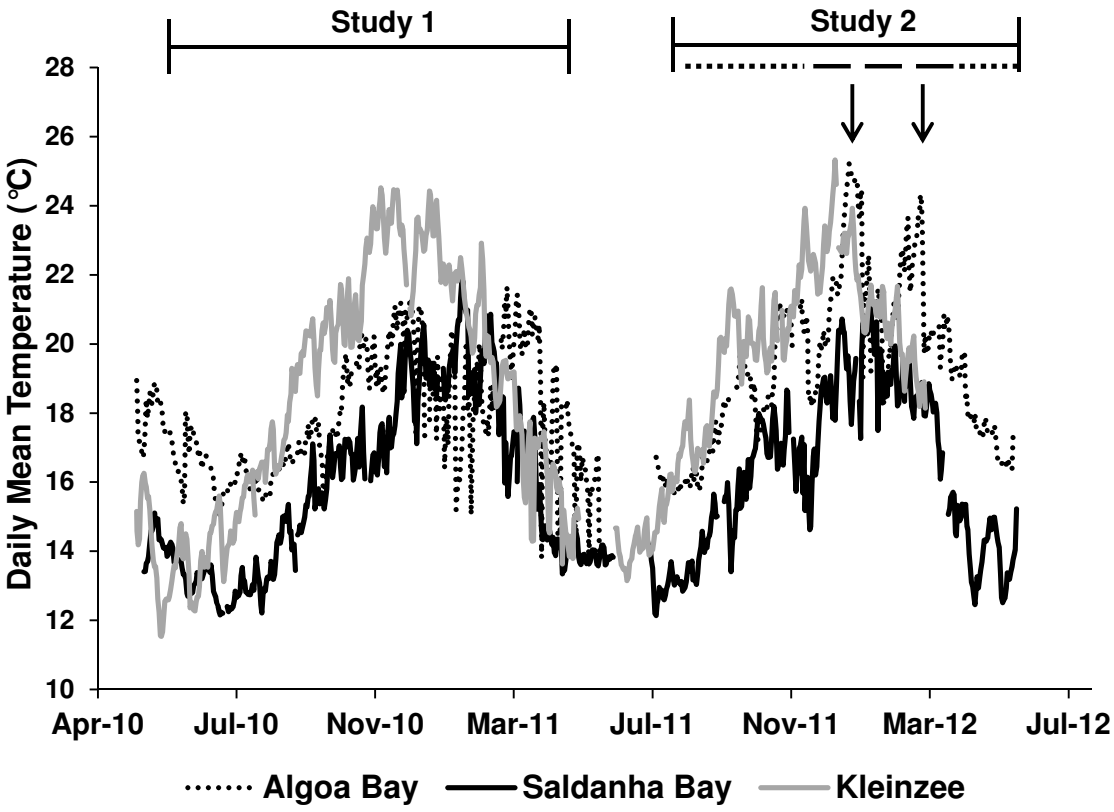


Figure 1: Daily mean seawater temperatures are displayed here for both Study 1 and Study 2. Summer for Study 2 is indicated by a dashed line, while winter is indicated by dotted lines before and after summer. The two arrows for this study indicate two peaks of daily-averaged temperature ranges in Algoa Bay which were absent in the previous year: the first peak was from 9 – 23 January 2012, when daily mean temperatures ranged from 24 – 25°C, and the second peak was on 14 – 15 March 2012 when the daily means were about 24°C. These sharp temperature increases are within the grow-out periods in which the highest mortalities occurred at Algoa Bay and Kleinzee (see Appendix 11).

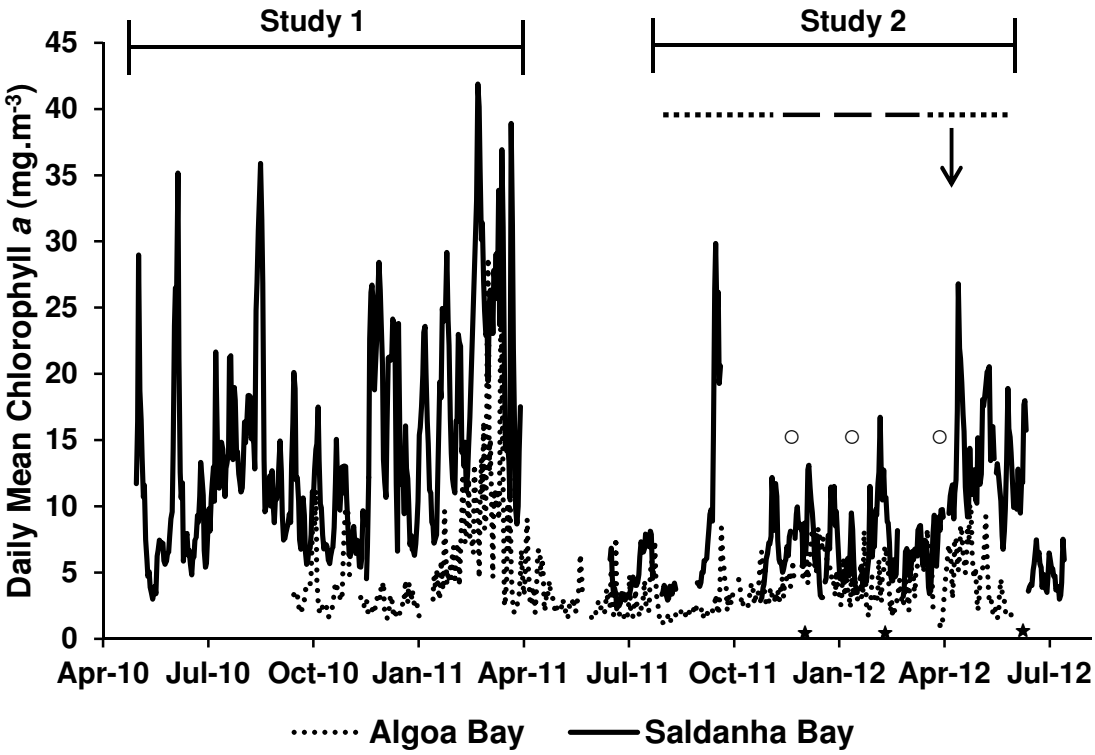


Figure 2: Saldanha Bay maintained chlorophyll *a* concentrations two times higher than those of Algoa Bay for Study 2. Summer for Study 2 is indicated by a dashed line and ended just before the big phytoplankton bloom at Saldanha Bay in April (arrow), while winter is indicated by dotted lines before and after summer. Dates for seawater filter samples were 30 Nov 2011, 1 Feb 2012, and 2 May 2012 for Algoa Bay, and 25 Nov 2011, 20 Jan 2012 and 5 Apr 2012 for Saldanha Bay and are indicated in Fig. 2 (with * on the x-axis for Algoa Bay and ○ above the graph for Saldanha Bay).

3.2. Fatty acids

Comparison of FA composition (Fig. 3) showed a significant difference between Algoa Bay and Saldanha Bay (one-way ANOSIM comparison with 9999 permutations: R = 0.5, p = 0.0005). The difference between samples within farms, collected in different months was not significant. A SIMPER analysis showed that 16:1ω7, 14:0, DHA, 18:0 and EPA (in descending order of percentage contribution) accounted for most of the variation between Algoa Bay and Saldanha Bay (Table 1).

For all the samples combined for each farm, Saldanha Bay had proportionally more bacterial fatty acids than Algoa Bay (19.5 and 16.4% for Saldanha Bay and Algoa Bay respectively), while Algoa Bay had proportionally more essential fatty acids (21.9 and 19.4% for DHA and EPA combined for Algoa Bay and Saldanha Bay respectively). Algoa Bay also had a higher proportion of ARA and therefore a lower (EPA+DHA): ARA ratio, although ARA only constituted 0.6% and 0.4% of all the fatty acids in the combined samples for Algoa Bay and Saldanha Bay respectively. Algoa Bay had proportionally more DHA (12.7%) which reflect high proportions of dinoflagellates, while Saldanha Bay had proportionally more EPA (10.8%) which reflects high proportions of diatoms (Parrish *et al*. 2000; Kharlamenko *et al*. 2008). Algoa Bay also had proportionally more PUFAs than Saldanha Bay (31.7 and 29.8% for Algoa Bay and Saldanha Bay respectively).

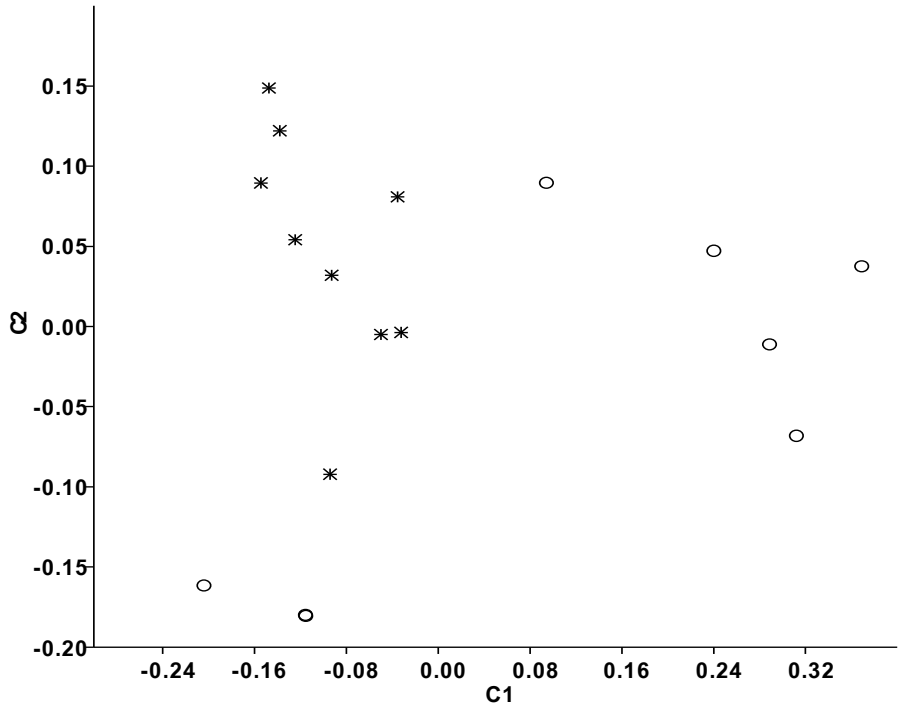


Figure 3: A two-dimensional nMDS scatterplot of the first and second dimension of FAs, for all water samples. Algoa Bay samples are represented by stars (★), while Saldanha Bay samples are represented by hollow circles (○) which grouped together, separately from Algoa Bay samples. The stress-value for this plot is 0.05 and R^2 values are 0.57 and 0.14 for axis 1 and 2 respectively. C1 = coordinate 1 and C2 = coordinate 2 and sample sizes are 9 and 8 for Algoa Bay and Saldanha Bay respectively.

Table 1: The individual FA peak areas as a percentage of the combined area for all FA in each sample are displayed, with common names (where they exist) given for those involved as phytoplankton biomarkers and their precursors. The FAs which contributed most to the difference between Algoa Bay and Saldanha Bay samples are typed in bold (SIMPER analysis), and statistics for each FAs contribution to this observed difference are displayed as their percentage contribution (% contr.). The diatom biomarkers EPA and 16:1ω7 are more prominent in Saldanha Bay and underlined with a double line, and the dinoflagellate biomarkers DHA, stearidonic acid (SDA; 18:4ω3) and oleic acid (OA; 18:1ω9) are more prominent in Algoa Bay and underlined with a single line. Bacterial FAs are underlined with a dashed line. SDA and a-linolenic acid (ALA; 18:3ω3) are precursors of EPA and DHA and were found at both sites, but linoleic acid (LA; 18:2ω6) which is the precursor of ARA was not found within the water samples.

	Saldanha Bay								**Algoa Bay**								
	Nov		Jan			Apr			Nov			Jan			May		
Sample number	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
FAs																	**% Contr.**
ai-14:0	0.7	0.6	0.5	0.4	0.4	0.7	1.7	0.8	1.6	0.9	0.5	0.3	0.8	0.1	0.6	1.4	0.2	1.1
14:0	**18.2**	**18.4**	**21.2**	**19.2**	**23.4**	11.1	11.0	5.9	11.1	10.9	10.9	10.6	9.8	10.2	10.6	9.3	11.3	**14.7**
i-15:0	0.5	0.2	0.2	0.5	0.0	0.1	0.1	0.0	0.6	0.6	0.8	0.9	0.4	0.1	1.0	1.0	1.2	1.2
ai-15:0	0.7	0.6	0.6	0.8	0.5	0.9	0.7	0.8	0.9	0.9	1.0	0.8	0.8	0.8	1.2	1.0	1.2	0.6
15:0	0.3	0.5	0.6	2.1	1.1	0.2	1.1	0.3	1.9	1.8	1.6	1.7	1.9	1.1	3.4	7.2	3.4	4.3
16:0	25.0	25.1	25.4	27.8	22.2	28.4	27.4	26.2	28.2	26.8	28.3	27.5	28.9	27.5	27.2	24.0	26.8	4.6
16:1ω7	**20.8**	**19.2**	**15.2**	**7.9**	**19.4**	4.0	2.2	5.4	4.6	8.7	3.9	3.5	4.0	5.6	5.1	8.1	8.1	**16.7**
16:1ω5	2.2	2.2	1.0	1.1	1.1	2.3	2.3	2.4	1.7	1.4	1.7	0.6	0.5	0.5	1.4	1.3	1.6	1.9
ai-17:0	0.2	0.5	0.2	0.9	0.3	0.0	0.3	0.2	0.8	0.9	0.8	0.7	0.4	0.6	0.4	1.1	1.2	1.0

Table 1 continued:

	Saldanha Bay								Algoa Bay									
	Nov		Jan			Apr			Nov			Jan			May			
Sample number	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
FA																		**% Contr.**
16:2ω4	2.0	1.8	1.5	1.4	2.1	1.5	1.3	1.7	1.8	1.7	1.6	0.9	0.7	0.8	1.5	1.5	1.6	1.0
ai-18:0	0.0	0.0	0.2	0.4	0.2	0.0	0.0	0.0	0.2	0.1	0.1	0.1	0.2	0.2	0.6	0.6	0.3	0.5
16:4ω1	3.9	3.7	2.0	2.5	2.1	3.3	2.7	3.5	2.8	1.9	2.6	2.7	2.1	3.1	5.3	4.2	5.2	2.5
18:00	3.7	5.3	4.2	6.0	3.8	2.1	3.0	2.5	**9.2**	**8.9**	**8.0**	**9.0**	**9.0**	**8.1**	**6.3**	**7.4**	**6.9**	**9.3**
OA	4.1	4.0	3.6	4.8	3.1	2.5	2.6	2.3	3.7	3.4	4.0	6.7	5.0	5.3	7.4	6.1	6.4	4.6
18:1ω5	2.3	2.2	1.5	1.9	1.1	3.8	3.9	3.7	2.9	2.5	2.8	3.2	2.4	2.7	4.4	3.5	3.0	2.4
ALA	2.9	2.5	3.3	3.0	2.4	11.9	12.0	9.9	4.2	4.3	5.3	3.2	3.0	3.2	3.0	2.6	2.6	**7.5**
SDA	0.0	0.6	0.0	0.5	0.0	0.0	0.0	0.2	0.0	0.7	0.0	0.8	0.5	1.3	0.0	1.6	1.1	1.3
20:0	0.0	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.3	0.2	0.4	0.8	0.5	0.9	0.8
ARA	0.2	0.2	0.4	0.7	0.9	0.0	0.4	0.2	0.3	0.4	0.7	0.9	0.3	0.5	0.8	0.7	0.6	0.7
EPA	9.1	8.2	7.5	7.6	8.3	**15.3**	**15.3**	**19.4**	9.1	7.9	8.8	8.9	9.0	9.0	11.7	9.8	9.3	**7.8**
22:0	0.0	0.5	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.4	0.0	0.0	0.6	0.7	0.6
22:5ω6	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.4	0.1	0.2	0.4	0.2	0.3	0.2	0.2	0.2	0.5
22:5ω3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.0	0.0	0.0	0.2
24:0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.2	0.2	0.4	0.2	0.4	0.8	0.5	0.6	0.8
DHA	3.1	3.3	10.8	9.4	7.3	12.0	12.0	14.5	**13.4**	**14.1**	**16.0**	**15.9**	**18.8**	**17.9**	6.6	5.7	5.5	**13.5**

3.3. Oyster growth

3.3.1. Growth differences between farms

For individually weighed oysters (including oysters in the top, middle and bottom cage layer), Algoa Bay oysters displayed higher whole masses as a function of time than Saldanha Bay oysters ($p < 0.0001$, Appendix 1), although this trend was probably strongest within winter (Fig. 4.). When a GLS analysis was applied to percentage mass gain of marked oysters from the top and bottom cage-layers, Saldanha Bay oysters had higher instantaneous growth rates than Algoa Bay oysters (Coefficient: 0.68, S.E.: 0.089, $t = 7.69$, $p < 0.000001$). Kleinzee oysters displayed the lowest whole mass growth rate ($p < 0.0001$, Appendix 1). Interestingly, estimated dry meat mass growth rate was higher at Saldanha Bay compared to Algoa Bay ($p < 0.0001$, Fig. 5, Appendix 2), despite higher mass growth rates at Algoa Bay. In Study 1, Algoa Bay oysters had higher whole live mass growth rates but almost equal dry meat mass growth rates compared to oysters at Saldanha Bay (Chapter 2, Fig. 4). This study (Study 2) indicates that higher growth rates at Algoa Bay are not as pronounced as in Study 1 and can be contributed to faster growth of shell only.

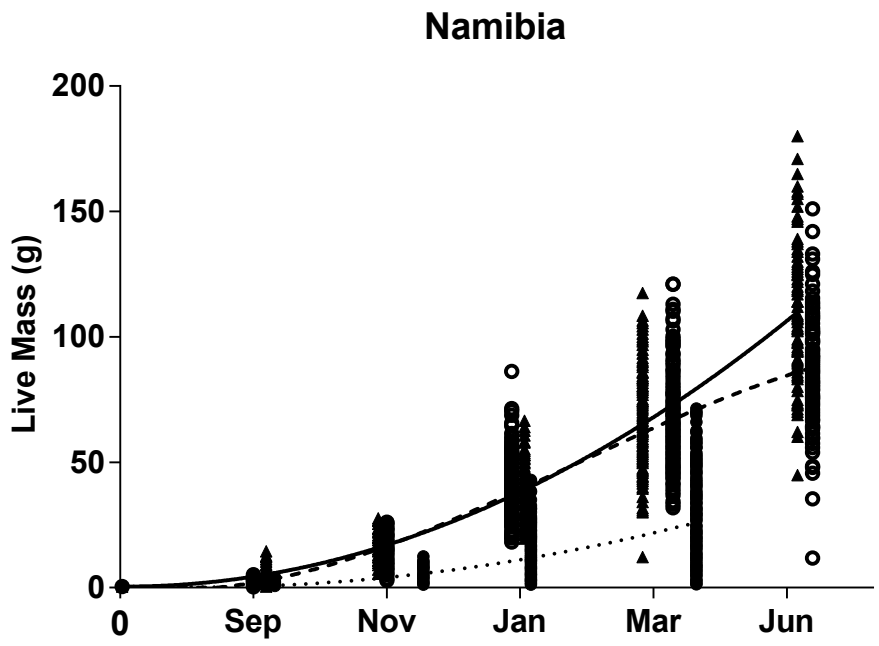


Figure 4: Gain in whole live mass as a function of time was fastest at Algoa Bay (triangles ▲ and solid lines), followed by Saldanha Bay (hollow circles ○ and dotted lines and), and then Kleinzee (solid circles ● and dashed lines) within both cohorts as is displayed here for the Namibian cohort ($F_{8, 6959} = 947.2$, $p < 0.0001$). Sample sizes per cohort and farm ranged from 750 – 55 due to mortality and the discardment of oysters. Saldanha Bay is fitted with a 3rd order polynomial, while Algoa Bay and Kleinzee are both fitted with 2nd order polynomials. Polynomial parameter estimates are given in Appendix 1.

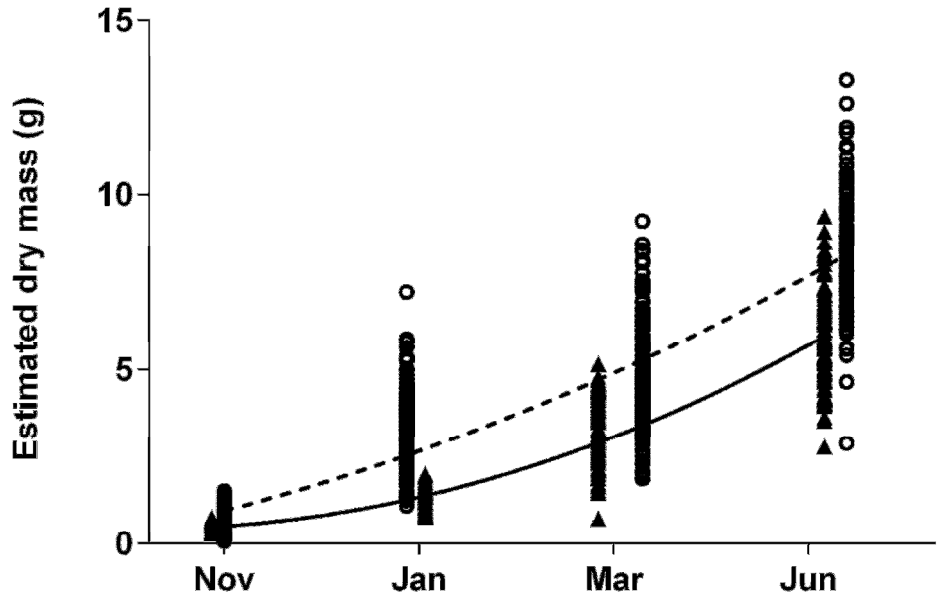


Figure 5: For both cohorts, gain in dry meat mass was fastest for oysters from Saldanha Bay (hollow circles ○ and a dashed line), compared to oysters from Algoa Bay (triangles ▲ and a solid line), as is shown here for estimated dry mass as a function of time for the Namibian cohort ($F_{3, 1601} = 270.4$, $p < 0.0001$). Both fits are second order polynomial. Polynomial parameter estimates are given in Appendix 2.

3.3.2. Growth differences between cohorts within all individuals

The Chilean cohort had higher live masses as a function of time than the Namibian cohort within Algoa Bay and Kleinzee ($p < 0.0001$, Appendix 3), while the opposite was true for Saldanha Bay ($p < 0.0001$, Appendix 3) (Fig. 6) within all the measured individual oysters (measured from Jul 2011 – Jun 2012). Polynomial regressions showed that higher mass gain within the top cage layer was mostly found within the Namibian cohort for all three sites ($p < 0.0001$), and within the Chilean cohort at Algoa Bay and Saldanha Bay ($p < 0.001$) (Appendix 4 – 6) (Fig. 7 – 9).

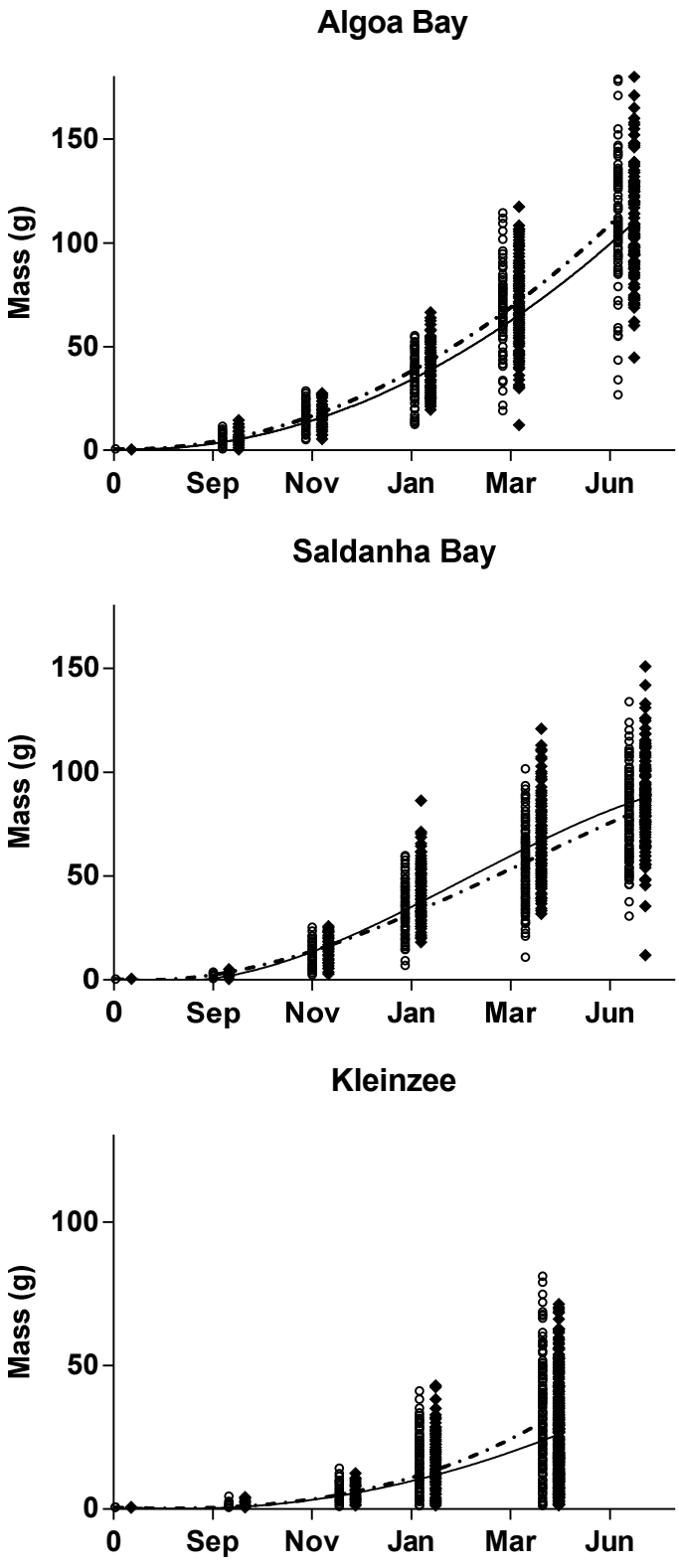


Figure 6: For oysters from Algoa Bay ($F_{3, 4362} = 46.9$, $p < 0.0001$) and Kleinzee ($F_{3, 4687} = 47.23$, $p < 0.0001$), the Chilean cohort (hollow circles ○ and dotted lines and) had a higher live mass gain than the Namibian cohort (♦ and solid lines), although the opposite trend was observed for oysters from Saldanha Bay ($F_{3, 4622} = 19.83$, $p < 0.0001$). Polynomial parameter estimates are given in Appendix 3.

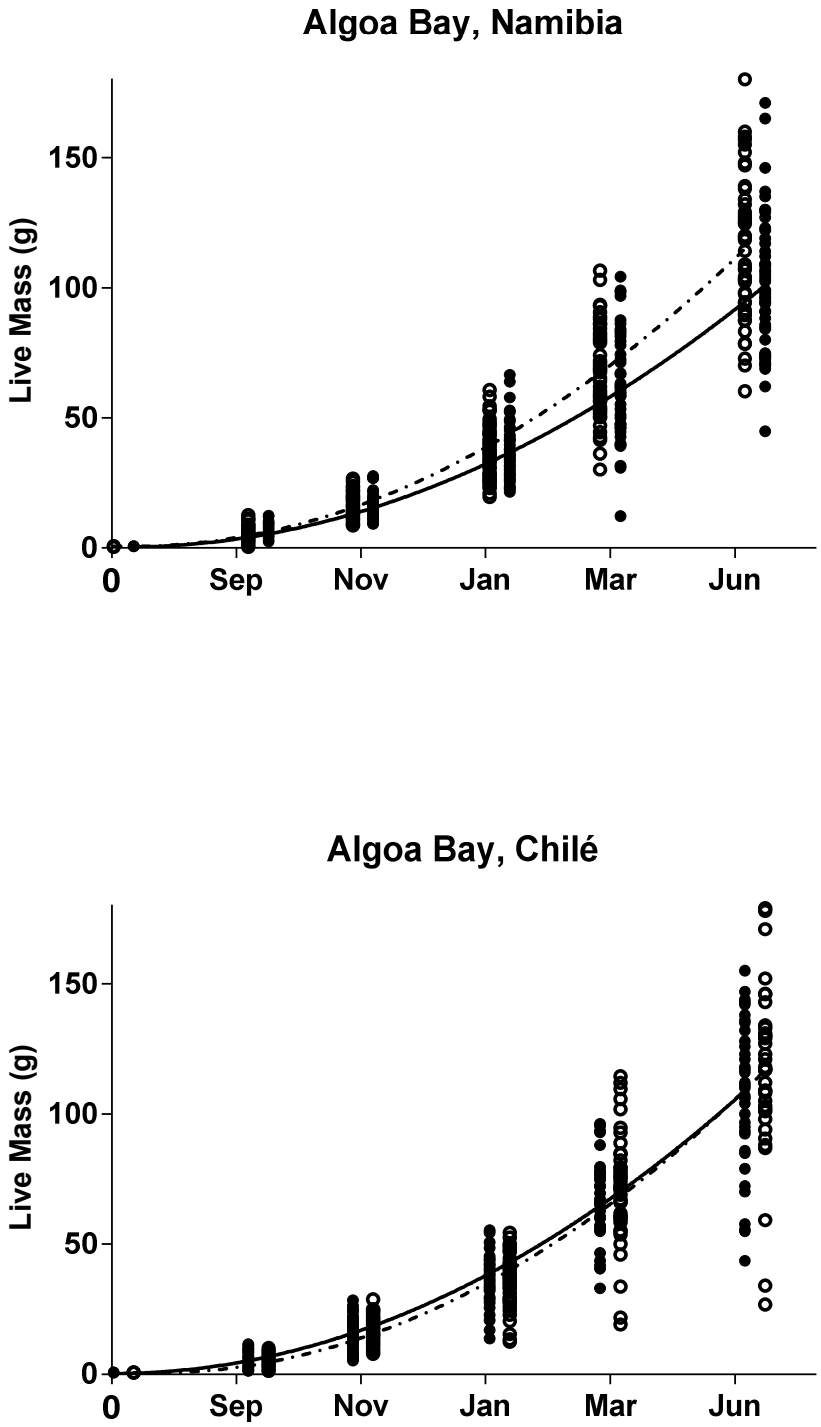


Figure 7: Individual live masses as a function of time for comparison between depth showed that the Namibian ($F_{3, 1530} = 72.2$, $p < 0.0001$) cohort differed more between the top (hollow circles ○ and dashed lines) and the bottom cage layer (solid circles ● and solid lines) than did the Chilean cohort ($F_{3, 1436} = 5.4$, $p = 0.001$) at Algoa Bay. Live masses at Algoa Bay were heavier in the top layer for the Namibian cohort. Polynomial parameter estimates are given in Appendix 4.

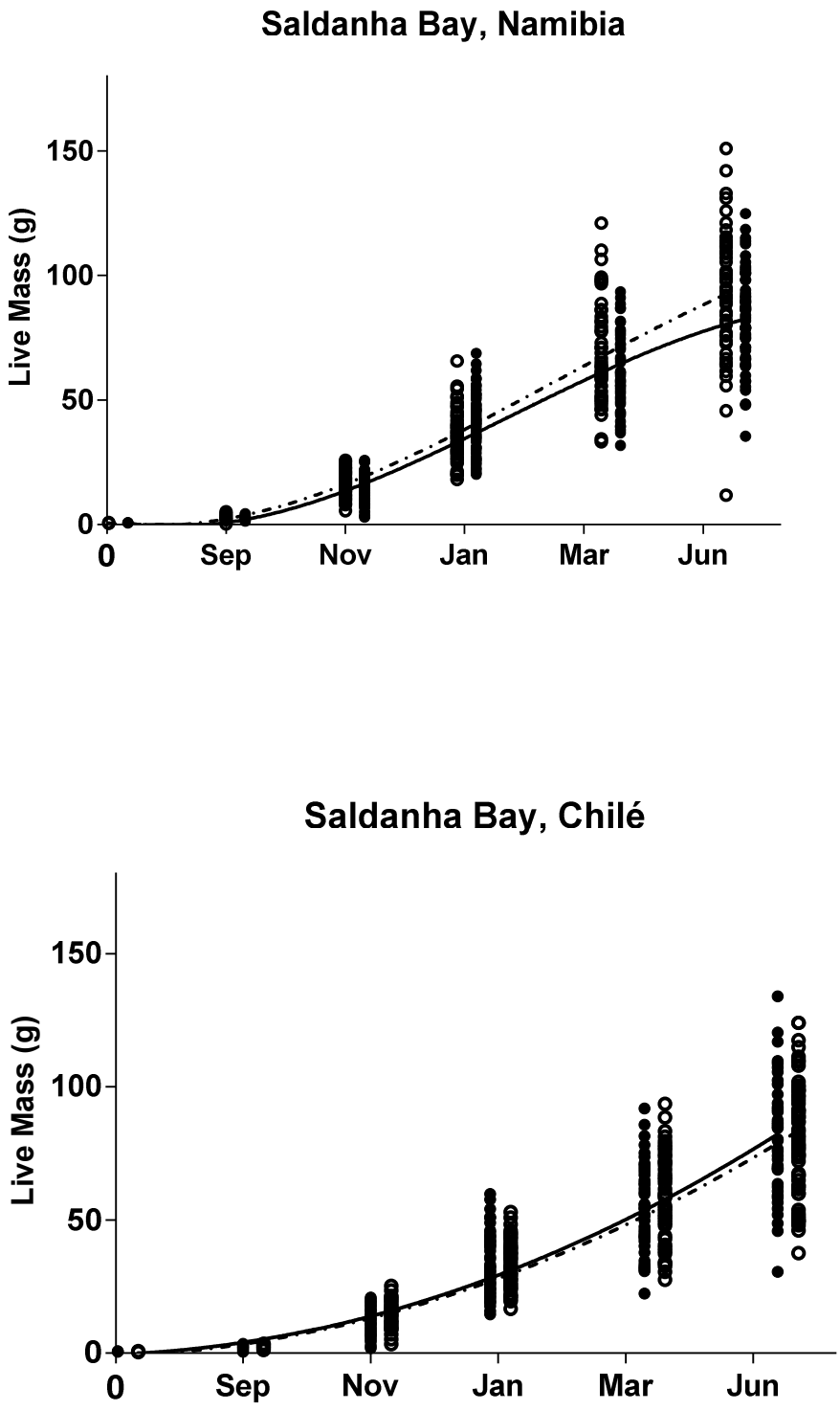


Figure 8: Individual live masses as a function of time for comparison between depth showed that the Namibian cohort ($F_{3, 1586} = 36.8$, $p < 0.0001$) differed more between the top (hollow circles ○ and dashed lines) and the bottom cage layer (solid circles ● and solid lines) than did the Chilean cohort ($F_{3, 1580} = 3.47$, $p = 0.02$) at Saldanha Bay. The top layer had a higher live mass gain within the Namibian cohort. Polynomial parameter estimates are given in Appendix 5.

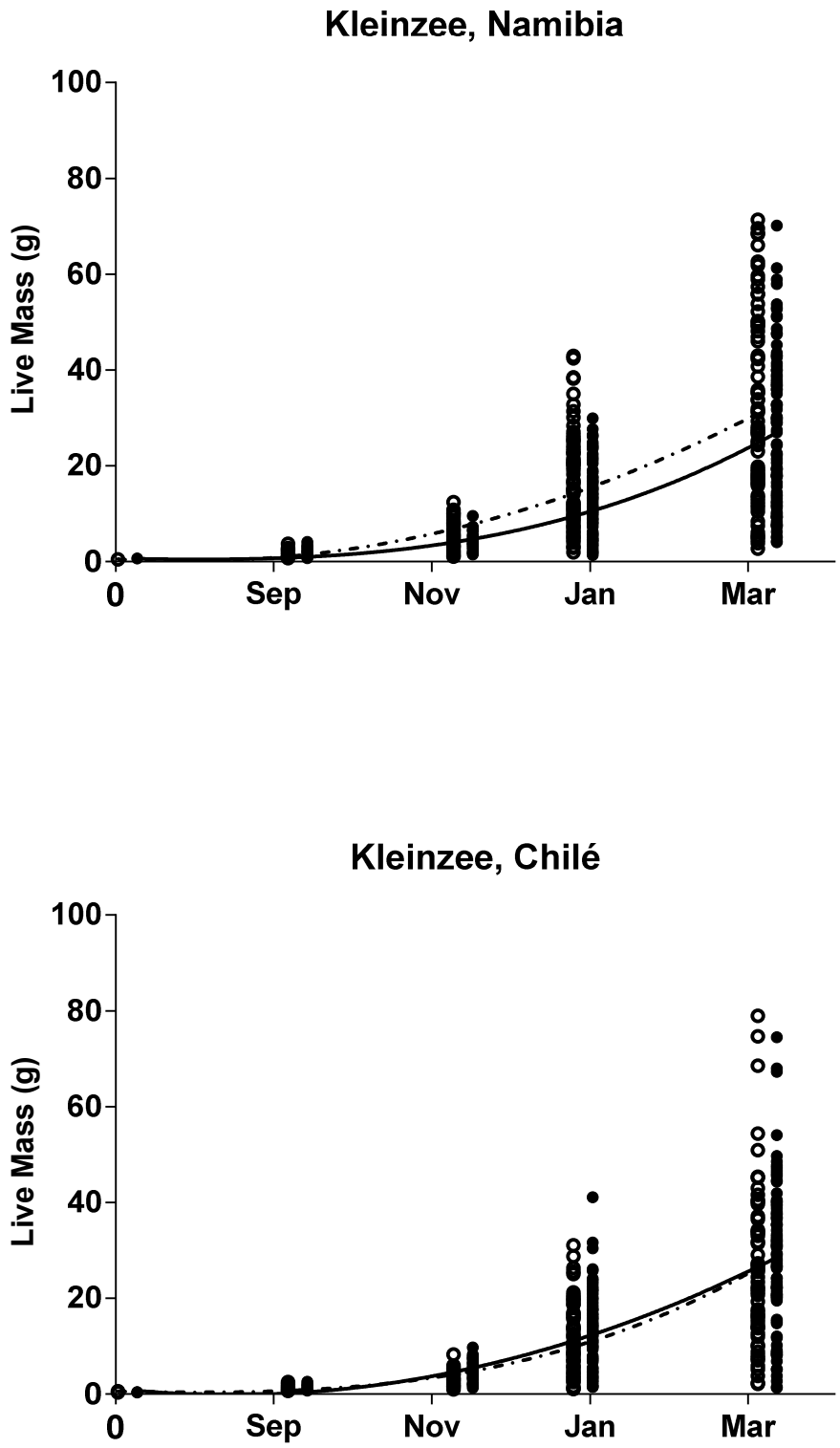


Figure 9: Individual live masses as a function of time for comparison between depth showed that only the Namibian cohort differed between the top (hollow circles ○ and dashed lines) and the bottom cage layers (solid circles ● and solid lines) ($F_{3, 1571} = 22.9$, $p < 0.0001$) at Kleinzee. Live masses at Kleinzee were heavier in the top layer for the Namibian cohort. Polynomial parameter estimates are given in Appendix 6.

3.3.3. Growth rates within the marked sub-sample of individuals

GLS analyses on the marked individuals (measured from Nov 2011 – Jun 2012) revealed that only temperature ($F = 89.84$, coefficient = 0.22, S.E. = 0.023, $t = 9.52$, $p < 0.00001$) and chlorophyll *a* ($F = 5.12$, coefficient = 1.02, S.E. = 0.45, $t = 2.27$, $p = 0.024$) contributed significantly to instantaneous growth rate at Algoa Bay. Temperature and chlorophyll *a* both had a positive effect. Cohort did not have a significant effect on instantaneous growth rate within marked (top and bottom "individuals" from Nov onwards) oysters from Algoa Bay ($N_{Chilé} = 55$, $N_{Namibia} = 101$). Mean instantaneous growth rates for these marked individuals at Algoa Bay were 1.42 % $g.day^{-1}$ and 1.48 % $g.day^{-1}$ for the Chilean and the Namibian cohort respectively. The difference within the Namibian cohort for growth between the top and bottom cage layer (Fig. 7) within all the individuals could not be found in the sub-sample ($N_{Top} = 54$, $N_{Bottom} = 47$).

At Saldanha Bay, only temperature ($F = 318.55$, coefficient = -4.21, $t = -17.89$, $p < 0.0001$) and chlorophyll *a* ($F = 338.53$, coefficient = -3.37, S.E. = 0.18, -18.44, $p = 0.01$) had significant effects on instantaneous growth rate. GLS analyses on the marked individuals ($N_{Chilé} = 102$, $N_{Namibia} = 100$) also showed no difference in percentage growth between the Chilean (1.3 % $g.day^{-1}$) and the Namibian cohort (1.31 % $g.day^{-1}$), even though polynomials (Fig. 6) revealed a difference in absolute mass as a function of time between cohorts.

3.4. Oyster condition

3.4.1. Differences in condition between cohorts

Where differences in DWCI or shell density occurred, Chilean oysters had a higher DWCI while Namibian oysters had a higher shell density within each farm. Within Saldanha Bay, DWCI was highest for the Chilean cohort (Fig. 11; Appendix 7), while shell density was highest for the Namibian cohort (Fig. 10; Appendix 9) within winter (Mann-Whitney $U_{71, 80} = 2006$, $z = -3.11$, $p = 0.002$; and Mann-Whitney $U_{79, 79}=1948$, $z = 3.2$, $p = 0.0009$ for DWCI and shell

density respectively). No difference between cohorts was found for either DWCI or shell density in Saldanha Bay within summer.

Within Algoa Bay, there was no difference in DWCI between cohorts for neither winter or summer, but the Namibian cohort had a higher shell density than the Chilan cohort (Fig. 10; Appendix 9) for both seasons (Mann-Whitney $U_{67, 36} = 336$, $z = 6.01$, $p < 0.0000001$; and Mann-Whitney $U_{80, 49} = 1404$, $z = 2.7$, $p = 0.007$ for winter and summer respectively). Within Kleinzee, there was no difference in DWCI or shell density between cohorts for winter, but the Namibian cohort had a higher shell density than the Chilean cohort (Fig. 10; Appendix 9) within summer (Mann-Whitney $U_{80, 80} = 2261$, $z = 3.2$, $p = 0.00014$).

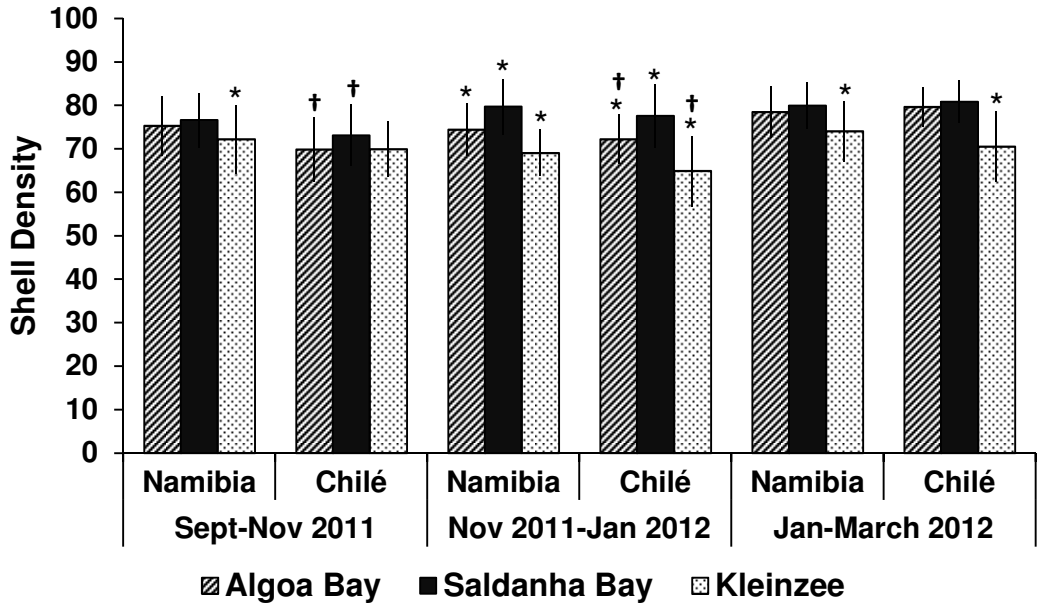


Figure 10: Saldanha Bay oysters had significantly higher shell densities than both other farms from Nov 2011 – Jan 2012 and Kleinzee oysters had significantly lower shell densities than both other farms from Sep 2011 – Mar 2012 (statistics in Appendix 10; * denotes a significant difference from other farms) as is shown here with medians and quartile ranges. From Sep 2011 – Jan 2012, shell densities within the Chilean cohort were often lower than those for the Namibian cohort (statistics in Appendix 9; significant differences are shown with †).

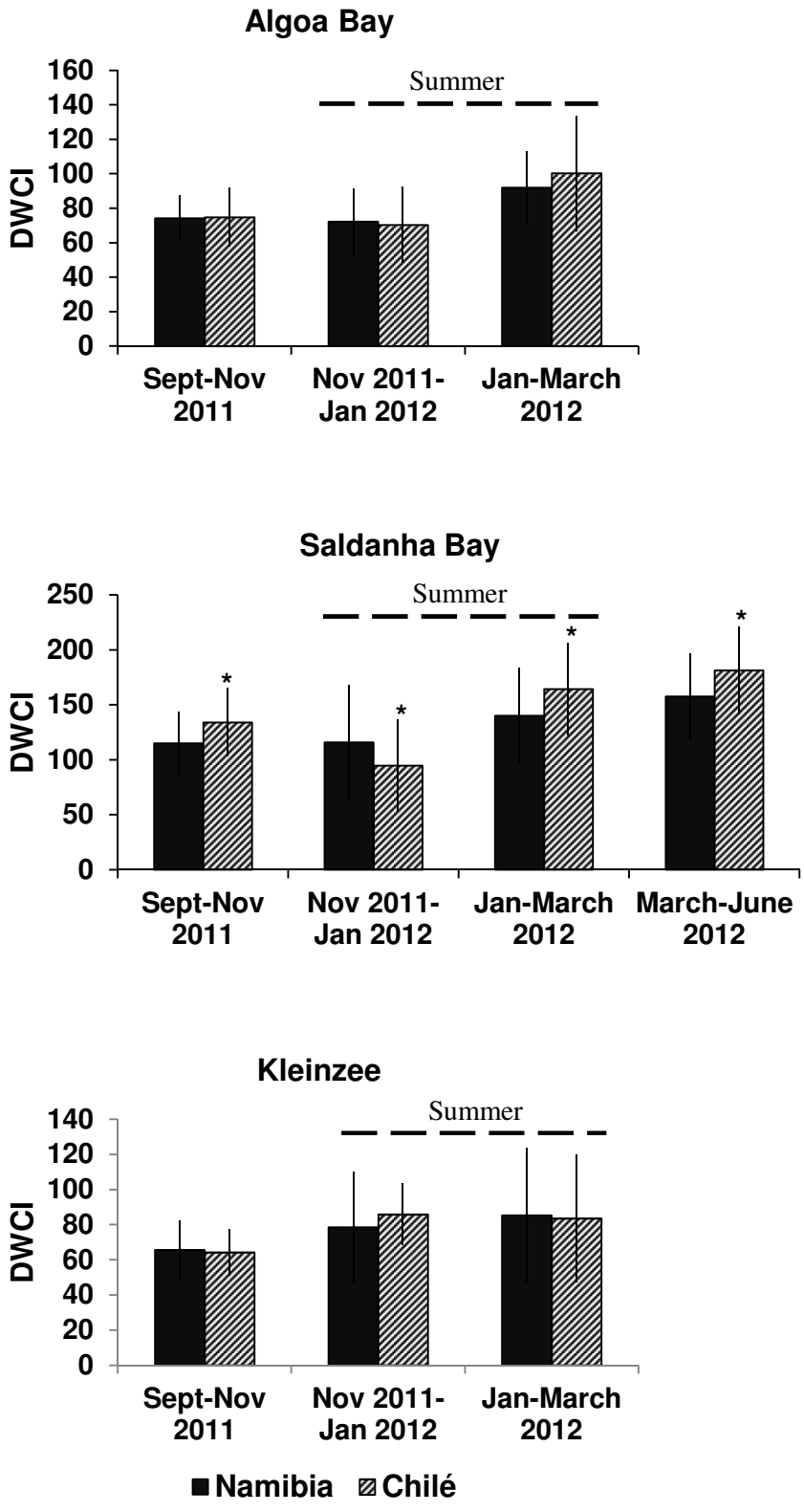


Figure 11: The Chilean cohort often had a higher DWCI than the Namibian cohort (Appendix 7) at Saldanha Bay, as is shown here with medians (with quartile ranges) for each cohort and grow-out period. Where one cohort is marked with an *, its DWCI is significantly higher than the other cohort for that grow-out period.

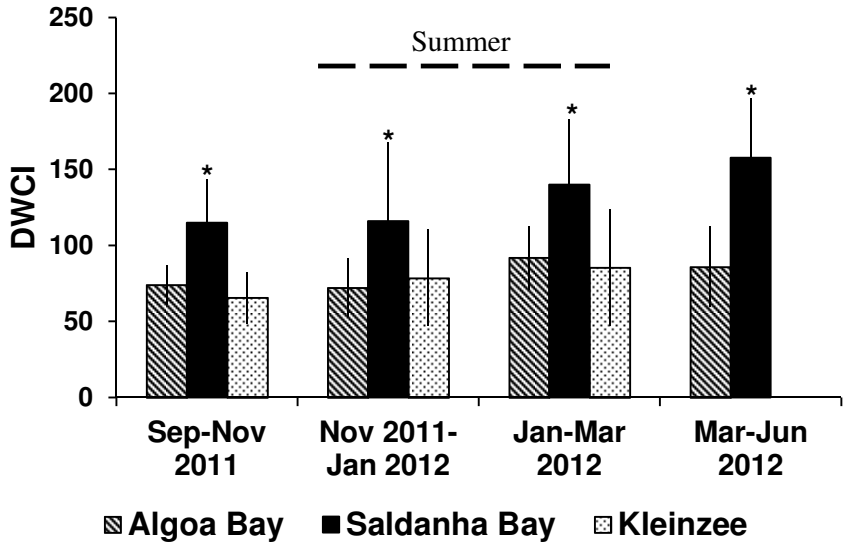


Figure 12: Saldanha Bay oysters had a significantly higher DWCI than both other farms from Sep 2011 – Mar 2012 and also compared to Algoa Bay in Mar – Jun 2012 (statistics in Appendix 8). DWCI medians and quartile ranges are displayed here for the Namibian cohort and * denotes a significant difference from other farms.

3.4.2. Differences in condition between farms

In both winter and summer DWCI was highest for Saldanha Bay oysters within both cohorts ($p < 0.001$ for all pairwise comparisons). No difference was found in DWCI between Algoa Bay and Kleinzee oysters within both seasons for both cohorts.

In winter and within the Namibian cohort, shell densities for oysters at both sea-based farms were higher than those of Kleinzee oysters, but there was no difference between the two sea-based farms (Kruskal-Wallis: $H_{2, 178} = 45.22$, $p < 0.0000001$ for both pairwise comparisons; Fig. 10). Within the Chilean cohort (also in winter), shell densities of Saldanha Bay oysters were higher than those of both other farms, with no difference between Algoa Bay and Kleinzee oysters (Kruskal-Wallis: $H_{2, 156} = 40.71$, $p < 0.001$ for both pairwise comparisons).

Within summer, shell densities for Saldanha Bay oysters were higher than those of Algoa Bay oysters, which in turn had higher shell densities than those of Kleinzee oysters for both cohorts (Kruskal-Wallis: $H_{2, 239} = 87.2$, $p < 0.00003$ for both pairwise comparisons and $H_{2, 208} =$

105.9, $p < 0.0002$ for all pairwise comparisons, for the Namibian and Chilean cohort respectively).

3.5. Mortality

The Chilean cohort had higher overall mortalities than the Namibian cohort within Algoa Bay and Kleinzee (Mann-Whitney $U_{100, 102} = 3793$, $z = -3.14$, $p = 0.00041$; and Mann-Whitney $U_{96, 96} = 3893$, $z = -2.28$, $p = 0.022$ for Algoa Bay and Kleinzee respectively). These differences occurred within the summer period (Appendix 11 – 12), when mortalities were higher than those of the winter period at each farm (Appendix 13). Total percentage mortality showed that the difference between farms was more pronounced than the difference between cohorts, and that Algoa Bay had the highest overall mortality (Table 2).

Within the Namibian cohort, Algoa Bay had a higher overall percentage mortality than Saldanha Bay ($z = 2.82$, $p = 0.014$), with no difference between Kleinzee and either of the sea-based farms (Kruskal-Wallis ANOVA: $H_{2, 295} = 14.68$, $p = 0.0007$). Within the Chilean cohort, Algoa Bay had higher overall percentage mortality than both Saldanha Bay ($z = 5.45$, $p < 0.0000001$) and Kleinzee ($z = 2.74$, $p = 0.019$), while Kleinzee had a higher percentage mortality than Saldanha Bay ($z = 2.64$, 0.025) (Kruskal-Wallis ANOVA: $H_{2, 298} = 42.3$, $p < 0.00001$).

Differences in percentage mortality between the top, middle and bottom positions in the cage occurred only at Algoa Bay within summer and only in the Namibian cohort, when the bottom layer seems to have been less favourable for survival: Within Nov 2011 – Jan 2012, percentage mortality was lower in the middle position than both the top ($z = 3.24$, $p = 0.0037$) and the bottom position ($z = 2.65$, $p = 0.024$) (Kruskal-Wallis ANOVA: $H_{2, 24} = 12.01$, $p = 0.003$), with no difference between the top and bottom position. Within Jan – Mar 2012,

percentage mortality in the bottom position was higher than that of the top position ($z = 2.6$, $p = 0.028$), with no difference between the middle and either the top or bottom layer (Kruskal-Wallis ANOVA: $H_{2, 24} = 7.76$, $p = 0.021$).

The final percentage mortality for each cohort and farm was 21%, 0.3% and 0.6% for Algoa Bay, Saldanha Bay and Kleinzee respectively within the Namibian cohort. Within the Chilean cohort final percentage mortality was 64.4%, 2.2% and 12.7% for Algoa Bay, Saldanha Bay and Kleinzee respectively.

Table 2: The total percentage mortality is displayed here and was calculated from the added number of dead oysters per batch, cohort and farm over the whole study period (Jul 2011 – Jun 2012), as a percentage of the added start numbers of oysters for all batches, grow-out periods for each cohort and farm.

		Algoa Bay	**Saldanha Bay**	**Kleinzee**
Chilé	Total start #	5765	5987	6356
	Total # dead	464	16	128
	Total Mortality (%)	**8.0**	**0.3**	**2.0**
Namibia	Total start #	5959	5965	6338
	Total # dead	155	16	38
	Total Mortality (%)	**2.7**	**0.3**	**0.6**

4. Discussion

More than half of the daily seawater temperatures at Algoa Bay and Kleinzee were above the 19°C limit for optimal oyster growth during this study. Temperature and chlorophyll *a* data indicate a further divergence of high temperature and low abundances of food, compared to Study 1, especially within Algoa Bay. Although chlorophyll *a* measurements at Algoa Bay were higher during summer than in winter, lower chlorophyll *a* measurements coupled with higher seawater temperatures compared to Study 1, resulted in Algoa Bay being a less favourable grow-out environment compared to conditions in Study 1 (Chapter 2). The higher chlorophyll *a* concentrations at Saldanha Baywithin winter suggests that phytoplankton abundance is driven by a factor other than temperature, and the phytoplankton blooms that occurred in winter may have coincided with upwelling within the Benguela current system.

Inspection of the FA nutritional status of Algoa Bay and Saldanha Bay revealed that these farms differed in essential FA composition and the biomarkers for dinoflagellates and diatoms. Both 16:1ω7 and EPA were among the top five FAs which contributed to the FA difference between Algoa Bay and Saldanha Bay, and both these FAs are biomarkers for diatoms and were measured at higher proportions within Saldanha Bay (Parrish *et al*. 2000; Kharlamenko *et al*. 2008). Also among the top five FAs which contributed to the FA difference between Algoa Bay and Saldanha Bay was DHA (especially in January), which was measured at greater proportions within Algoa Bay, compared to Saldanha Bay. Proportions of DHA, SDA and OA were elevated at Algoa Bay compared to Saldanha Bay, and these FAs are biomarkers for dinoflagellates, which occurred at higher proportions at Algoa Bay compared to Saldanha Bay (Parrish *et al*. 2000; Kharlamenko *et al*. 2008).

Combined essential FAs and overall PUFAs were proportionally higher at Algoa Bay compared to Saldanha Bay for the periods sampled and Algoa Bay therefore offers food sources high in nutritional value, despite low abundances of phytoplankton in general. Cassis *et al*. (2011) found that a favourable phytoplankton composition rather than a high phytoplankton

abundance of less favourable phytoplankton species influenced *C. gigas* growth most: growth rate was positively correlated with phytoplankton groups which were in the minority. If Algoa Bay contains a high proportion of phytoplankton species which have optimal FA ratios suited to the metabolic and growth requirements of *C. gigas*, this could promote growth at Algoa Bay (Rico-Villa *et al.* 2006; Pettersen *et al.* 2010). This would help explain why growth at Algoa Bay is generally fast when oysters are not exposed to prolonged high temperatures (Study 1).

Growth differences between the top (1 – 3 m below the water surface) and the bottom (2 – 5 m below the water surface) cage layer, especially at Saldanha Bay, are possibly related to higher temperature-associated phytoplankton distribution levels close to the surface (Toro *et al.* 1999). These temperature differences were very slight and are unlikely to have had differential effects on oyster metabolism and growth between top and bottom cage layers. More likely, the depth effect on growth was due to differences in fouling between the different cage layers (B. Havenga, J. Jonker, and S. Jackson, unpublished data).

Since conditions at Algoa Bay for Study 2 did not encourage oyster growth, resources were allocated to shell growth. In addition, the faster growth at Algoa Bay can be explained by faster growth during the winter, when the temperature range at Algoa Bay (15.6 – 21.2°C) was low enough to prevent stress, but still relatively high (compared to winter temperature at Saldanha Bay, range: 12.1 – 18.7°C) to steer a fast metabolism to accelerate functions involved in shell formation, but not high enough to stop growth. At both Algoa Bay and Kleinzee, it seems that shell growth was given preference over meat growth, since accelerated shell growth is promoted by increased temperatures and stress (Walne and Mann 1975; Shpigel and Baylock 1991). Saldanha Bay reared healthy oysters with a fast dry meat mass growth and high shell densities, probably due to adequate nutritional requirements and a low exposure (14% of all daily mean temperatures from July 2011 – June 2012) to temperatures beyond the 19°C thermal threshold for optimum growth. Oyster growth at Saldanha Bay was driven by cohort in addition

to temperature and chlorophyll *a*, which directly affects metabolism and were the main drivers for oyster growth at Algoa Bay.

Although meat and shell growth fdiffered more between environments than between cohorts, it seems that survival also differed strongly between cohorts relative to environments. These findings are in agreement with Degremont *et al*. (2005) and Evans and Langdon (2006), despite the fact that half-sib oysters were used for this study. The Chilean and Namibian cohort responded differently to stress induced by high and variable temperatures, especially at Algoa Bay and Kleinzee. At temperatures above 19°C, induced gonad maturation increases the utilization of energy reserves (stored glycogen), which is coupled by an increase in metabolic demand and lower dissolved oxygen in warmer waters to fuel the increased metabolism (Moal *et al*. 2007). The combination of high temperatures and low food availability becomes emphasized when filtration rate decreases at temperatures above 20°C, and food supply to meet the demands of increased metabolism becomes further limited (Bougrier *et al*. 1995).

Oysters are well adapted to these stressful summer conditions (Moal *et al*. 2007; Zhang *et al*. 2012), as is seen within the Namibian cohort, which had a third of the mortalities found within the Chilean cohort at Algoa Bay and Kleinzee. Additional stress is caused by sudden increased temperature fluctuations which induce spawning in oysters with mature gonads (Moal *et al*. 2007), which probably occurred at Algoa Bay during mid-January and March when oyster spat were found to have settled onto the large oysters. During these periods, higher mortalities were found within the Chilean cohort compared to the Namibian cohort. Since the highest mortalities were found at Algoa Bay and Kleinzee, the low food availability during high temperatures here might have limited resources available for adjustment to higher temperatures during possible spawning. During Nov 2011 – Jan 2012 the Chilean cohort probably spawned at Saldanha Bay as is reflected by a dip in DWCI, which was not found for the Namibian cohort. If oysters from the Chilean cohort are more sensitive to spawning queues, this would make them more vulnerable to environmental stress.

In addition to differences in mortality, the Chilean cohort often had a higher DWCI than the Namibian cohort. The Namibian cohort had a higher shell density than the Chilean cohort, which contributes to the hardy character of the Namibian cohort, since dense shells protect oysters from the outside environment. The growth differences between cohorts, suggested by polynomials on overall mass gain of individual oysters which included oysters from the top, middle and bottom cage layers (Jul 2011 – Jun 2012) were probably slight. The GLS analyses on individual oysters from the top and bottom cage layers (Nov 2011 – Jun 2012), however, proved that cohort differences in percentage growth rates, were non-significant. It is possible that mortalities within the Chilean cohort at Algoa Bay were higher because of stress associated with reproductive activity during high temperatures. The Namibian cohort responded more in mass as a function of time to differences in depth than the Chilean cohort, and this might be due to a better adjustability to micro-habitats.

DWCI for both cohorts at Saldanha Bay (mean: 134 and 145 for the Namibian and Chilean cohort respectively) and Algoa Bay (mean: 81 and 76 for the Namibian and Chilean cohort respectively) were similar to DWCI-values for Saldanha Bay and Algoa Bay oysters in Study 1 (Chapter 2). DWCI for both cohorts at Kleinzee (mean: 77 and 79 for the Namibian and Chilean cohort respectively) were higher than those in Study 1, and similar to the highest values of oysters reared in suspended pond-culture near two estuaries in northern Portugal (Almeida *et al*. 1997). At these three seawater ponds, oysters planted at 0.4 g (20 mm shell height) had a mean shell height of ~65 mm after 9 months, compared to oysters at Kleinzee which were planted at 0.5g (shell height 16.3 mm) and had reached a mean shell height of 65 mm after 9 months.

The final percentage mortality, for oysters over the whole study, reflects the fact that within Algoa Bay and its exposure to particularly high temperatures during the summer of 2012, 64% of the “harvestable crop” were lost within the Chilean cohort, compared to 22% within the Namibian cohort. At the end of the study period and during the second sharp increase in

temperature in March, oysters were already market size (60 g). It is possible that mortality in oysters at Algoa Bay was promoted by spawning in Nov 2011 – Jan 2012, since DWCI was low for this period and *C. gigas* spat were found to have settled on the bigger oysters (after a few temperature spikes had already occurred for that period).

5. Summary

In addition to constant differences in seawater temperature and phytoplankton abundance between Algoa Bay and Saldanha Bay, these farms possibly differ in phytoplankton composition. Instantaneous growth rate at both Saldanha Bay and Algoa Bay was influenced most by chlorophyll *a* and temperature. The main differences between the Chilean and the Namibian cohort were that the Chilean cohort had higher mortalities within environments that induced physiological stress during summer (Algoa Bay and Kleinzee). Across all environments, the Chilean cohort had the highest DWCI but also the lowest shell density, although the latter was still higher within the Chilean cohort than shell densities at other farms. Although both cohorts displayed favourable phenotypic traits, the fact that the Namibian cohort had a lower loss of production at grow-out environments with relatively high temperature ranges, makes it more suited for commercial purposes in such grow-out environments. Both cohorts are probably equally suited to Saldanha Bay for optimal performance.

6. References

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Appendix 1: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of individual Namibian oysters (Fig. 4) and statistics for the extra sums-of-squares F-test.

	Algoa Bay	**Saldanha Bay**	**Kleinzee**
$F_{8, 6959} = 947.2$, $P < 0.0001$			
B0	0.54 ± 0.31	0.78 ± 0.3	0.64 ± 0.25
B1	0.01 ± 0.01	- 0.12 ± 0.01	- 0.03 ± 0.01
B2	0.001 ± 0.0001	$0.0025 \pm 9.8E^{-5}$	0.0005 ± 0.0001
B3	$3.96\ E^{-8} \pm 2.3\ E^{-7}$	$-3.99\ E^{-6} \pm 2.2\ E^{-7}$	$-1.61\ E^{-7} \pm 3.2\ E^{-7}$
df	2270	2320	2369
r^2	0.91	0.91	0.61
Absolute SS	161688	155899	107548
$S_{y.x}$	8.44	8.2	6.74

Appendix 2: Best-fit polynomial parameter estimates (± 1 S.E.) for oyster dry meat mass growth curves (Fig. 5) and statistics for the extra sums-of-squares F-test.

	Algoa Bay	**Saldanha Bay**
$F_{3, 1601} = 270.4$, $P < 0.0001$		
B0	1.55 ± 0.22	-0.9 ± 0.42
B1	-0.02 ± 0.0023	0.0064 ± 0.0043
B2	$0.0001 \pm 5.5\ E^{-6}$	$6.85\ E^{-5} \pm 9.9\ E^{-6}$
df	774	827
r^2	0.89	0.83
Absolute SS	312.5	1093
$S_{y.x}$	0.64	1.15

Appendix 3: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of individual oysters (Fig. 6) and statistics for the extra sums-of-squares F-test.

	Namibia	**Chlié**
Algoa Bay: $F_{3, 4362} = 46.9$, $P < 0.0001$		
B0	0.64 ± 0.34	0.54 ± 0.27
B1	- 0.03 ± 0.0063	- 0.02 ± 0.006
B2	0.001 ± 2.1 E^{-5}	0.001 ± 2.06 E^{-5}
df	2271	2091
r^2	0.91	0.91
Absolute SS	162779	125153
$S_{y.x}$	8.5	7.74
Saldanha Bay: $F_{3,4622} = 19.83$, $P < 0.0001$		
B0	-1.6 ± 0.35	-0.45 ± 0.27
B1	0.04 ± 0.006	0.03 ± 0.005
B2	0.0008 ± 2.05E^{-5}	0.0007 ± 1.8 E^{-5}
df	2321	2301
r^2	0.9	0.89
Absolute SS	177915	138847
$S_{y.x}$	8.76	7.8
Kleinzee: $F_{3, 4687} = 47.23$, $P < 0.0001$		
B0	1.0 ± 0.28	0.9 ± 0.2
B1	-0.04 ± 0.005	- 0.05 ± 0.005
B2	0.0005 ± 1.9 E^{-5}	0.0006 ± 1.8 E^{-5}
df	2370	2317
r^2	0.6	0.69
Absolute SS	108329	88083
$S_{y.x}$	6.76	6.2

Appendix 4: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of Algoa Bay individual oysters (Fig. 7) and statistics for the extra sums-of-squares F-test.

	Top	**Bottom**
Namibia: $F_{3, 1530} = 72.2$, $P < 0.0001$		
B0	0.82 ± 0.55	0.5 ± 0.63
B1	- 0.03 ± 0.01	- 0.01 ± 0.01
B2	0.001 ± 3.58 E^{-5}	0.001 ± 3.65 E^{-5}
df	771	759
r^2	0.93	0.9
Absolute SS	62214	61606
$S_{y.x}$	8.98	9.01
Chilé: $F_{3, 1436} = 5.4$, $P = 0.001$		
B0	1.02 ± 0.67	0.3 ± 0.49
B1	- 0.05 ± 0.01	- 0.003 ± 0.0099
B2	0.001 ± 4.1 E^{-5}	0.001 ± 3.4 E^{-5}
df	726	710
r^2	0.9	0.92
Absolute SS	65985	45853
$S_{y.x}$	9.5	8.04

Appendix 5: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of Saldanha Bay individual oysters (Fig. 8) and statistics for the extra sums-of-squares F-test.

	Top	**Bottom**
Namibia: $F_{3, 1586} = 36.8$, $P < 0.0001$		
B0	-0.87 ± 0.57	-2.05 ± 0.58
B1	0.04 ± 0.01	0.06 ± 0.01
B2	0.0008 ± 3.6 E^{-5}	0.0006 ± 3.2 E^{-5}
df	794	792
r^2	0.9	0.905
Absolute SS	71770	55652
$S_{y.x}$	9.5	8.38
Chilé: $F_{3, 1580} = 3.47$, $P = 0.02$		
B0	-1.29 ± 0.57	- 0.47 ± 0.49
B1	0.034 ± 0.01	0.03 ± 0.0095
B2	0.0007 ± 3.1 E^{-5}	0.0007 ± 3.18 E^{-5}
df	797	783
r^2	0.9	0.89
Absolute SS	54150	52317
$S_{y.x}$	8.24	8.17

Appendix 6: Best-fit polynomial parameter estimates (± 1 S.E.) for live mass growth curves of Kleinzee individual oysters (Fig. 9) and statistics for the extra sums-of-squares F-test.

	Top	**Bottom**
Namibia: $F_{3, 1571} = 22.89$, $P < 0.0001$		
B0	0.56 ± 0.46	1.3 ± 0.42
B1	- 0.03 ± 0.01	- 0.05 ± 0.008
B2	0.0005 ± 3.7 E^{-5}	0.0005 ± 2.98 E^{-5}
df	788	783
r^2	0.64	0.66
Absolute SS	44209	28587
$S_{y.x}$	7.49	6.04
Chilé: $F_{3, 1536} = 0.5$, $P = 0.68$		
B0	0.88 ± 0.34	1.16 ± 0.4
B1	- 0.05 ± 0.0072	- 0.053 ± 0.008
B2	0.0005 ± 2.85 E^{-5}	0.0006 ± 2.88 E^{-5}
df	755	781
r^2	0.65	0.71
Absolute SS	22559	26171
$S_{y.x}$	5.47	5.79

Appendix 7: Z-values (with p-values in parenthesis) from a Mann-Whitney U DWCI comparison between cohorts are displayed for each farm and grow-out period. Significant differences (typed in bold) were only found within Saldanha Bay (p-values typed in bold). The Chilean cohort had a significantly higher DWCI for all grow-out periods (z-values with a minus sign indicate a higher DWCI for the Chilean cohort) except for Nov 2011 – Jan 2012 when DWCI was higher for the Namibian cohort.

	Algoa Bay	**Saldanha Bay**	**Kleinzee**
Sep – Nov 2011	$U_{36,36} = 612$	$U_{40,40} = 587$	$U_{40,40} = 785$
	0.4 (0.7)	**-2.04 (0.04)**	-0.14 (0.89)
Nov 2011 – Jan 2012	$U_{40,39} = 750$	$U_{38,39} = 516$	$U_{40,40} = 618$
	0.29 (0.77)	**2.3 (0.02)**	-1.75 (0.08)
Jan – Mar 2012	$U_{40,9} = 127$	$U_{40,40} = 531$	$U_{40,40} = 763$
	-1.4 (0.18)	**-2.6 (0.01)**	-0.35 (0.73)
Mar – Jun 2012		$U_{31,40} = 323$	
		-3.4 (0.0006)	

Appendix 8: Z-values for between-farm differences in DWCI are displayed here with p-values in parenthesis are displayed here for the Namibian cohort, with significant z- and p-values typed in bold. Sample sizes ranged from 38 – 41 for each farm for all grow-out periods except the last one (Mar – Jun 2012), when 31 oysters were measured for both Algoa Bay and Saldanha Bay. The Chilean cohort showed the same trend for between-farm comparisons.

		Saldanha Bay	**Kleinzee**
Sep – Nov 2011	**Algoa Bay**	**5.76 (< 0.0000001)**	2.1 (0.11)
$H_{2,116} = 69.7$	**Saldanha Bay**		**8.1 (< 0.0000001)**
Nov 2011 – Jan 2012	**Algoa Bay**	**6.2 (< 0.0000001)**	1.15 (0.75)
$H_{2,118} = 42.6$	**Saldanha Bay**		**5.02 (0.000002)**
Jan – Mar 2012	**Algoa Bay**	**6.56 (< 0.0000001)**	1.47 (0.43)
$H_{2,120} = 73.0$	**Saldanha Bay**		**8.02 (< 0.0000001)**
Mar – Jun 2012	**Algoa Bay**	**6.74 (< 0.0000001)**	
$U_{31,31} = 1$			

Appendix 9: Z-values from Mann-Whitney U test shell density differences between cohorts within each farm and grow-out period are displayed, with p-values in parenthesis. Where significant differences between cohorts occurred, the Namibian cohort usually showed a higher shell density than the Chilean cohort (where the Namibian cohort has a higher shell density z-values have a positive sign). Significant differences between cohorts are typed in bold.

	Algoa Bay	**Saldanha Bay**	**Kleinzee**
Sep – Nov 2011	**3.9 (0.00006)**	**3.21 (0.001)**	1.1 (0.29)
(Winter)	$U_{36, 36} = 301$	$U_{40, 40} = 466$	$U_{40, 40} = 688$
Nov 2011 – Jan 2012	**2.49 (0.012)**	1.52 (0.13)	**3.41 (0.00052)**
(Summer)	$U_{40, 39} = 526$	$U_{38, 39} = 608$	$U_{40, 40} = 445$
Jan – March 2012	-1.66 (0.1)	-1.36 (0.17)	1.8 (0.07)
(Summer)	$U_{40, 10} = 131$	$U_{40, 40} = 658$	$U_{40, 40} = 612$
March – June 2012		**5.03 (< 0.0000001)**	
(Winter)		$U_{31, 40} = 186$	

Appendix 10: Z-values are displayed for shell density comparisons between farms with p-values in parenthesis. Since both cohorts showed the same trend for between-farm comparisons for each grow-out period (Fig. 10), statistics are displayed for the Namibian cohort only.

		Saldanha Bay	**Kleinzee**
Sep – Nov 2011 (Winter)	**Algoa Bay**	0.88 (1.0)	**4.14 (0.005)**
Kruskal-Wallis ANOVA:	**Saldanha Bay**		**3.15 (0.0001)**
$H_{2, 116} = 18.85$			
Nov 2011 – Jan 2012	**Algoa Bay**	**3.91 (0.00028)**	**3.65 (0.0008)**
(Summer)			
$H_{2, 118} = 56.74$	**Saldanha Bay**		**7.53 (< 0.0000001)**
Jan – March 2012	**Algoa Bay**	2.06 (0.12)	**3.66 (0.00075)**
(Summer)			
$H_{2, 120} = 33.7$	**Saldanha Bay**		**5.73 (< 0.0000001)**
March – June 2012	**Algoa Bay**	-1.18 (0.24)	
(Winter)			
Mann-Whitney $U_{31, 31} = 1$			

Appendix 11: Medians for the mortality (%) for all batches within each cohort, farm and grow-out period are displayed with lower and upper quartiles in parenthesis. Mortality (%) was calculated as a the number of dead oysters per batch at the end of each grow-out period, as a percentage of the total starting number of oysters per batch at the beginning of that grow-out period. The batch sample sizes for cohort within each farm and grow-out period is 24, except for the last grow-out period (Mar – Jun 2012) when only 4 batches were planted for each cohort at the two sea-based farms. Differences between cohorts only occurred within summer at each farm (Table 13), when the highest mortalities overall mortalities were highest and significantly higher mortalities compared to other farms are typed in bold (statistics given in Table 14).

		Algoa Bay	**Saldanha Bay**	**Kleinzee**	
					Season
Jul - Sep 2011	Namibia	0 (0-0)	0 (0-0)	0 (0-0)	Winter
	Chilé	0 (0-0)	0 (0-0)	0 (0-0)	Winter
Sep - Nov 2011	Namibia	0 (0-0)	0 (0-0)	0 (0-0)	Winter
	Chilé	0 (0-0)	0 (0-0)	0 (0-0)	Winter
Nov 2011 - Jan 2012	Namibia	**9.5** (4.9-17.5)	0 (0-2.5)	0 (0-2.0)	Summer
	Chilé	**34.2** (27.8-52.5)	0 (0-0)	0 (0-2.0)	Summer
Jan - Mar 2012	Namibia	**1.7** (0-5.5)	0 (0-0)	**2.6** (0-5.0)	Summer
	Chilé	**13.4** (7.9-21.1)	0 (0-3.3)	**8.2** (5.2-12.5)	Summer
Mar - Jun 2012	Namibia	0 (0-0)	0 (0-0)		Winter
	Chilé	0 (0-3.1)	0 (0-0)		Winter

Appendix 12: Mann-Whitney U test z- and p-values are given for differences in percentage mortality between cohorts within summer and winter. Bold values indicate significant differences and a negative sign for z-values indicate that the Chilean cohort had higher mortalities than the Namibian cohort.

	Algoa Bay	**Saldanha Bay**	**Kleinzee**
Winter	-1.4 (0.74)	0.85 (0.73)	-1.41 (0.73)
	$U_{52, 52} = 1300$	$U_{52, 52} = 1299$	$U_{48, 48} = 1104$
Summer	**-5.77 (< 0.0000001)**	-0.79 (0.57)	**-3.01 (0.0035)**
	$U_{48, 50} = 390.5$	$U_{47, 48} = 1050.5$	$U_{48, 48} = 756$

Appendix 13: Z- and p-values (in parenthesis) are displayed for comparisons of percentage mortality between summer and winter for each cohort and farm. Significant differences are typed in bold, and a negative sign for z-values indicate that mortalities were higher in summer.

		Algoa Bay	**Saldanha Bay**	**Kleinzee**
		Summer	Summer	Summer
Namibia	Winter	**-6.9 (< 0.0000001)**	-1.8 (0.29)	**-5.4 (0.000035)**
		$U_{52, 48} = 416$	$U_{52, 47} = 1071$	$U_{48, 48} = 600$
Chilé	Winter	**-8.93 (< 0.0000001)**	-3.15 (0.057)	**-6.54 (< 0.0000001)**
		$U_{52, 50} = 57$	$U_{52, 48} = 972$	$U_{48, 48} = 381$

Chapter 4

Flexibility of feeding-rate and the size of the feeding organs within a single Pacific oyster (*Crassostrea gigas*) cohort grown at two environmentally distinct sites

ABSTRACT

Differences in clearance rates and the size of gills and labial palps were investigated for the Pacific oyster, *Crassostrea gigas* from a single cohort obtained from a Namibian hatchery, grown-out in long-line culture at two distinct environments in South Africa: sites include a Saldanha Bay sea-based farm within a cool, nutrient-rich upwelling system, and the warmer, but less productive Algoa Bay sea-based farm. Oysters exhibit phenotypic plasticity with regard to gill and palp sizes and their adjustment to different environments to maximize food intake. Relatively large gills maximize intake when seston quantity and food availability is low. The first hypothesis states that oysters grown in Algoa Bay would have higher clearance rates to compensate for their oligotrophic environment. The second hypothesis states that oysters at Algoa Bay have relatively larger gills than those of Saldanha Bay. Both hypotheses were confirmed: In a flow-through clearance rate experiment using a standardized phytoplankton mix at 4 600 cells.ml^{-1}, and in the dissection of experimental oysters grown out at both farms to establish their relative gill and labial palp sizes. Oysters had higher clearance rates in Algoa Bay than in Saldanha Bay (Mann-Whitney $U_{10, 9} = 10$, $z = -2.82$, $p = 0.003$), but this was not related to gill size. Oysters from Algoa Bay also had a higher gill: palp ratio than oysters from Saldanha Bay (gill: palp wet mass ratio: Mann-Whitney $U_{31, 31} = 343$, $z = -1.93$, $p = 0.054$; gill: palp surface area ratio: Mann-Whitney $U_{31, 31} = 236$, $z = -3.4$, $p = 0.0004$). Oysters from Algoa Bay displayed high clearance rates in an environment different to their own. More knowledge is needed on other possible physiological functions of compensatory feeding before we can know whether oysters from Algoa Bay are more efficient feeders.

1. Introduction

Pacific oysters (*Crassostrea gigas*) from the same cohort, grown-out at different environments in South Africa, differ in growth rates between the different environments (Chapter 2). Oysters from the warmer, but comparatively nutrient-poor sea-based Algoa Bay farm on the East coast displayed faster growth rates in whole oyster mass than oysters grown at the cooler, and comparatively nutrient-rich, sea-based Saldanha Bay farm. The Benguela current with its strong upwelling cycles on the West Coast is responsible for the nutrient-rich environment at Saldanha Bay. Algoa Bay is situated in the warmer Agulhas current system which lacks the strong upwelling of the Benguela current. The same phenomenon found for oysters grown at Algoa Bay, with high growth rates despite low chlorophyll *a* concentrations, was also found in the Thau lagoon in France (Dupuy *et al*. 2000). Because growth rate is correlated with feeding rate (Bayne *et al*. 1999) which is in turn correlated with oyster gill and labial palp sizes (Honkoop *et al.* 2003; Dutertre *et al.* 2007), and because oysters under similar conditions with regard to food abundance at the Thau lagoon displayed high clearance rates, we assessed the feeding-rate and morphology of gills and labial palps for oysters from Algoa Bay and Saldanha Bay to explain reasons for geographical differences in growth rates.

Oysters are suspension-feeding bivalves with heterorhabdic pseudolamellibranch gills, where lamellibranchs refer to organisms which feed by using their gills to obtain suspended food from the incoming water current, and this includes all bivalves (Gosling 2003). Bivalves, particularly the Pacific oyster, exhibit considerable phenotypic plasticity in morphological and physiological traits involved in feeding behaviour (Barillé *et al.* 2000; Honkoop *et al*. 2003; Bayne 2004; Ward and Shumway 2004; Benninger *et al*. 2008). Plasticity permits animals of the same genotype to develop differential physiological and morphological responses to the environment (West-Eberhard 1989). This short-term adaptability of sessile bivalves improves their selection efficiency through their feeding

organs and processes to provide them with a high quality diet, with optimal nitrogen to carbon ratios and proportions of essential nutrients, out of the substantially variable seston quantity and quality (Ward *et al*. 2003; Benninger *et al*. 2008; Bayne 2009). Selection efficiency is pre-ingestive; through preferential retention of particles on the gills and on the labial palps at which pseudofaeces particles of low nutritional value are ejected, and also post-ingestive through variable absorption favouring organic rich particles (Shumway *et al*. 1985; Ward and Shumway 2004).

Gills of *Crassostrea gigas* can sort particles into two groups; live, nutrituous particles of phytoplankton, bacteria and particulate organic matter (POM) which are transported to the labial palps along dorsal tracts on the gills, and dead detritus particles and particulate inorganic matter (PIM) which are transported along ventral tracts on the gills and ejected (Ward *et al*. 1997; Ward and Shumway 2004). Particles transported along the ventral tracts are bound in high-viscosity mucus strings, while particles transported along the dorsal tracts are bound in mucus slurry and are moved by cilia at a faster rate than particles in the ventral tracts (Ward *et al*. 2003). Labial palps are feeding organs that direct food particles to the mouth (see below) and their relative sizes can also be modified to optimize particle selection (Barillé *et al.* 2000; Honkoop *et al*. 2003). In *C. gigas*, relatively large gills improve selection efficiency when seston concentrations are low, while large palps improve the selection capacity per unit time when seston concentrations are high and food quality is low and (Barillé *et al*. 2000; Honkoop *et al*. 2003). Lighter oysters have a relatively large gill to palp size ratio, when compared to heavier oysters (Honkoop *et al*. 2003), and in addition to the fact that smaller oysters have higher metabolic rates, this could explain faster clearance rates in younger oysters at normal seston concentrations.

Feeding rates in bivalves can be increased through gill modifications in two ways: 1) through increased clearance rates facilitated by increases in gill surface area when more

particles are selectively retained per unit time (Barillé *et al.* 2000; Honkoop *et al*. 2003), 2) and through increased filtration rates or pumping rates by adjusting the rate of ciliary movement on the gill and through the movements of valve opening (Gosling 2003; Ward *et al*. 2003; Ward and Shumway 2004). Clearance rate in oysters refer to the combined function of filtration rate and retention efficiency at the gills, which can be influenced by current speed and the degree of valve-opening, and particle-size respectively (Bayne 2009). In *Crassostrea gigas*, the rate of gill-ciliary movement is naturally high and remains relatively constant for increases in particle concentration (Ward *et al*. 2003). Increased feeding efficiency is achieved through the combination of a high clearance rate and selection efficiency, which is enhanced in *C. gigas* by the combined selection capacity of two sets of feeding organs, namely the gills and labial palps, and additional pathways (dorsal tracts) for particle transport to the labial palps (Ward *et al*. 2003).

Gosling (2003) describes the morphology of feeding organs of oysters in detail and it can be summarized as follows: each gill constitutes two demibranchs which are a series of V-shaped ciliated filaments (composed of two lamellae arms). Two sets of V-shaped filaments are stacked to shape a W (of which only the outer lamellae arms are exposed to the incoming current), and each W is arranged adjacent to another at either side and they are joined together by intrafilament junctions. This specific gill type is termed a eulamellibranch gill, and oysters have pseudo-eulamellibranch gills because these junctions are less expansive than those of lamellibranchs. The filaments are heterorhabdic in oysters and are composed of two different types for different functions, where one type is situated in the troughs created by folds (plica) of the gill surface. Cilia on the filaments create water currents to move particles to selection sites and to transport particles along different tracts on the gills. On the anterior side of the oyster, at the end of each gill are two triangular-shaped labial palps with ciliated

and grooved inner surfaces for particle sorting, from where selected particles are directed to the mouth.

Fast growth in Pacific oysters is achieved by a high metabolisable energy intake, a low energy expenditure on maintenance relative to growth and a low metabolic cost of growth through higher metabolic efficiencies (Bayne *et al*. 1999; Bayne 2002; Bayne 2004). Energy intake is dependent on the following physiological efficiencies: clearance rate, proportion of particles rejected, selection efficiency, absorption efficiency and metabolic efficiency (Bayne 2004). Energy intake is maximized by a high abundance of phytoplankton and other organic suspended particles with optimal proportions of nutritional phytoplankton species within a size range which are efficiently retained by oysters, combined with high selection efficiency and clearance rates (Shumway *et al*. 1985; Barillé *et al*. 1993; Bougrier *et al*. 1997; Cranford 1998; Cognie *et al*. 2001). High clearance rates maximize the number of particles retained on the gills per unit time, and high selection efficiencies improve the capacity of oysters to select particles of high nutritional value for ingestion. In *C. gigas*, clearance rate and selection efficiency can be optimized under suboptimal and unfavourable environmental conditions to a certain extent, beyond which their functionality becomes impaired (Bougrier *et al*. 1995; Barillé *et al*. 1997).

Clearance rate in *C. gigas* increases with increased temperature (from 5°C to 20°C) and current speed (Walne 1972; Bougrier *et al*. 1995). Clearance rate decreases with increasing oyster size and seston loads above 100 $mg.l^{-1}$ and an unfavourable detritus: organic content ratio (Bougrier *et al*. 1995; Barillé *et al*. 1997). Selection efficiency at the gills is directly related to seston quantity and inversely related to seston quality for particles small enough to enter the gill principal filament acceptance tracts, situated in the plical troughs (gill folds) (Benninger *et al*. 2008). Selection efficiency at the labial palps is also inversely related to seston quality at an intermediate seston quantity, above which selection efficiency by the

labial palps decreases (Benninger *et al*. 2008). Selection efficiency is also influenced by filtration rate, and is optimal at a relatively low filtration rate of seston rich in organic matter (Bayne 2009). Filtration rate in turn is positively influenced by the seston load up to a certain threshold (Barillé *et al*. 1997).

Selection of organic particles by *C. gigas* is not only dependent on mechanical selection based on size and shape, and active phytoplankton species-specific selection can be related to essential nutrients and their ratios, such as the carbon: nitrogen ratio in phytoplankton cells (Bougrier *et al*. 1997; Cognie *et al*. 2001; Ward and Shumway 2004; Bayne 2009*)*. As was found for *C. virginica*, *C. gigas* oysters probably select for specific phytoplankton species through the recognition of carbohydrates bound to phytoplankton cell surfaces, by lectins (sugar-binding proteins) within the mucous covering of the gills and labial palps (Espinosa *et al.* 2009, 2010). Since phytoplankton species differ in nutritional value, specific species are possibly selected by oysters due to their fatty acid ratios in addition to selection based on nitrogen ratios. Small particles < 4 µm are usually retained with a low efficiency in bivalves, and they are mostly sieved through the interfilamentar openings of the gills without retention (Haven and Morales-Alamo *et al*. 1970; Shumway *et al.* 1985; Barillé *et al*. 1993; Ropert and Goulletquer 2000). The Pacific oyster, however, can adjust particle retention efficiencies in response to food quantity, and the threshold for particles retained can therefore be increased or decreased over short periods of time (Barillé *et al*. 1993).

Since oysters grown at Algoa Bay had higher growth rates (whole oyster mass gain.day^{-1}) than oysters grown at Saldanha Bay despite chlorophyll *a* concentrations being 3.8 times higher in Saldanha Bay during study 1 (Chapter 2), this study aimed to examine and compare clearance rates in oysters between these environments. Fast growing oysters at Algoa Bay might be more efficient feeders. Adjusted to their relatively phytoplankton-poor environment, Algoa Bay-grown oysters might have developed the capacity to maximize

intake of available food particles. To maximize clearance rate in environments with low phytoplankton abundances, oysters would need relatively large gills to retain more particles per unit time to promote high clearance rates. The first hypothesis would therefore state that clearance rates in Algoa Bay oysters are higher compared to those in Saldanha Bay oysters. Due to Algoa Bay's low food abundance, the second hypothesis states that oysters from Algoa Bay have relatively larger gills and possibly a higher gill to palp ratio compared to oysters from Saldanha Bay, since oysters enlarge their labial palps in response to a relativly high food abundnce.

If oyster clearance rates are higher at Algoa Bay, it is also possible that high clearance rates at Algoa Bay may have been facilitated externally by higher temperatures or a higher current speed. Clearance rates were compared for both oyster groups at the same temperature, water flow rate and phytoplankton composition and concentration. First, a cross-over closed system incubation experiment was performed, in which clearance rates were measured in closed systems containing natural seawater, harvested either from Algoa Bay or from Saldanha Bay. An open flow-through experimental system supply oysters with a constant phytoplankton concentration, which eliminates the effect of food concentration on clearance rates (Riisgard 2001; Gosling 2003; Cranford *et al.* 2011), and was used for the second set of feeding experiments. Seawater enriched with a cultured phytoplankton mix at a standard concentration was pumped through oyster chambers at a constant rate, which was high enough to prevent the recirculation of incoming water currents (containing suspended particles) already filtered by the oysters (Ren *et al*. 2000; Filgueira *et al.* 2006). Gill and palp sizes (masses and areas) were compared within the same cohort of oysters from Algoa Bay and Saldanha Bay.

2. Materials and methods

2.1. Incubation clearance rates

Cross-over field incubation experiments to test particle depletion by oysters from Algoa Bay and Saldanha Bay in a closed system with natural seawater, were conducted at both sites during autumn (March – April 2012). The experiment was repeated once at each site under similar light intensity conditions as the previous experiment. At both Algoa Bay and Saldanha Bay, fresh seawater was harvested around the oyster cages at culture depth (~1 m) three to ten hours before the experiment, and cooled to 18°C while being kept in a dark place. Simultaneously, oysters from the "Chilé" cohort imported from Tongoy in Chilé and planted for grow-out at both Algoa Bay and Saldanha Bay in July 2011 (Chapter 3), were collected at both farms from the middle position in the cage (1.7 – 2.3 m below the sea surface).

2.1.1. Experimental oysters

Oysters selected at both farms were already 11 months old and were from the same long-line cages as those used in Chapter 3. These oysters were cleaned of epifauna with a paint scraper, and weighed live to size-match experimental oysters within a narrow range (out of oysters selected at 60 – 80 g). At the end of the last grow-out period before the experiment (in March – April 2012), oysters from the Chilean cohort from both Algoa Bay and Saldanha Bay were selected to match experimental oysters within a ~5 g size range.

2.1.2. Oyster transport

For the Algoa Bay field experiment, oysters from Saldanha Bay were kept on ice in closed styrofoam boxes (340 mm width x 690 mm breadth x 180 mm depth) for five days during transport and put into Algoa Bay water for ~8 hours. These oysters were then transferred to ice again along with the Algoa Bay oysters ~5 hours prior to the experiment. For the Saldanha Bay field experiment, Algoa Bay oysters were on ice for five days during

transport, while the Saldanha Bay oysters were on ice for one day. Both groups were then put into seawater at the Fisheries Research and Development Institute of the Department of Agriculture, Forestry and Fisheries, based in Sea Point, Cape Town for one day until 1– 5 hours before stabilization and the experiment within water from Saldanha Bay. This Sea Point research facility was used for the Saldanha Bay field experiment for which seawater from Saldanha Bay was used. This facility was used because of its relatively close proximity to Saldanha Bay, because it is fully equipped and after transport both oyster groups could stabilize in seawater distinct from both origin environments.

2.1.3. Experimental design and protocol

Two styrofoam fish boxes (340 mm width x 690 mm breadth x 180 mm depth) were used as water-jackets for temperature control. Each had an outflow ~130 mm from the bottom and was placed in series so that outflow from the top box flowed into the bottom box. The outflow from the bottom box flowed into a reservoir containing a submersible pump. Water from the reservoir was pumped through a custom-made chiller accurate to 1°C, and into the top box through hosepipe tubing (20 mm diameter). Each water jacket contained a total of six cylindrical 2 l polyethylene oyster chambers each (radius: 80.6 mm); three chambers for oysters from each farm. Each oyster chamber contained an air stone (~20 mm long) connected to a Tetra APS 400 aquarium air pump (D-49304 Melle, Germany) to aerate and mix the water. Air bubbles within each chamber were equally strong.

Oysters were stabilized within the chambers to the experimental water temperature (set to reach 18°C before the onset of the stabilization period) for 1– 2 hours just prior to each experiment. This temperature was chosen because it falls within the range of summer temperatures at both farms (Chapter 2), and therefore oysters were not subjected to an experimental temperature which differed drastically from temperatures exposed to

previously. Temperatures between containers were also measured with a glass thermometer during and before these experiments and also during the flow-through experiments (see below). During this period oysters were observed to determine whether all oysters had their valves open to allow feeding, and oysters that were closed were replaced with other weighed and cleaned oysters. This was also done to ensure that oysters were not still sleeping due to tidally programmed circadian patterns. After stabilization, which refers to a short period of acclimitization, oysters were removed from the water and placed in a specific order next to their chambers. The water in each chamber was replaced with another batch of harvested seawater, which was brought to 18°C by chilling of the water-jacket, and by adding warmer tap water when necessary.

As soon as the required temperature was reached, oysters were lightly cleaned again with seawater and a nailbrush to remove particles that have settled on them during the stabilization period. They were then placed into their respective chambers, and simultaneously, the water was lightly mixed and a 140 ml water sample was taken for an initial concentration measurement for each chamber. Each oyster chamber contained one oyster. The experiments commenced when all initial samples were taken, and lasted 1 hour and 30 minutes at both sites, after which another sample was taken to represent the end concentration of each chamber.

2.1.3. Water sample analyses

Samples were analyzed with a Multisizer™ 4 Beckman Coulter counter® (Fullerton, USA) with a 280 µm aperture tube, covering a particle diameter range of 7.5 – 168 µm (divided into 400 size bins). The coulter counter measured 40 ml of the 140 ml sample by counting particles in 2 ml aliquots for 20 replicates, for which the particles in each size bin were added for all the replicates, and divided by two times the number of replicates to obtain

the number of particles for 1 ml. Beginning and end concentrations were compared for each chamber for which percentage particle reduction was calculated as: $((C_{initial} - C_{end})/C_{initial}) \times 100$ (Filgueria *et al.* 2006).

2.2. Flow-through clearance rates

For clearance rate experiments a flow-through system was built, where phytoplankton-enriched seawater was pumped by a submersible pump through a chiller and upwards to a pressure reservoir, which fed enriched and chilled seawater to oyster containers through gravity-flow.

2.2.1 Oyster selection

Within June 2012 (winter), the individually-marked 13-month old oysters from the "Namibian" cohort, imported from Namibia as spat and planted for grow-out in July 2011 in long-line cages (Chapter 3), were harvested from Algoa and Saldanha bays three days before the experiment. At both farms oysters were harvested within the Namibian cohort from the same cage-layer (top position in the cage, ~1.5 m below the sea surface). They were then kept in on-farm holding tanks for two days between length and weight measurements, using digital calipers (accurate to the nearest 0.1 mm) and the Denver MAXX 120 g balance used in Chapters 2 and 3 (accurate to 0.01 g). Sea and holding tank temperatures ranged from 15 – 17°C and 12 –15°C for Algoa Bay and Saldanha Bay respectively during the last few weeks at each site.

Temperature is directly related to clearance rates in *C. gigas* up to a certain point (Bougrier *et al*. 1995, Ren *et al.* 2000), and therefore a single standard experimental temperature range of 14 – 15°C was used, as close as possible to winter seawater temperatures at both farms. Two weeks prior to harvesting (from the 20 May – 2 June 2013), the local seawater temperature range was 16.2 – 19.4°C (median: 16.5°C) and 12.3 – 14.5°C

(median 13.2°C) at Algoa Bay and Saldanha Bay respectively. For each experimental repeat, a new set of oysters from both Algoa Bay and Saldanha Bay were selected within a live mass range of ~5 g, to compare clearance rates between oysters from a different origin. Experimental oysters for all repeats ranged from 78.1 – 121.7 g (87 – 117 mm).

2.2.2. Experimental setup

Prior to the start of the experiment, water was pumped out of a 200 l polyethylene reservoir tank through a chiller (Hailea® HC-250 A; Guangzhou, China) set at 14°C, and back into the reservoir tank. Outflow from the pump was divided by a T-joint to diverge into two pipes, and prior to the experiment, outflow of one pipe flowed back into the reservoir tank, while the other pipe led water through the chiller and back into the reservoir to keep the reservoir cool. During the experiment, one pipe supplied the pressure reservoir and oyster chambers with chilled water, while the other pipe circulated water through the chiller and back into the reservoir. The 200 l reservoir tank was fitted with an aquarium tank propeller (JVP 101 with a 3 000 $l.h^{-1}$ capacity), fastened near the bottom of the tank to stir the water in a circular motion.

The chilled and enriched reservoir seawater was pumped upwards to a 20 l PVC pressure buffer reservoir (radius: 135 mm) with a fixed water level (135 mm), which was maintained through an outflow back into the 200 l reservoir tank. The 20 l buffer tank was placed approximately 1 m above the individual oyster chambers, so that water flowed downwards through 20 mm and 12 mm hoses, and through five cylindrical polyethylene 2 l individual oyster chambers (radius: 80.6 mm). The hosepipes connecting the buffer tank with the experimental chambers were each fitted with PVC adjustable valves to regulate flow-rate. Each chamber contained an individual outflow opposite and upper to the inflow (Filgueira *et al*. 2006). Outflow from each chamber, including the empty control chamber, was

accumulated in cleaned individual 100 l catchment chambers for measurement of particle concentrations. The experiments were conducted at the Fisheries Research and Development Institute of the Department of Agriculture, Forestry and Fisheries, based in Sea Point (same facility as that mentioned in Section 2.1.2).

2.2.3. Experimental conditions

Two commercial algal strains, the diatom species *Chaetoceros mullerei* (3 – 30 µm) and the flagellate mix *Isochrysis sp.* (4 – 8 µm) were cultured in F/2 and Walnes medium under dark and refrigerated conditions and incubated for the experiment. The cultures were obtained from the same premises where the experiment was conducted. Both strains were chosen because diatoms and flagellates are prominent at both sites and are good food sources for oysters. Concentrations for each cultured strain were measured with a Beckman Z™2 Coulter counter® (Fullerton, USA) within a 2.8 – 60 µm cell diameter range. Concentrations for each algal strain were calculated to obtain a summed concentration (the added concentration of both strains) equal to concentrations of Saldanha Bay water fed to Algoa Bay and Saldanha Bay oysters during the incubation experiments. A concentration of ~2 300 cells.ml^{-1} was calculated for each strain to obtain a total concentration of 4 600 cells.ml^{-1} which was added to natural seawater (filtered through a 18 µm filter) to obtain a 280 – 340 l oyster mix fed to the oysters over three hours. The seawater mix was mixed in a large drum, scooped into the 200 l supply tank, chilled prior to placement of oysters in the chambers, and topped up as the water-level dropped. Seawater concentrations without phytoplankton enrichment ranged from approximately 2 000 – 7 000 cells.ml^{-1}.

Before the experiments, flow-rates through each oyster chamber were adjusted manually in the range of 270 – 320 ml.min^{-1} (16.2 – 19 l.h^{-1}) and maintained in that range throughout each experiment, while flow-rates were recorded approximately every 20 minutes for each chamber. Flow rates were recorded by measuring the volume of seawater acquired

within a volumetric flask within a one-minute time period from the outflow of the oyster chamber. This water was poured back into the catchment chamber and the flask was rinsed in seawater from the adjacent holding tank before the next chambers' flow rate was measured. This range was chosen to avoid backflow at the inflow of the chamber and recirculation of water in the oyster chamber which reintroduces oysters with particles already filtered (Filgueria *et al.* 2006; Ren *et al*. 2000), and to aim for a particle reduction not higher than 20 – 30% which was found to be the maximum particle reduction range at which no recirculation occurred in mussels (Filgueria *et al.* 2006).

Following the first experiment, each prospective experimental oyster batch was stabilized in the holding tank for five to fifteen hours prior to each trial (temperatures in the holding tank were close to the experimental temperatures and ranged between 14 – 15°C), while the rest of the oysters were put in styrofoam boxes (340 mm width x 690 mm breadth x 180 mm depth) with ice bricks. Temperatures in these styrofoam boxes ranged from 5 – 10°C (median: 7.2°C) in between opening of the box to switch ice bricks, and with opening of boxes the inside temperature reached up to 14°C before the median temperature was reached again after ~ 140 min. Water temperatures over the experiment ranged from 14.4 – 16°C (median: 14.9°C, C.V.: 2.6%). This alternation of oysters between ice and experimental-temperature seawater allowed both for the stabilization of oysters, and time out of the local seawater environment to ensure that oysters did not adjust their feeding behaviour or morphology over the four days which spanned all experimental repeats. On average, each experimental oyster spent 10 ± 6 hours (±1 S.D.) in seawater in the holding tank, and 11± 5 hours on ice before stabilization in the holding tank prior to experiments. The experiment spanned four days.

2.2.4. Experimental protocol

Algoa Bay is situated 750 km from Sea Point and 880 km from Saldanha Bay and therefore oysters needed to be transported under cool conditions. The oysters from the Algoa Bay Namibian group were put on ice for approximately fifteen hours, and after travelling these oysters were put into the experimental holding tank for stabilization at 14.5°C approximately five hours before the first experiment, along with the oysters from the Saldanha Bay Namibian group. The Saldanha Bay oysters were on ice for approximately five hours during travelling, prior to stabilization. Each experimental repeat batch contained two Saldanha Bay and two Algoa Bay oysters, each with a designated chamber, and one empty control chamber. The total experimental sample size for each group was 10 oysters each, out of five repeats. This is the same sample size used in Ren *et al.* (2000) for different diets.

The timing for each experimental repeat was planned to fit into the daily rhythms of oysters from each origin, since oysters open their valves at high tide and close them at low tides in a rhythm following that of their local environment (Tran *et al*. 2011). Sea Point is situated between the two local environments from which the two groups originated, and therefore the tides at Sea Point could be used as an approximate reference for both groups. In addition to prior removal of all epifuana through scraping, all experimental oysters were scrubbed with a nailbrush to remove all particles settled on the oysters before the experiment. After stabilization, selection and cleaning, oysters were put into their experimental oyster chambers for 30 minutes shortly after the experimental enriched seawater started pumping through the system and water temperatures were measured.

Each oyster was placed with its ventral-posterior side towards the inflow so that the inflow-current side of the gills was directed towards the incoming flow of water and food. Feeding behaviour was observed, and where oysters were not open, they were replaced with other acclimatized and size-matched oysters. After the 30 minutes and another three hours of

feeding, 200 ml water samples were taken from the well-mixed outflow-catchment containers of each oyster chamber and the control chamber. After the experiment, oysters were shucked, and body components weighed wet, then dried at 50°C for 6 days to obtain dry meat mass.

2.2.5. Particle concentration analyses

Of the 200 ml water samples, 50 – 90 ml was taken and topped up with filtered (0.2 µm) seawater to obtain a 140 ml diluted sample for measurement with the coulter counter. Samples were counted for frequency of different sized particles, within a diameter size range of 2.8 – 60 µm (divided into 400 size bins) by means of a Multisizer™ 4 Beckman coulter counter® (Fullerton, USA) with a 140 µm aperture tube. The coulter counter measured 20 ml of the 140 ml sample by counting particles in 2 ml aliquots for 10 replicates, for which the particles in each size bin were added for all the replicates and divided by two times the number of replicates to obtain the number of particles for 1 ml. The count per 1 ml of each size bin and replicate was multiplied by the dilution factor, and the "undiluted" total count per 1 ml for each size bin was added for all the replicates to obtain the total number of particles per ml for each sample.

The outflow concentration from the empty control chamber was used for representation of the inflow concentration in each trial, and percentage particle reduction was calculated as: $((C_{inflow} - C_{outflow})/C_{inflow}) \times 100$ for each oyster-containing chamber. C_{inflow} and $C_{outflow}$ are the control chamber concentration (after 3 hours) and the oyster chamber concentration (after 3 hours) respectively. The clearance rate was calculated for each chamber as $((C_{inflow} - C_{outflow})/C_{inflow}) \times V$, where V is the flow rate ($l.h^{-1}$) through the chamber (Bougrier *et al.* 1995, Ren *et al.* 2000). Each clearance rate was adjusted to a constant flow rate by multiplying it with the adjusted flow rate, and then dividing it by the actual flow rate.

2.2.6. Validation

Two validation experiments on equal concentration supply between experimental chambers, and one on equal concentration supply over time was conducted with empty oyster chambers. For the concentration over time validation, the same algal strains, concentration, time-span, flow-rates and sampling protocol was used as that of the experiment. Samples were taken from each chamber after 30 minutes, 1 hour 30 minutes and after 2 hours 30 minutes, and analyzed on the coulter counter. Variation in concentration through each chamber over time was more prominent than variation between chambers. Prior to two hours of water flow through each experimental chamber; samples showed that each oyster chamber usually had higher particle concentrations than the control chamber. Within the system, the three chambers on the left (two Saldanha and one Algoa Bay oyster chamber) were directly under the reservoir, and pipes through these chambers allowed a steeper flow and a shorter travelling distance.

In a clearance rate flow-through system study, Ren *et al.* (2000) found the outflow chambers to be mixed well after three hours and therefore our sampling was also conducted at the end of a three hour trial. Samples taken after 2 hours and 30 minutes were analyzed and the percentage concentration difference between each chamber and the control chamber was calculated in the same way as for the experiment. The perentage particle reduction for each chamber was 4.5%, 14.6%, -9.1% and 8.6% for AB sample 1, SB sample 1, AB sample 2 and SB sample 2 respectively (AB = Algoa Bay, SB = Saldanha Bay). Note that the AB sample 2 chamber had a higher concentration than the control chamber, hence the negative percentage reduction value. The experimental percentage reduction for Saldanha Bay oysters was 10.8 ± 7.8% (± 1 S.D.), and for Algoa Bay oysters it was 32.5 ± 13.8% (Section 3.2 below). Algoa Bay oysters displayed at least two times higher percentage reductions than any concentration differences found between chambers without oysters.

For the concentration of supply experiments, each algal strain was supplied once separately at the same concentration it would be supplied in the experiment, and at the same flow-rate and temperature. This was done to compare concentration supply between chambers and to look at the size distribution for each strain separately. After 30 minutes of water supply (enriched with each algal strain separately) the average percentage particle reduction for the two different algal strain trials combined, was -13.1 ± 7.2% and -16.31 ± 7.8 5% for the Saldanha Bay "oyster chambers" and the Algoa Bay "oyster chambers" respectively. Note that the oyster chambers had a higher concentration of particles after 30 minutes of algal supply to each chamber; this was probably due to the control chambers' position in the circuit – furthest away from the algal supply reservoir and its pump.

The same chambers always contained oysters from a specific farm, meaning that the order of oysters from the two different groups placed into the chambers was consistent for each repeat. Concentration differences found after 2 hours and 30 minutes in the validation run (Section 2.2.6 above) from sampling the specific chambers without oysters were used to adjust particle reduction and clearance rates from the experiments. To see if this variation influenced the trend found for the experimental oysters, the "% particle reduction" (from the difference of a specific oyster chambers' concentration from the control chamber, as a percentage of the concentration from the control chamber) in the validation study was subtracted from the percentage particle reduction of the experimental oysters for their specific chambers for each repeat. This was done only to see if variation in particle concentration without oyster feeding changed the general trend for both farm groups found in the feeding experiment, which it did not. Therefore the non-adjusted clearance rate values and their comparison between oyster groups are reported in Section 3.2.

2.3. Gill morphology

Thirty-one oysters from Saldanha Bay (62 – 143.8 g whole live mass; 6 – 14.1 g meat dry mass) and Algoa Bay (78 – 153 g whole live mass; 3.8 – 8.2 g meat dry mass) were collected from the Namibian cohort from the top position in the cage, and these included the oysters used in the flow-through experiments. Oysters were selected according to length. Prior to dissection, the oysters were alternated between storage on ice and hydration within flowing seawater at Sea Point (along with those used in the flow-through feeding experiment). Following the flow-through experiment, these oysters were then kept live on ice for one to three days during the process of shucking them individually and removing their gills and palps through dissection (after each oyster was weighed for whole live mass). The four gill demibranchs were dissected as a unit (Honkoop *et al*. 2003), of which the wet mass was measured, and the four labial palps were also weighed together immediately after dissection. The residual wet meat masses without the gills and palps were weighed to relate gill and labial palp masses to the residual meat masses without gills and labial palps respectively. The outline of the largest and the smallest demibranch and each of the four labial palps were traced on a transparent paper. The tracings were photocopied onto graph paper with 1 mm^2 blocks, which was totalled for blocks within the outline of each palp tracing and for tracings of two of the demibranchs (of which the average was multiplied by four) to derive the different surface areas.

2.4. Statistical analyses

All variables were tested for normality with Shapiro-Wilks tests and analysed with Statictica 10.0 (Statsoft, Tulsa, Oklahoma, U.S.A.), nonparametric tests were applied to data which failed the normality test. Statistically significant differences are reported for when $p <$

0.05, although some differences where $p < 0.09$ are also reported where the possibility exists that differences observed can be of biological significance.

The percentage reduction in particles for both the incubation- and flow-through experiments and the clearance rates from the flow-through experiments were compared between Algoa and Saldanha Bay oysters by means of a Mann-Whitney U test. Linear regressions were done on both clearance rate and percentage reduction of particles in the flow-through experiment, with flow-rate, whole oyster mass, dry meat mass (and DWCI), gill surface area and palp surface area as independent variables. Linear regressions were also done on gill and palp wet and dry masses, with oyster length, gill and palp surface areas, residual masses and whole oyster live mass as independent variables. GLZ-ANCOVAs and Mann-Whitney U tests were used to compare gill and labial palp wet masses, gill and labial palp surface areas, gill: palp wet mass ratios, gill: palp surface area ratios and clearance rate between oysters from Algoa and Saldanha bays.

3. Results

3.1. Incubation clearance rates

For both Saldanha Bay oysters (N = 6 for Saldanha Bay water and N = 4 for Algoa Bay water) and Algoa Bay oysters (N = 5 oysters for both water treatments) no difference was found for their feeding response (in percentage particle reduction) between the water at Saldanha Bay and the water at Algoa Bay for an autumn incubation trial. No difference was found for pooled water treatments (incubations in both Saldanha Bay and Algoa Bay water) for comparison of total percentage reduction and percentage reduction within different size classes (5 –10 µm, 10 –15 µm, 10 – 15 µm and > 20 µm diameter) between Algoa Bay and Saldanha Bay oysters (N = 10 for both groups). Within Algoa Bay water, the difference between oyster groups was more pronounced; with Algoa Bay oysters (N = 5) having a

higher particle reduction than Saldanha Bay oysters (N = 4), although this difference was not significant. For the incubation experiments, percentage particle reduction was in the range of 60.5 – 70.8% for both oyster groups and water treatments.

3.2. Flow-through clearance rates

Algoa Bay oysters had significantly higher percentage particle reductions ($z = -2.98$, $p = 0.001$) and clearance rates than Saldanha Bay oysters (Fig. 1) for the values which were not adjusted for empty-chamber concentration differences (Section 2.2.6.). One outlier data point for percentage reduction and clearance rates from the Saldanha Bay group was > 2 S.D. away from the mean and was removed. This outlier did not affect the difference between oyster groups, but influenced analyses on correlations. The percentage particle reduction was 9.2% (median, with a quartile range of 7.3 – 11.9%) for Saldanha Bay oysters (N = 9), and 32.5% (26.2 – 40.8%) for Algoa Bay oysters (N = 10). No difference was found for percentage reduction between particles within the < 5µm range and particles within the > 5µm range for both Algoa Bay and Saldanha Bay oysters. No particle selection (based on size) could therefore be measured.

No correlation was found between clearance rate (or percentage reduction of particles) and either whole oyster live mass, flow rate, dry meat mass, length, DWCI or gill surface area for both oyster groups within the experiment, but within the Algoa Bay group clearance rate was positively related to palp surface area (Fig. 2). A same-slopes identity-linked GLZ ANCOVA comparison of clearance rates between the Algoa Bay and Saldanha Bay group with palp surface area as a covariate, revealed that there was no difference in clearance rates between oysters from Algoa Bay and Saldanha Bay ($X^2 = 0.99$, $p = 0.75$). Clearance rate was negatively related to gill: palp surface area for Algoa Bay oysters ($y = 8.9124 - 0.672$, $R^2 = 0.39$, $p = 0.053$), and a same-slopes identity-linked GLZ ANCOVA comparison with gill:

palp surface area as a covariate showed that clearance rates were still higher for Algoa Bay oysters compared to Saldanha Bay oysters ($X^2 = 9.8$, $p = 0.002$).

Experimental Algoa Bay oysters had a mean growth rate (whole live mass) similar to that of their population (N = 156; Chapter 3); with a mean percentage mass.day^{-1} growth rate of 3.12 ± 0.55%.day^{-1} (± 1 S.D.) from July 2011 – June 2012, compared to a population mean of 3.05 ± 1.07%.day^{-1}. Experimental Saldanha Bay oysters had a growth rate of 2.82 ± 0.76%.day^{-1}, which was higher than that of their population (N = 203), which was 2.47 ± 0.76%.day^{-1}. Experimental Saldanha Bay oysters were among the fast growers from their cohort and the size-matching between Algoa Bay and Saldanha Bay experimental oysters was successful within the selected range (Mann-Whitney $U_{9,10} = 44$, $z = -0.04$, $p = 0.097$). Saldanha Bay oysters, however, had a higher DWCI than the selected Algoa Bay oysters (Mann-Whitney $U_{10,10} = 0$; $z = 3.74$, $p = 0.00001$).

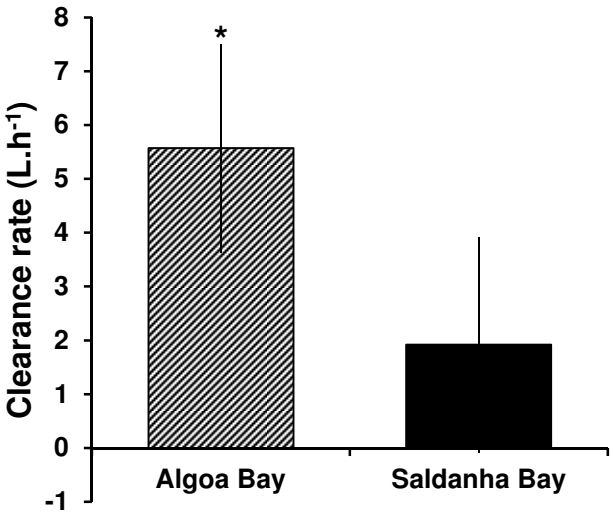

Figure 1: Clearance rates ($l.h^{-1}$) as medians with quartile ranges for the flow-through experiment oysters from Algoa Bay (with a coefficient of variation of 43.9%) and Saldanha Bay (138.6% C.V.). * Denotes a significant difference ($p < 0.05$) for all figures, where Algoa Bay oysters had higher clearance rates compared to Saldanha Bay oysters (Mann-Whitney $U_{10, 9} = 10$, $z = -2.82$, $p = 0.003$).

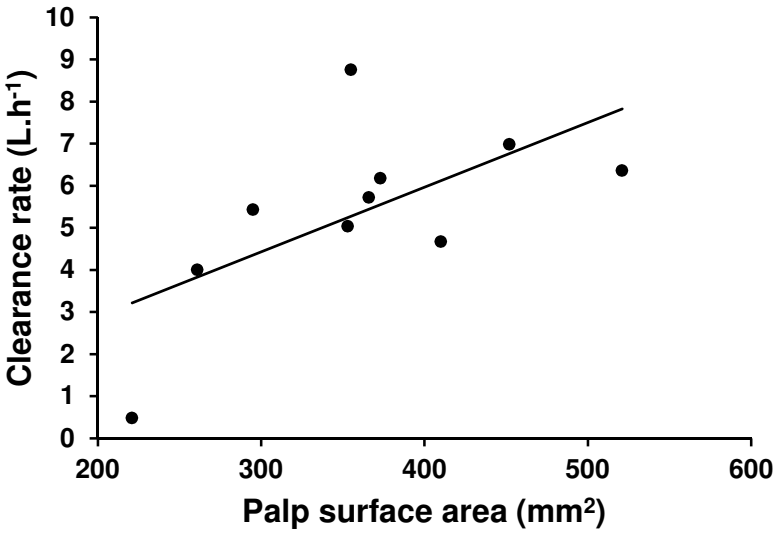

Figure 2: Clearance rate was almost positively related to palp surface area for the Algoa Bay (● with a solid line) group ($N = 10$, $R^2 = 0.39$, $p = 0.052$).

3.3. Gill morphology

Gill and palp wet and dry masses, their surface areas and ratios were compared between farms with different oyster size variables as covariates (correlations given in Table 1), unless non-significant correlations between covariates necessitated the use of Mann-Whitney U tests. Among the oysters selected for dissection, Algoa Bay oysters had heavier whole live masses (Mann- Whitney $U_{31, 31} = 302$, $z = -2.51$, $p = 0.01$), but due to condition, had lighter dry meat masses than the Saldanha Bay oysters (Mann-Whitney $U_{31, 31} = 86$, $z = 5.5$, $p < 0.000001$), but there was no difference within wet meat mass variables. For farm-comparisons of gill and palp variables in Fig. 3 and 4, the three smallest oysters from the Saldanha Bay group and the two largest oysters from the Algoa Bay group were removed from the data set. These "size-matched" oysters differed between sites only in dry mass variables (there was no difference between whole oyster mass or wet meat mass variables).

Comparison of gill wet and dry mass (the total mass of the four gill lamellae) with the inclusion of significant covariates (Table 1), showed that no difference was found for gill wet mass when compared between groups (Fig. 3) with same-slopes GLZ ANCOVAs with either whole live mass ($X^2 = 0.14$, $p = 0.7$) or residual wet meat mass ($X^2 = 0.3$, $p = 0.57$) as a covariate. No difference between sites was found for gill dry mass (Fig. 3) with total dry meat mass as a covariate ($X^2 = 0.00005$, $p = 0.99$).

For significant covariates (Table 1), no difference was found in palp wet mass with GLZ ANCOVA comparisons between groups (Fig. 3) with either whole live mass (same-slopes model; $X^2 = 0.18$, $p = 0.67$) or residual wet meat mass (separate slopes; $X^2 = 4.01$, $p = 0.13$) as a covariate. A Mann-Whitney U comparison on size-matched oysters showed that palp wet masses were almost significantly heavier for Saldanha Bay oysters ($p = 0.052$; Fig.3), and this difference is possibly too strong to not be of biological significance. For significant covariates (Table 1), no difference was also found for palp dry mass between

oysters from Algoa Bay and Saldanha Bay (Fig. 3) when compared with same-slopes GLZ ANCOVA, with either residual dry meat mass ($X^2 = 0.00007$, $p = 0.99$) or whole oyster dry mass ($X^2 = 0.48$, $p = 0.49$) as a covariate.

For size-matched oysters, oysters from Saldanha Bay had heavier dry gill and palp masses (Fig. 3) than oysters from Algoa Bay (Mann-Whitney $U_{29, 28} = 140$, $z = 4.2$, $p = 0.000009$; and Mann-Whitney $U_{29, 28} = 183$, $z = 3.55$, $p = 0.0003$ for gill dry masses and palp dry masses respectively). This was possibly due to a higher DWCI for oysters from Saldanha Bay (Mann-Whitney $U_{31, 31} = 1$; $z = 6.7$, $p < 0.000001$) which was more pronounced than the three previous grow-out periods. These differences fell away when dry masses were corrected for whole oyster and residual meat masses.

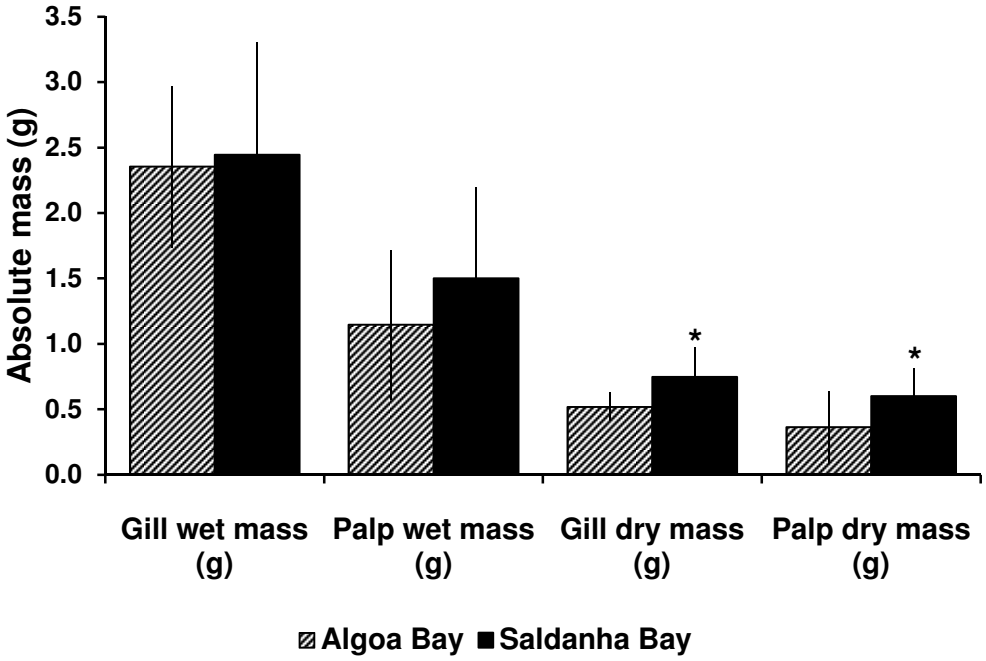

Figure 3: Gill and palp wet and dry masses are displayed as medians with quartile ranges for size-matched oyster groups, and shows that oysters from Saldanha Bay had significantly heavier dry masses for both sets of feeding organs (marked with *). Palp wet masses were also heavier for oysters from Saldanha Bay.

Table 1: Gill and palp variables and their relationship with body variables are displayed with significant correlations typed in bold. S. area = surface area, w. live mass = whole live mass and r. = residual for wet or dry meat mass without the set of feeding organs measured. W. dry mass = whole dry mass (dry shell mass + dry meat mass). For Algoa Bay oysters, gill and palp surface areas were often negatively related to gill wet mass and total palp wet mass.

Farm		**Equation**	**R^2**	***P***
Algoa Bay	Gill s. area vs gill wet mass	2376.5 - 262.1x	0.11	0.07
	Gill s. area vs gill dry mass	1335.9 + 723.8x	0.15	**0.03**
	Palp s. area vs palp wet mass	613.0 - 120.5x	0.18	**0.02**
	Palp s. area vs palp dry mass	587.1 - 312.0x	0.15	**0.03**
	Gill s. area vs w. live mass	2189.01 - 4.1x	0.07	0.15
	Palp s. area vs w. live mass	595.1 - 1.3x	0.04	0.31
	Gill wet mass vs w. live mass	1.1 + 0.01x	0.42	**0.00008**
	Gill wet mass vs r. wet meat mass	1.7 + 0.02x	0.51	**0.004**
	Gill dry mass vs r. dry meat mass	0.6 + 0.003x	0.00	0.9
	Palp wet mass vs w. live mass	0.4 + 0.01x	0.12	0.06
	Palp wet mass vs r. wet meat mass	1.3 + 0.001x	0.00	0.9
	Palp dry mass vs r. dry meat mass	0.2 + 0.04x	0.07	0.15
	Palp dry mass vs w. dry mass	0.1 + 0.01x	0.2	**0.009**
Saldanha Bay	Gill s. area vs gill wet mass	1118.8 + 118.9x	0.1	0.09
	Gill s. area vs gill dry mass	1274.3 + 170.2x	0.03	0.39
	Palp s. area vs palp wet mass	185.0 + 192.4x	0.63	**< 0.00001**
	Palp s. area vs palp dry mass	344.1 + 214.3x	0.11	0.08
	Gill s. area vs w. live mass	1102.3 + 3.3x	0.14	**0.04**
	Palp s. area vs w. live mass	208.3 + 2.9x	0.23	**0.01**
	Gill wet mass vs w. live mass	1.2+ 0.01x	0.31	**0.001**
	Gill wet mass vs r. wet meat mass	1.5 + 0.03x	0.27	**0.003**
	Gill dry mass vs r. dry meat mass	0.5 + 0.03x	0.1	0.08
	Palp wet mass vs w. live mass	0.2 + 0.01x	0.35	**0.0005**
	Palp wet mass vs r. wet meat mass	0.4 + 0.03x	0.34	**0.0006**
	Palp dry mass vs r. dry meat mass	0.2 + 0.05x	0.23	**0.006**

Although gill and palp wet masses did not differ significantly between sites when compared with either ANCOVAs or Mann-Whitney U tests (Fig. 3), the gill: palp wet mass ratio was higher for Algoa Bay oysters (Fig. 4). Gill and palp surface areas (the total surface area of the four gill lamellae and the total inside surface area of the four palps) were positively related to whole oyster live mass for Saldanha Bay oysters but not for Algoa Bay oysters. Algoa Bay oysters had bigger gill surface areas than Saldanha Bay oysters (Fig. 5), with no difference in palp surface areas. The gill: palp surface area ratio was significantly higher for Algoa Bay oysters (Fig. 6).

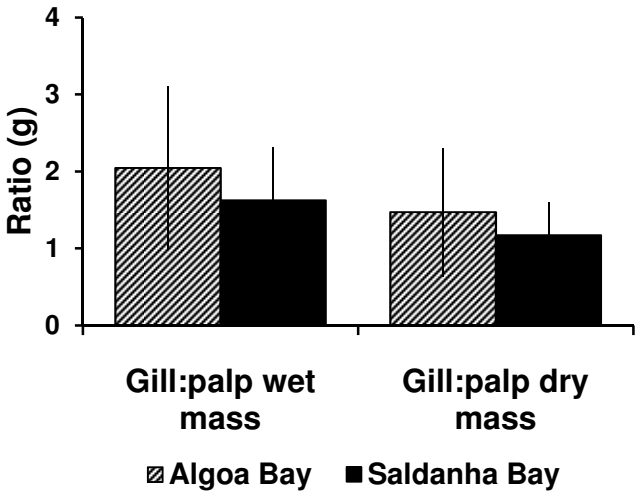

Figure 4: Ratios of gill: palp mass values for individual oysters, displayed here as the median of all ratios with quartile ranges. Algoa Bay oysters had a higher gill: palp wet mass ratio (Mann-Whitney $U_{31,31} = 344$, $z = -1.93$, $p = 0.053$) than Saldanha Bay oysters although this was not statistically significant, and there was no difference between Algoa and Saldanha Bay for the gill: palp dry mass ratio (Mann-Whitney $U_{31, 31} = 430$, $z = -0.7$, $p = 0.48$).

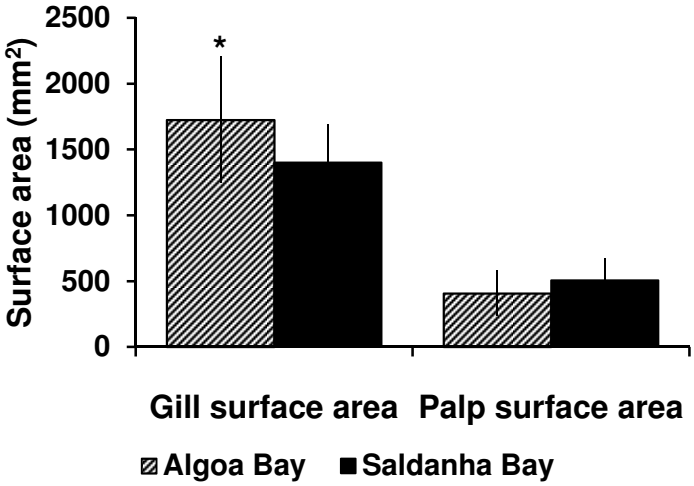

Figure 5: Gill and palp surface areas displayed as medians with quartile ranges. For size-matched oyster groups, Algoa Bay oysters had bigger gill surface areas (Mann-Whitney $U_{29, 28} = 154$, $z = -4.0$, $p = 0.00003$) and that there was no difference in palp surface areas.

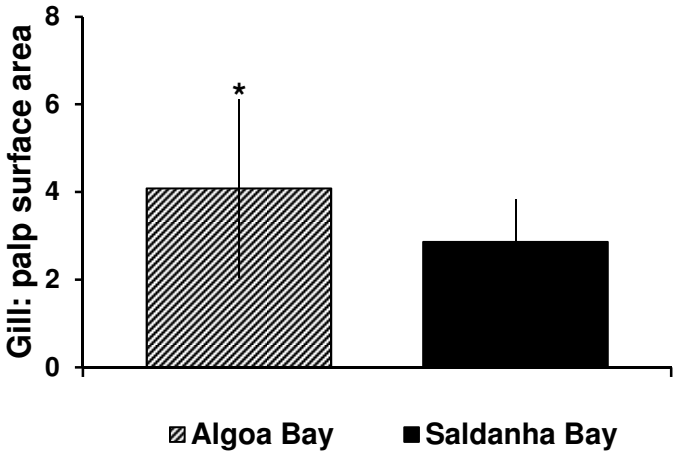

Figure 6: Gill: palp surface area ratios for individual oysters, displayed here as medians with quartile ranges. Algoa Bay oysters had a higher gill: palp surface area ratio (Mann-Whitney $U_{31, 31} = 236$, $z = -3.4$, $p = 0.0004$) than Saldanha Bay oysters.

4. Discussion

The Algoa Bay oysters had faster feeding rates during the flow-through feeding experiment and this confirms the first hypothesis. Due to the selection of experimental oysters within a narrow size range, clearance rates showed no correlation with whole live mass, length or dry meat mass, and measures of oyster size could not be used as a covariate for the comparison of clearance rates between oysters from Algoa Bay and Saldanha Bay. Since oyster size had no effect on clearance rates during the experiment, obtained clearance rates were not standardized to dry meat mass as was done for other studies that measured clearance rates in oysters (Bougrier *et al.* 1995; Ren *et al.* 2000). The difference in clearance rate between Algoa Bay and Saldanha Bay oysters fell away when clearance rate was corrected for differences in palp surface area. This reveals that the difference in clearance rate can be explained by palp surface areas, where clearance rate for Algoa Bay was positively related to palp surface areas. Palp surface area relates to particle concentration (of available food) and would only have benefitted oysters from Algoa Bay instead of those at Saldanha Bay if Algoa Bay oysters had palp surface area ratios specifically suited to the experimental food concentrations. Saldanha Bay oysters had bigger palp surface areas than Algoa Bay oysters, but this did not seem to benefit them during the flow-through experiment.

Relatively large palp surface areas would have been optimal for relatively high seston concentrations (Payne *et al*. 1995; Barillé *et al*. 2000; Honkoop *et al*. 2003; Dutertre *et al.* 2007), and this suggests that Saldanha Bay oysters were adjusted to particle concentrations which were higher than those found in the flow-through experiment. Experimental particle concentrations might have been somewhere between conditions found at Algoa Bay and those of Saldanha Bay, but without seston data at either Algoa Bay or Saldanha Bay, this can not be known. This would explain why high clearance rates found for the Algoa Bay oyster group during the flow-through experiment were related to oysters with larger palps and not to

oysters with larger gills. Oysters from Algoa Bay had relatively larger gill sizes than those of Saldanha Bay, but gill: palp surface area was negatively related to clearance rates for Algoa Bay oysters.

These findings do not establish whether Algoa Bay oysters would have higher clearance rates within particle concentrations found at Saldanha Bay. Although no statistically significant difference in particle depletion occurred between Saldanha Bay and Algoa Bay oysters during the incubation experiments, this was probably due to a combination of small sample sizes and variable feeding rates induced by diminishing particle concentrations. It was observed that Algoa Bay oysters were consistently quicker to open their valves to facilitate feeding. Due to low food concentrations at Algoa Bay, these oysters might go into a state of compensatory feeding when exposed to higher food concentrations, and may simply feed faster than the Saldanha Bay oysters due to hunger. Since Saldanha Bay oysters failed to display higher clearance rates within their own environment (incubation experiment) and because Algoa Bay oysters displayed an active feeding response beyond adjustment to their own environment within the flow-through experiment, further confirmation for the first hypothesis is established.

Algoa Bay oysters caused slightly higher particle depletions relative to Saldanha Bay oysters within their own water environment. Oysters at Algoa Bay have therefore adapted to their oligotrophic environment which would explain why oysters at Algoa Bay had higher gill: palp surface areas compared to Saldanha Bay oysters. The second hypothesis, which states that Algoa Bay oysters have larger gills than Saldanha Bay oysters, and its relation to the first hypthesis, has also been proved. Both gill and palp surface area was negatively related to their respective masses for the Algoa Bay oysters. This is possibly due to resorption of gills (Honkoop *et al.* 2003) and palps at Algoa Bay or due to the confounding factor of meat condition (Filgueira *et al*. 2008).

Gill: palp dry mass ratio values at Algoa Bay (1.47 median, quartile range 0.83) and Saldanha Bay (1.2 median, quartile range 0.4) were lower than ratios for the ash-free dry mass (AFDM) of gills to the AFDM of palps of Honkoop *et al*. (2003) which ranged from ~1.7 – 6.5 for adult *C. gigas* with ash-free dry meat body masses of 0.4 – 2.4 g. Ash-free dry masses refer to mass values calculated by subtracting the ashed masses of soft tissues (ashed for 4 hours at 560°C) from the dried soft tissue masses (dried for 48 hours at 80°C). Honkoop *et al.* (2003) also found that only lighter oysters had a relatively high gill: palp ratio, whereas this ratio ranged from ~1.7 – 2.5 for heavier oysters, and these ratios were very variable over time for *C. gigas*.

Food supply at Algoa Bay seems to be limited, due to low chlorophyll *a* concentrations and the fact that oysters grown there are thin with fast shell growth – characteristic of food-deprived oysters (Brown and Hartwick 1988; Løfstedt 2010), and also because Algoa Bay oysters show signs of hunger when supplied with a relatively rich food source. Food supply increases with optimal water movement which contributes to the delivery of food to oysters, when new food particles are carried through the oysters' mantle cavity within a stream of incoming water to replenish depleted food in the immediate vicinity of the oyster (Walne 1972; Wilson-Ormond *et al*. 1997). Although Algoa Bay displayed low food concentrations, a high nutritional value or good dispersal of available food particles through water movement, could compensate in oyster growth for the deficiency in food particle abundance. Advantages for growth are more likely to be induced by a high nutritional value. If essential fatty acid proportions are suited to the requirements of oysters at Algoa Bay (Chapter 3) in order to meet the requirements of a metabolism steered upward by high temperatures, this could explain fast growth in addition to the high feeding efficiency found for Algoa Bay oysters in this study (Malouf and Breese 1977).

Despite low food abundance in the Mediterranean Thau Lagoon in France, similar to Algoa Bay, *C. gigas* oysters showed high growth rates (Dupuy *et al.* 2000). Although it was suggested that oysters have adapted to source picophytoplankton (plankton < 3µm) for feeding, fast growth was instead related to high clearance rates due to adjustments in feeding efficiency (Le Gall *et al*. 1997; Dupuy *et al.* 2000), results which are in agreement to those found for this study.

5. Summary

It is evident that Algoa Bay oysters feed faster than Saldanha Bay oysters when exposed to the same environment. Algoa Bay oysters had relatively large gills compared to Saldanha Bay oysters, but clearance rate did not show a positive relationship with gill surface area for Algoa Bay oysters under experimental conditions. Algoa Bay oysters were therefore not specifically adjusted to conditions within the flow-through experiment and the difference in clearance rate was accounted for instead by those oysters with larger palp surface areas. Since Saldanha Bay oysters displayed relatively low clearance rates despite large palp surface areas, the difference in clearance rate cannot fully be explained by differences in experimentally-suited morphological adaptations. Differences in clearance rates must therefore involve an active response beyond morphological adaptations, and more information is needed on the feeding response of adult *C. gigas* oysters after a period of food deprivation. At both Algoa Bay and Saldanha Bay, feeding organs were adapted to what we expect for the specific seston quantity conditions at both sites, but more information is needed on the seston composition and quality at both sites in order to asses further morphological adaptations. To assess the potential for selection efficiency at both sites, more information on the seston quantity, current speed and the nutritional quality of the seston is needed for both sites. Further studies on the utilization of picophytoplankton at Algoa Bay through ciliates and protists may also explain feeding efficiency and constant high growth rates of oysters in this oligotrophic bay.

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Chapter 5

General conclusion

The important environmental parameters affecting oyster growth, such as sea temperature, phytoplankton abundance, phytoplankton composition and seston concentration (suspended particulate organic and inorganic matter) are highly variable over time scales ranging from daily to inter-annual. Continuous measurements of seawater temperature and phytoplankton abundance during this study confirm that monitoring over a number of years will be required to establish the suitability of an environment for oyster grow-out. Nonetheless, it is clear that Saldanha Bay displayed relatively high phytoplankton abundance and moderate temperatures relative to Algoa Bay, and it is this combination of conditions which suggests that Saldanha Bay is the most favourable environment among those studied for oyster growth in South Africa. The lack of extreme fluctuations in temperature in Saldanha Bay, which were consistently found at Kleinzee, and also at Algoa Bay during the second year of the study, accounted for its relatively low oyster mortalities (Pauly *et al.* 1988; Cassis *et al.* 2011). Chlorophyll *a* concentration was a reasonable proxyfor food abundance, since it was significantly correlated with oyster growth rate at both sea-based farms (Chapter 3).

Algoa Bay appeared to be a favourable grow-out environment during the first year of the study, when oysters displayed growth in meat mass as fast as oysters at Saldanha Bay, despite chlorophyll *a* measurements almost three times lower than those of Saldanha Bay. This phenomenon was similar to that found for oysters in the Thau Lagoon in France which also displayed high clearance rates (Dupuy *et al*. 2000). Likewise the surprisingly fast growth for oysters at Algoa Bay was probably due to high clearance rates, as those that were observed during the second year of the study (Chapter 4), in combination with an increased metabolism driven by relatively high temperatures. In the first year of the study oysters at Algoa Bay displayed relatively fast meat growth and low mortalities, but this pattern changed in the second year of the study when temperatures increased with sharp fluctuations and

phytoplankton abundance decreased and consequently, slow meat mass growth and high mortalities occurred. Algoa Bay thus became a relatively poor and stressful grow-out environment, which would be even more detrimental to fast-growing and therefore vulnerable oyster spat (García-Esquivel *et al*. 2000) during the summer period if Algoa Bay became an oyster nursery. To establish whether the enduring high temperatures (consistent for more than a few days on end) and sharp fluctuations in high temperatures are found within Algoa Bay once every couple of years, or more often, would require long-term temperature monitoring.

Algoa Bay oysters adapted to their environment with low food abundance by displaying a high gill: palp ratio, which would maximize clearance rates within such conditions (Barillé *et al.* 2000; Honkoop *et al*. 2003; Dutertre *et al.* 2007; Chapter 4). Although no differences in particle depletion occurred between Algoa Bay and Saldanha Bay oysters within cross-over incubation experiments, a trend of comparatively higher clearance rates for Algoa Bay oysters developed within seawater from Algoa Bay. These differences might have been significant within bigger sample sizes, or within depletion measurements of phytoplankton particles within a smaller size range (< 7 µm), especially if proportionally more phytoplankton within this size range is found at Algoa Bay compared to Saldanha Bay. Retention efficiency of smaller particles is likely to be higher for oysters at Algoa Bay within low seston concentrations (Barillé *et al.* 1993), but more information is needed on seston concentrations and phytoplankton size ranges within Algoa Bay.

Within the flow-through feeding experiment (Chapter 4), oysters from Algoa Bay displayed high clearance rates within an environment they were not specifically adapted to, even after the stress induced by travelling. Although oysters from both farms adapted to their specific origin environments with regards to their gill: palp size ratios, no correlation was found between clearance rates and Algoa Bay oysters' relatively large gills, and also none for

clearance rates and Saldanha Bay oysters' relatively large palps. Therefore both oyster groups were probably adapted to seston concentrations other than those found in the flow-through experiment, of which the seston concentrations were probably somewhere between conditions at both farms. Future analysis on the morphology of feeding organs can be made in conjunction with measurements on the seston concentrations of oyster origin environments. Within both feeding trials, Algoa Bay oysters were open quicker and for longer than oysters from Saldanha Bay, and therefore showed a great capacity in feeding behaviour to compensate for prior food limitations.

Nutritional quality of phytoplankton with regards to fatty acid proportions (Burnell and Allan 2009), in addition to feeding efficiency and temperature-driven high metabolic rates, adds another factor to explain high growth rates at Algoa Bay during the first year of the study. Phytoplankton fatty acid (FA) composition, which is species-specific (Langdon and Waldock 1981; Rico-Villa *et al.* 2006), differed between Algoa Bay and Saldanha Bay despite small sample sizes. Algoa Bay had favourable FA ratios with regards to high essential FA ratios. Comparison of FA differences between impending and existing oyster grow-out environments is a potential area for future research to determine whether favourable FA proportions are constant throughout an annual cycle. FA analysis can be supplemented with the microscopic identification of specific species found within the same samples to confirm which species account for favourable FA proportions.

Measurement of growth rates, condition index and shell density at Saldanha Bay reflected favourable environmental conditions, including a positive nutritional status which further confirms the suitability of Saldanha Bay as an oyster grow-out environment. Growth rates for *Crassostrea gigas* in whole oyster mass are among the highest in the world for oysters at both sea-based farms (Chapter 2). Algoa Bay might be viable as a nursery environment to promote fast initial growth rates prior to subsequent fattening at Saldanha

Bay, but suitable oyster cohorts, relatively resistant to fluctuating high temperatures would be required for optimal oyster survival at both Algoa Bay and Kleinzee. Also, the feasibility of transportation costs between farms, in addition to a risk assessment of the biosecurity and biodiversity implications of moving oyster between farms, should be measured up against the benefits of fast initial oyster growth.

Generally, *C. gigas* oysters are well-adapted to high temperature fluctuations due to flexibility of thermal limits (Hamdoun *et al*. 2003). The Chilean cohort in Chapter 3, which displayed high mortalities at Algoa Bay and Kleinzee compared to the Namibian cohort, was not as well suited to these environments with regards to survival and stress adaptation traits as was the Namibian cohort. Analysis of the interaction between genotypes and environment within South Africa is the next step for studies on full-sib *C. gigas* families. Such interaction studies would explain whether the Namibian cohort performed well at Saldanha Bay due to genotype or also because these oysters were subjected to developmental effects at the Namibian hatchery, an environment belonging to the same current system. Study of a combination of commercially important traits, including growth, hardiness and survival, suited to different South African environments also offers great potential for future research. Establishment of a local hatchery will provide the scope for such studies on full-sib oyster families.

Depth was the other factor which influenced oyster growth in this study, where according to polynomial curves, mass gain was often faster within the top cage layer, ~1.4 m above the bottom cage layer and ~1.5 m below the sea surface (Chapter 3). Although these differences were not found to be very pronounced, they are in agreement with other studies (King *et al*. 2006; Cassis *et al*. 2011) which found that *C. gigas* grows faster closer to the sea surface. These findings did not agree though with previous results for oyster growth in cages with only 0.75 m between the top and bottom cage layers (Chapter 2), where the bottom layer

displayed faster growth, probably due to small-scale differences in phytoplankton abundance and a closer distance to the sea surface compared to the bottom cage layers for Study 2. Future studies on the correlation between depth and fouling, and the effect of fouling organisms at different culture depths are important. Comparison of shell-boring polychaete abundance and consequent damage of oyster shells between oyster culture sites, and possibly depths, will add to our knowledge of favourable oyster grow-out environments.

In conclusion, based on results found within this study, Saldanha Bay is an optimal environment for oyster growth in South Africa, and is suited to large culture operations which rely on relatively low mortalities. More information is needed on the advantages of diatoms compared to dinoflagellates within specific South African environmental conditions, and also on phytoplankton composition at both sea-based farms throughout the year. Temperature and phytoplankton abundance, which were more favourable at Saldanha Bay, were the main determinants of growth, possibly in addition to phytoplankton composition. In response, *C. gigas* oysters showed considerable adjustment to local conditions during the first year of the study, and compensated in growth at Algoa Bay to the extent of catching up with oysters at Saldanha Bay. As was found within the second year of the study, Algoa Bay oysters probably managed to do this by adjusting their feeding rates and morphology to keep up with the demands of a temperature-driven increased metabolism. This study confirms the phenotypic plasticity of *C. gigas* feeding organs and processes, as has been found for this species in studies done all over the world.

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