PH.D. THESIS

Population Dynamics of two Oyster Species *Pinctada capensis* (Sowerby, 1872) and *Saccostrea cucullata* (Born, 1778) with potential for Aquaculture in Maputo Bay

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Declaration of originality

I declare that this thesis has never been presented for the fulfilment of any degree or in any other context and that it constitutes the result of my individual work. This thesis is submitted in partial fulfilment of the requirements for obtaining the degree of Doctor in Biosciences, from the Eduardo Mondlane University.

Signed:

Maputo, 2024

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Abstract

Oysters are important resources for the daily diet, a source of economic income for many coastal communities and a delicacy for the tourism industry. In Maputo Bay, these invertebrates species are intensively exploited for food consumption or as ornamental objects for peoples inhabiting coastal areas, tourists and other resident and non-resident communities. The high demographic pressure and easy access of the coastal communities to the oyster banks, allows high captures of these organisms, causing intense exploitation in the wild banks. This practice can lead to over-exploitation and eventual risk of depletion in some areas around Maputo Bay. Thus, the goal of this thesis is to evaluate the population dynamics with a focus on the oyster's exploitation, reproductive activities and larvae settlement as input for management measures to prevent over-exploitation and as a baseline for future cultivation of oyster's species in large scale at Inhaca Island, southern Mozambique.

The thesis includes four (I-IV) studies that culminated with 5 published papers. **Study I** (Paper **I**) aimed at assessing the catch per unit effort, density and size composition of oysters *Pinctada capensis* and *Saccostrea cucullata*. The study was conducted over a three-year period on eight locations at Inhaca Island. Transects, quadrats and daily fisheries catches approaches were used. Results show that *P. capensis* is the most exploited species on the island. Oyster densities, sizes and catches per unit effort were higher in the less accessible areas only for *P. capensis*. **Study II** (Paper **II**) describes reproductive aspects of these two species on Inhaca Island. Adult oysters were collected monthly over two years period within seagrass banks for *P. capensis* and rocky shore habitats for *S. cucullata*. Animals were evaluated using biometric and histological analysis of the gonads. Females were predominant among larger individuals (>55 mm) while males were more dominant among smaller individuals (<55 mm) for both species. Five gonad maturation stages were identified: indifferent, developing I, developing II, ripe and spent. The size at first maturity was 27 mm and 26.2 mm for pearl oyster females and males, respectively, whereas for rocky shore oysters was 32.8 and 28.3 mm for females and males, respectively. Overall, the reproduction of *S. cucullata* and *P. capensis* occurs mainly in summer, with a short resting period in winter. **Study III** (Paper **III**) the combined effects of temperature and salinity on the embryonic and larval development of the rocky oyster were investigated in laboratory conditions. A factorial experimental design tested three temperatures (24, 30 and 34 ºC) and three salinities (30, 35 and 40 parts per thousand) over a seven-day period. Larval survival and growth were assessed by regular sampling by counting and measurement of larvae under an optical microscope equipped with a micrometric scale. Significantly higher larval survival was observed at the combination of 30 °C and 35 ‰ salinity. The lowest temperature (24 ºC) negatively affected growth regardless of salinity level and survival decreased linearly with increasing salinities. **Study IV** (Paper **IV** and **V**), spat of *Pinctada capensis* and *Saccostrea cucullata* and fouling fauna were collected using artificial substrate from four (4) selected sites at Inhaca Island, during a field experiment of 8 months to assess the effect of different factor (season, location, surface and duration) on spat settlement and abundance of fouling fauna. Ceramic tiles were deployed from surface to 3 m depth and replaced monthly. Overall, settlement in ceramic tile were higher for *S. cucullata* than pearl oyster *P. capensis*. There was a tendency of higher number of spat settled during summer than winter and on rough surface than smooth surface for both species. The results have also indicated that the ceramic tiles could be used as substrate for rocky shore oyster *S. cucullata* settlement in later summer (Paper **IV**). Five groups of fouling

fauna were recorded on the oyster collectors: barnacles, gastropods, mussels, Polychaeta and algae. The diversity of fouling fauna was higher on collectors deployed in seagrass habitat than on rock shores. Barnacles dominate among fouling fauna on collectors. For both rocky shore and seagrass habitat, there was a tendency to higher numbers of fouling organism in summer and in rough surface. Artificial tiles used as oyster collectors, have also shown to attract a variety of fouling fauna when deployed in rocky and seagrass habitats (Paper **V**).

Abstract in Portuguese

As ostras são recursos importantes para a dieta diária, uma fonte de rendimento económico para muitas comunidades costeiras e uma etiqueta para a indústria do turismo. Na Baía de Maputo, estas espécies de invertebrados são intensamente exploradas para consumo alimentar ou como objectos ornamentais pelas populações que habitam as zonas costeiras, e outras comunidades não residentes. A elevada pressão demográfica e o fácil acesso das comunidades costeiras aos bancos de ostras, permite elevadas capturas destes organismos nas áreas, causando uma intensa exploração nos bancos naturais. Esta prática pode levar à sobre-exploração e eventual risco de diminuicão ou esgotamento nalgumas áreas em torno da Baía de Maputo. Assim, o objetivo desta tese é avaliar a dinâmica populacional com foco no nível de exploração das ostras, do ciclo reprodutivo e do assentamento de larvas como contributo para definir medidas adequadas de gestão destes recursos para evitar a sobre-exploração e que servirá como subsídio para o futuro cultivo de espécies de ostras em grande escala na Ilha de Inhaca, sul de Moçambique.

A tese inclui quatro estudos (I-IV) que culminaram com 5 artigos publicados. O **estudo I** (Artigo I) teve como objetivo avaliar a captura por unidade de esforço, a densidade e a composição de tamanhos das ostras *Pinctada capensis* e *Saccostrea cucullata*. O estudo foi efectuado durante um período de três anos em oito locais da Ilha da Inhaca. As amostragens consistiram em transectos, quadrículas e capturas diárias de pesca. Os resultados mostram que a *P. capensis* é a espécie mais explorada na ilha. As densidades de ostras, os tamanhos e as capturas por unidade de esforço foram mais elevados nas zonas menos acessíveis apenas para a *P. capensis*. O **estudo II** (Artigo II) descreve os aspectos reprodutivos destas duas espécies na ilha da Inhaca. Ostras adultas foram colhidas mensalmente durante um período de dois anos em bancos de ervas marinhas para *P. capensis* e em habitats de costa rochosa para *S. cucullata*. Os animais foram avaliados através de análises biométricas e histológicas das gónadas. As fêmeas foram predominantes entre os indivíduos maiores (>55 mm), enquanto os machos foram mais predominantes entre os indivíduos mais pequenos (<55 mm) para ambas as espécies. Foram identificados cinco estádios de maturação das gónadas: indiferente, em desenvolvimento I, em desenvolvimento II, maduro e desovado. O tamanho da primeira maturação foi de 27 mm e 26,2 mm para fêmeas e machos de ostras produtoras de pérola, respetivamente, enquanto que para ostras das rochas foi de 32,8 e 28,3 mm para fêmeas e machos, respetivamente. De um modo geral, a reprodução de *S. cucullata* e *P. capensis* ocorre principalmente no verão, com um curto período de repouso no inverno. **Estudo III** (Artigo III) os efeitos combinados da temperatura e da salinidade no desenvolvimento embrionário e larvar da ostra-das-rochas foram avaliados em condições laboratoriais. O delineamento experimental testou três temperaturas (24, 30 e 34 ºC) e três salinidades (30, 35 e 40 partes por mil) durante um período de sete dias. A sobrevivência e o crescimento das larvas foram avaliados por amostragem regular através da contagem e medição das larvas num microscópio ótico equipado com uma escala micrométrica. Observou-se uma sobrevivência larvar significativamente mais elevada na combinação de 30 °C e 35 de salinidade. A temperatura mais baixa (24 ºC) afectou negativamente o crescimento, independentemente do nível de salinidade, e a sobrevivência diminuiu linearmente com o aumento da salinidade. No **estudo IV** (Artigo IV e V), foram obtidos sementes de *P. capensis* e *S. cucullata* e fauna incrustante utilizando substrato artificial em quatro (4) locais seleccionados na ilha da Inhaca, durante uma experiência de campo de 8 meses, para avaliar o efeito de diferentes factores (estação, localização, superfície e duração da colocação) no recrutamento das ostras e na abundância da fauna incrustante. As tijoleiras foram colocadas desde a superfície até 3 m de profundidade e substituídas mensalmente. De um modo geral, o assentamento nas tijoleiras foi mais elevado para a *S. cucullata* do que

para a ostra *P. capensis*. Verificou-se uma tendência para um maior número de larvas assentadas durante o verão do que no inverno e na superfície rugosa do que na superfície lisa da tijoleira para ambas as espécies. Os resultados também indicaram que as tijoleiras podem ser utilizados como substrato para a colonização da ostra do substrato rochoso *S. cucullata* no final do verão (Artigo IV). Foram registados cinco grupos de fauna incrustante nos colectores de ostras: cracas, gastrópodes, mexilhões, Polychaeta e algas. A diversidade da fauna incrustante foi mais elevada nos colectores colocados em habitat de ervas marinhas do que nas rochas. As cracas dominam a fauna incrustante dos colectores. Tanto no habitat rochoso como no habitat de ervas marinhas, verificou-se uma tendência para um maior número de organismos incrustantes no verão e em superfícies rugosas. As tijoleiras utilizadas como colectores de ostras também demonstraram atrair uma variedade de fauna incrustante quando colocados em habitats rochosos e de ervas marinhas (Artigo **V**).

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List of abbreviations

DCB – Department of Biological Science
^{o}C – Degrees Celsius
WIO JMS – Western Indian Ocean Journal of Marine Science
Lab – Laboratory
Ma – Mega annum (similar to one-million year)
IB – Pound Mass
µm – Micrometre
UNFSS – United Nations Forum on Sustainability Standards
SDG – Sustainable Development Goal
LDCs – Least Developed Countries
Mt – Million tonnes
Kg – Kilogram
ha – Hectare
S – South
E – East
km^{2} – Square Kilometre
m^{2} – Square Meter
P. capensis – Pictada capensis
S. cucullata – Saccostrea cucullata
EBMI – Inhaca Marine Biological Station
% - Percentage
ml-L – Millilitre per litre
CPUE – Catch per Unit Effort
SL – Shell length
SW$_{50}$ – Size at Sexual Maturity
SNK – Student Newman Keuls Test
CI – Condition Index
mm – Millimetres
2m, 3m, 4m – 2,3 and 4 months
PVC – Polyvinyl Chloride
$ - US Dollars
IDEPA – Instituto de Desenvolvimento de Pesca e Aquacultura

List of papers

This thesis is based on the following peer-reviewed articles, which are referred to in the table by their roman numerals.

Table 1: List of the papers published in scientific journals as part of this thesis

No.	Paper	Impact factor	Journal Name
I.	**Mafambissa, M.J**.; Gimo, C.A.; Andrade, C.P.; Macia, A.A. Catch per Unit Effort, Density and Size Distribution of the oysters *Pinctada capensis* and *Saccostrea cucullata* (Class Bivalvia) on Inhaca Island, Southern Mozambique. Life 2023, 13, 83. https://doi.org/ 10.3390/life13010083	**3.2**	**Life**
II.	**Mafambissa, M.**; Rodrigues, M.; Taimo, T.; Andrade, C.; Lindegart, M.; Macia, A. Gametogenic Cycle of the Oysters *Pinctada capensis* (Sowerby III, 1890) and *Saccostrea cucullata* (Born, 1778) (Class Bivalvia) in Inhaca Island, Southern Mozambique: A Subsidy for Bivalve Culture in the Region. Diversity 2023, 15, 361. https://doi.org/10.3390/d15030361	**2.4**	**Diversity**
III.	Marcelino JA, Macia A, **Mafambissa MJ**, Castejón D, Andrade C (2023) Combined effects of salinity and temperature on survival and growth during the early life cycle of the rock oyster *Saccostrea cuccullata* (Born, 1778). Western Indian Ocean Journal of Marine Science 22(1): 95-102 [doi: 10.4314/wiojms.v22i1.10]	**0.5**	**WIO JMS**
IV.	**Mafambissa, M**., Lindegarth, S., Lindegarth, M., Macia, A. Experimental spat collection of the pearl and rocky shore oysters on ceramic tile substrates. Aquac. Report 36. https://doi.org/10.1016/j.aqrep.2024.102089	**3.7**	**Aquaculture Report**
V.	**Mafambissa, M**., Lindegarth, M., Macia, A. Spatial and temporal variability of fouling communities on spat collectors at Inhaca Island Southern Mozambique: evidence of mild influence on the recruitment success of the oysters *Pinctada capensis* and *Saccostrea cucullata* Heliyon 10(2024)e35420https://doi.org/10.1016/j.heliyon.2024.e35420	**3.4**	**Heliyon**

CHAPTER 1

BACKGROUND AND AIMS

1. Background

Shellfish form an important dietary component in many developing countries, especially in the tropics (FAO 2016). Increased population pressure, whether from increased birth rates or migration, usually leads to increased pressure on such open-access resources. Shellfish resources in eastern Africa have been exploited for at least 100,000 years as evidenced by the presence of shell middens (Dye, 1990). However, coastal communities in Mozambique do not have a long history of fishing and exploiting the coastal resources (Pereira et al. 2014). Many people are recent migrants who moved to the coastal areas during periods of civil unrest (Bryceson & Massinga 2002). This means that there is little traditional knowledge about resources and sustainable areas. This often leads not only to overexploitation of resources, but also to conflicts harvesting.

Food security is an important issue in Mozambique as in most other countries in Africa (FAO 2016). Collection of molluscs, crustaceans and other marine invertebrates by coastal people provides a significant dietary supplement, especially animal protein, as well as a source of income for women and children (de Boer & Prins 2002). Intertidal areas are usually “open access”, meaning that anybody can collect anything anywhere and anytime except in a few restricted sanctuaries or totally protected areas (de Boer et al. 2000).

Most collection of shellfish in Mozambique is performed at a small scale and used for local consumption. In Maputo Bay, especially on Inhaca Island, bivalves are exploited for food consumption, sale, and production of ornamental objects by collectors (de Boer et al. 2000). Among these bivalves there are two oyster species collected by local communities living at Inhaca Island: the pearl oyster *Pinctada capensis* and the rock oyster *Saccostrea cucullata*. Despite being less commonly consumed than the pearl oyster by Inhaca Island residents, *S. cucullata* remains a valuable resource for coastal communities, who harvest it alongside other intertidal organisms for sustenance (Marcelino, 2014; Mafambissa et al. 2022). The high commercial value of these oysters species on the local and foreign markets, combined with high population pressure, allows these organisms to be intensively captured in natural banks (Mafambissa et al. 2022) and can lead to over-exploitation. In addition, other anthropogenic factors, such as marine pollution in Maputo Bay (Scarlet 2005), may exacerbate the conservation status of the oyster species and their abundance.

Both pearl oysters and rock oysters harvested in Maputo Bay are carried out by local communities as part of their subsistence and food security strategy. Consumption of oysters is part of their habit, however collection of oysters is not always done in a way that preserves the resource and its habitat, as there is no selectivity in the size of the oysters. Different tools are used to collect them, such as machetes, hoes etc. (Nrepo 2011, Marcelino 2014, Gimo 2016, Nafio 2019). Consequently, the way in which local communities exploit these oysters is potentially unsustainable for both the oysters and the ecosystem (Mafambissa et al 2022).

In recent years, the study of oyster population dynamics has intensified in different coastal areas with the objective to develop farming techniques appropriate to local conditions (Chávez-Villalba et al. 2022). Studies on survival and growth of oysters from the early life to adult stage have been conducted since this knowledge is vital for commercial purposes such as spat production and on growth in the sea, but also for replenishing natural stocks in the wild (Jordan & Coakley, 2004). Knowledge about reproductive aspects, such as gonadal maturation, spawning, larval development, recruitment and settlement, are extremely important for spat production and can provide the basis for oyster farming.

To this date, such knowledge has not been established in Mozambique. Oyster production has not yet started in the country, despite favourable environmental conditions. National aquaculture is still dominated by commercial shrimp production and small-scale tilapia culture initiatives (IDEPA 2020).

In general, there are two ways to obtain oyster seeds for aquaculture purpose: (1) production of larvae and seed in laboratories, called hatcheries, or (2) capture of larvae in the natural environment by placing appropriate structures (collectors) for the settlement (Taylor et al. 1998). The production of seeds in hatcheries is considered more complex and laborious process and requires a good maintenance of the production system, which generally requires qualified people. Hatchery production is justified when the capture in the wild is insufficient, or when the species to be cultivated is of high value, or when it is an exotic species, as is the case of the Pacific oyster *Crassostrea gigas* (Saucedo et al. 2005).

To mitigate the negative effects of unsustainable oyster harvesting, it is crucial to conduct thorough biological and ecological assessments of oyster populations in their natural habitats. This data can inform effective management strategies, including fishing regulations and on the other hand promote the development of large-scale aquaculture initiatives. Thus, in this thesis, aspects of population dynamics with a focus on exploitation of oysters, reproductive activities, larval development in lab condition, recruitment and settlement of the oyster species *P. capensis* and *S. cucullata* were evaluated on Inhaca Island southern Mozambique. Hopefully, this work will contribute for management plans and the development of local aquaculture aiming at preserving oyster populations in the wild and improving the income of local communities.

The thesis was designed to answer the following questions:

Are the oysters *Pinctada capensis* and *Saccostrea cucullata* a valuable resource for the communities living on Inhaca Island? What is the level of exploitation of this resource on the Island?

What is the optimum period for reproduction and larval settlement of these oyster species on Inhaca Island?

What are the optimum temperatures and salinities for the larvae development of the specie *S. cucullata* under controlled conditions?

Could leftover building material such as tiles be useful as a substrate for oyster larval settlement?

1.1. Aims

The thesis consists of five papers resulting from four field and laboratory studies. In study I (Paper **I**), aspects of trends in *P. capensis* and *S. cucullata* abundance, size distribution and catch-per-unit effort were evaluated to provide baseline information of the status of these species. Study II (Paper **II**), evaluated the reproductive activity, sex ratio and its relation to size and size at first maturity of these oysters. This was in order to provide information for possible use in the development of oyster culture in the region and also for management of fisheries in the area. Given the degree to which *S. cucullata* is resistant to exposure during low tide and the importance of this species to aquaculture in the region, in study III (Paper **III**) I experimentally evaluated in laboratory conditions, the combined effects of salinity and temperature on early larvae life cycle in order to assess and understand the optimal environmental factors behind the recruitment in the wild and the establishment of optimal environmental culture. In study IV (Papers **IV** and **V**) I evaluated the potential of using artificial collectors for spat settlement of *P. capensis* and *S. cucullata* to provide usefully information for future development of aquaculture of these species and to reduce the pressure of collection in the field (Paper **IV**). Paper **V** also evaluates the spatial and temporal variability of associated fouling communities on spat collectors, which were quantified to assess their influence on the recruitment success of the oysters *P. capensis* and *S. cucullata.*

CHAPTER 2

CONTEXTUALIZATION

2. Introduction

The bivalves (class Bivalvia), comprise of more than 15,000 species of clams, oysters, mussels, scallops and a range of other groups of two-shelled species of phylum Mollusca (Gosling 2003). The first bivalve molluscs appeared in the sea during the Cambrian period about 620 Ma (Ma is a date millions of years before the present), before organisms had colonised terrestrial environments (Angel 1986). Because their shells are made of calcium carbonate, bivalves are well represented in the fossil record and are often used as paleontological markers. They are gregarious in nature and are often found in association with corals and other reef-forming organisms (Stenzel 1971). The bivalves' success can be attributed to their metabolic rates being 3 to 10 times higher than those of brachiopods and to a more efficient calcium-carbonate buffering system (Knoll et al. 2007).

The bivalve molluscs are thought to have originated in warm shallow eurihaline coastal waters and gradually invaded estuaries and brackish systems, as well as reaching all of the ocean systems. Because the adult forms of the majority of these animals are benthic or bottom dwelling, many different evolutionary adaptations to the benthic habitat have occurred (Dame 2016, Smaal et al. 2018). Most common lifestyles include the following: (1) buried within burrows in unconsolidated soft sediments; (2) attached by byssal threads to pebbles (3) cemented to shells or rocks; and (4) as semi-mobile members of the epibenthos. Today these adaptations are exemplified by clams, mussels, oysters, and scallops, respectively (Gosling 2003). In natural shallow water habitats, there are often gradients of sediments from muddy unconsolidated materials to hard substrates that reflect a water dynamics-energy gradient from low to high energy environments, with different species of bivalves zoned accordingly (Norkko et al. 2001).

Among the group of bivalves with economic importance, oysters stand out for their high market value associated with their high content of essential nutrients and vitamins. Most commercially important species of oysters are classified in three major genera: *Ostrea*, *Saccostrea*, and *Crassostrea* and a number of minor genera (Carriker & Gaffney 1996) which are of interest to aquaculture.

Oysters are important components of benthic, marine ecosystems around the globe (Dame 2016, Greeve et al. 2023). Different species of oysters, often form dense beds, which have multiple effects on the structure, function and ecosystem services of coastal habitats (Smaal et al. 2018, Michaelis 2020). Oysters typically feed on suspended particles and thereby affect the water clarity (Peterson and Heck 2001, Newell 2004) and exert top-down control of phytoplankton communities (Riisgård et al. 2007, Grabowski et al. 2012). This means that nutrients are captured from the pelagic and transported to the benthos where they are further processed by bacterial communities supporting important benthic-pelagic processes (e.g. Kellogg et al. 2014, Ehrnsten et al. 2020, Yuan et al. 2016). Oysters also modify habitats for other species, thus supporting the maintenance of biodiversity (e.g. Norkko et al. 2001, Norling et al. 2015, McLeod et al. 2019) and may have a stabilizing effect on coastal sediments and therefore can mitigate coastal erosion (Wiberg et al. 2019). The reefs can help to absorb the impact of waves and reduce the force of storms, which can prevent damage to nearby coastal communities and infrastructure (Cochennec-Laureau 2010). Several studies conducted confirm that oysters could extract carbon ions from seawater to build their shells, in a process called calcification. In fact, a single oyster can filter and process up to 50 litres of water each day, effectively sequestering carbon within their shell structures (Parker & Ross 2010).

Oyster reefs increase nitrogen removal from the water column, reducing the likelihood of harmful algal blooms or local anoxic conditions. In addition, reducing nitrogen loads helps reduce the export of nitrogen into deeper offshore waters where it creates "dead zones." In bay waters, oyster reefs are estimated to remove between 127.006 and 1.886.944 kg of nitrogen per year (Cerco & Novel 2007). While this reduction is too small to noticeably affect nitrogen levels bay-wide, it nonetheless is likely to improve water quality in the vicinity of the reefs sufficiently to generate economic benefits from avoided algal blooms or fish kills and in the form of increased property values for coastal homes (Newell et al. 2005)

Nevertheless, the most obvious benefit for human well-being is the provision of nutritious food by harvesting or farming of oysters. Such activities further provide important income and cultural values to human societies in coastal areas around the world (van der Schatte Olivier et al. 2020). Shellfish collected from local intertidal and shallow subtidal areas in the coastal zone form an importantdietary component in many developing countries, especially in the tropics (Zhu et al. 2019). Oysters, mussels, and other bivalve shellfish are some of the most sustainable sources of animal protein. Among shellfish bivalves, oysters provide a significant dietarysupplement, especially of animal protein, as well as a source of income for many coastal inhabitants around the world specially women and children (Smaal et al. 2018; van der Schatte Olivier et al. 2020).

On the other hand, oyster cultivation provides significant economic value to local and regional coastal communities. Research has shown that oyster farming can also stimulate local economic development by attracting tourists, creating markets for related products, and generating income for local businesses (Coen et al. 1999). Pearls and pearl-oyster shells have long been used as jewellery items by humans. However, these are not the sole uses for this renewable resource (Saucedo et al. 2005).

The employment that oyster farming creates is one of the primary economic advantages. The cultivation and harvesting of oysters demand a competent crew and is labour-intensive. Oyster farming supports a variety of adjacent sectors, including distribution and processing, in addition to offering employment possibilities for people who are directly involved in the industry. This may contribute to the growth of regional economies and the creation of jobs nearby also through tourism (Coen et al. 1999, FAO 2012).

Today, shellfish aquaculture accounts for 76% of marine aquaculture production worldwide (FAO 2012). The oyster that makes up the majority of the production and harvest worldwide is *Magallana gigas* regionally referred to as the Pacific oyster. In addition to *Crassostrea*, there are several other families in the order Ostreidae, considered ‘true oysters’, including *Ostrea* and *Saccostrea*. *Pteroida* is another oyster order that represents the pearl oysters such as those in the genus *Pinctada* (Angel. 1986)

2.1 Distribution of oysters, worldwide and in Mozambique

Oysters are bivalve molluscs widely distributed globally in tropical and temperate shallow coastal and estuarine waters (Beck et al. 2011). They are found everywhere in the world except in the north and south poles. In tropical regions, oysters occur in rivers and coastal areas, with most species assembled in narrow bands or dense banks at a tidal range where desiccation, fouling and predation are minimized (Gosling 2003).

The pearl oysters *Pinctada capensis* (= *Pinctada imbricata* (Roding, 1798)) inhabit tropical and sub-tropical waters. Along the Eastern coast of Africa it is abundant in areas associated with seagrasses beds, and in Mozambique its distribution is mainly at the Bazaruto Archipelago and Maputo Bay. It thrives in shallow and relatively clear waters, generally fixed in hard substrates within seagrass beds, at depths from 5 to 30 m (Kimani 2006). The pearls oyster is an important commercial bivalve which produces marine cultured pearls (Martínez-Fernandéz et al 2003). *P. capensis* appears to be rare among the bivalves collected for food in Mozambique (De Boer & Prins 2002, Mafambissa et al. 2022), whereas the congeneric species *Pinctada nigra* is the most common bivalve species in the shell middens (De Boer et al. 2000).

The hooded rock oyster species *Saccostrea cucullata* has a wide Indo-Pacific distribution, ranging longitudinally from East (Branch & Grindley 1979) and South Africa (Dye 1990) to the Pacific Islands, and latitudinal from Japan (Torigoe 1981) to Australia (Roughley 1933) and New Zealand (Dinamani,1976). *S. cucullata* occurs all along the Chinese coast and the Philippines and is the most widely distributed of the species of Saccostrea (Angell 1986), widespread in the tropical Indo-Pacific region. In Mozambique, this species is found in Pemba Bay, Palma and Mocímboa da praia (northern Mozambique), Bazaruto Archipelago (Everett 2008), Xai-Xai beach as well as in Maputo Bay (de Boer & Prins 2002).

S. cucullata was first described by the Czech mineralogist, metallurgist, and malacologist Ignaz von Born in 1778. The taxonomy of this genus is unclear because of morphological plasticity among its constituent species. Dye et al. 1994 considered *S. cucullata* to represent a super species, with all other putative species of Saccostrea, including *Saccostrea mordax* and *Saccostrea glomerata*, as its component species (Dinamani 1976).

2.2 Life-history

Most coastal marine invertebrates begin life as dispersive planktonic larvae (Gerber et al 2014, Levin 2006). Subject to coastal currents and turbulence, these larvae can be transported dozens or hundreds of kilometers off- and alongshore of their parents' habitats (Shanks 2009, Shanks et al 2003). In contrast, adults of these species are generally slow-moving (e.g., crabs, urchins, some bivalves) or completely sessile (e.g., oysters, mussels, anemones) (Levin & Bridges 1995). Thus, movement during the larval stage comprises the majority of lifetime displacement for many such organisms and is the main driver of connectivity between coastal populations (Gerber et al 2014, Levin 2006, Pechenik 1999). The successful dispersal of a larvae typically begins with spawning from, and ends with settling into, a nearshore habitat (Shanks 2009). The period between spawning and settling, called the larval duration, lasts for as little as a few minutes (as in the tunicate *Ecteinascidia turbinata*) up to nearly one year (as in the giant triton, *Cymatium pathenopeum*) (Shanks et al 2003). During the larval period, individuals undergo developmental changes in preparation for metamorphosing into post-larvae upon settling. Larvae of some species feed to acquire energy for development and other processes (feeding, or planktotrophic, larvae), while larvae of others are spawned with maternally supplied energy sources and are unable to eat (non-feeding, or lecithotrophic, larvae; see Levin and Bridges (1995)). Regardless of their feeding modes, larvae must finish dispersal in suitable habitats and with sufficient energy to undergo metamorphosis (Elkin & Marshall 2007, Pechenik 1999).

Like others marine invertebrates, the life cycle of oysters begins with fertilization of eggs, usually in the water surrounding the adults (Barnes 1974). Obviously, for fertilization to be successful spawning of males and females must not only be timed to occur simultaneously; males and females also need to live in close proximity of one another (Bayne 2017). The eggs of oysters develop into free-swimming trochophore and then veliger larvae. The time taken for embryonic and larval development is species specific and temperature dependent (Gomes et al. 2014). Within 24 hours the fertilized egg has passed through the multicellular blastula and gastrula stages and in 24 to 36 hours has developed into a motile trochophore. The early larval stage is referred to as the straight-hinge, "D" or “Prodissoconch I” stage. Shell length of the initial straight-hinge stage varies with species, but it is generally 80-100µm (Nor Idayu et al. 2015, Syafriadiman 2017). The larva has two valves, a complete digestive system and an organ called the velum that is peculiar to bivalve larvae (Bayne 2017, Breton et al. 2018, Gomes et al. 2014). Larvae continue to swim, feed and grow and within a week the umbones, which are protuberances of the shell near the hinge, develop. Duration of the larval stage varies with species and environmental factors such as temperature, but it can be 18-30 days (Fig.1).

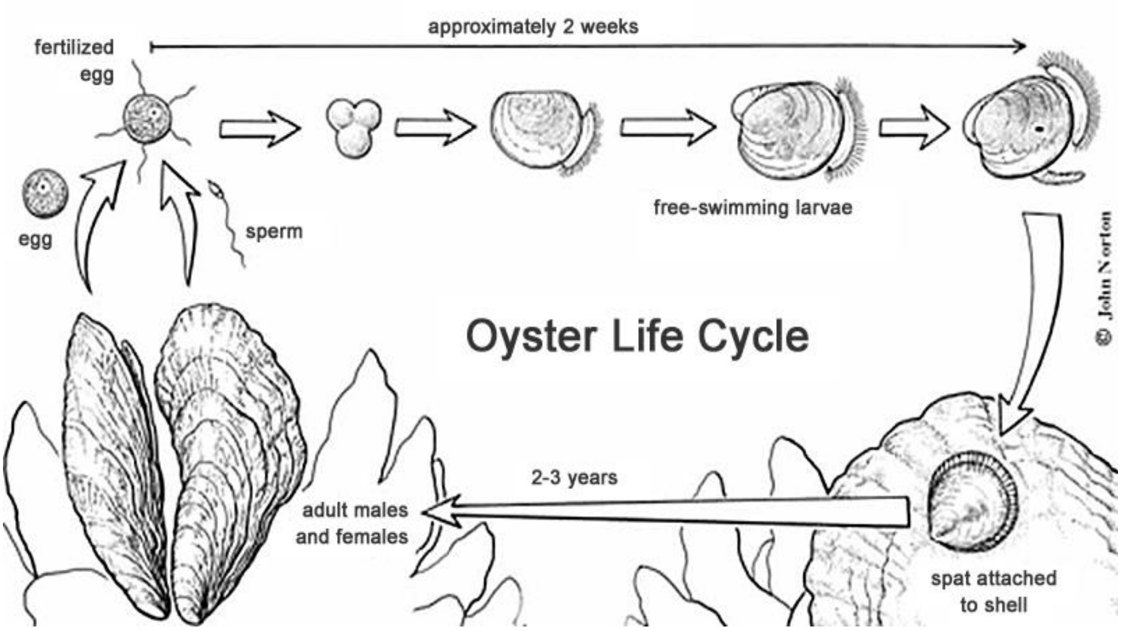

Figure 1: Oyster life cycle (from Dame 2016)

The larval stage is planktonic and subject to very high mortality. At metamorphosis, the larvae settle out of the plankton and the oysters spends its remaining life as a member of the benthos. Consequently, the life cycle of oysters can be separated into two very distinct stages: the planktonic pre-settlement–larval period and the post settlement juvenile–adult period (Marques-Silva et al. 2006). The pre-settlement period is important for dispersal of the species while the juvenile–adult period is important for reproduction (Fraschetii et al. 2002). The two stages are spatially and temporally separated for most of their existence, and that reduces the chances of adult-induced mortality on the larvae through adult filter feeding (Underwood & Fairweather 1989). The population dynamic of the pre-settlement stage is poorly studied probably because of its short duration and the greater logistical complications of sampling plankton. Thus, most studies of bivalve population dynamics focus on the post settlement stage and particularly on shallow-water, commercially valuable species (Hunt & Scheibling 1997).

Despite being focus of much research, the understanding of processes affecting recruitment in oyster species remain fragmentary, particularly those involving interspecific interactions. Among the most studied benthic invertebrates in this regard are reef-forming oysters in the genus *Crassostrea*, for which larval settlement preferences have been shown to be influenced by physical factors, such as temperature, light (e.g., Thorson 1964), and currents (Bushek 1988), as well as the interaction of several physical parameters (Hidu et al. 1978). Several studies have shown that *Crassostrea virginica* larvae preferentially recruit sub tidally, despite a primarily intertidal adult distribution throughout much of its range (Osman et al. 1989; Tamburri et al. 2008). Crisp (1967) found that oysters preferentially recruit to the smooth interior of shells. More recently, oyster larvae have been shown to avoid substrates covered by sediment (Tamburri et al. 2008). Gregarious settlement of oyster larvae, first reported by Cole & Knight-Jones (1939), in response to water soluble cues produced by conspecific and congeneric adults and juveniles have been reported in both still (Hidu 1969, Hidu et al. 1978, Tamburri et al. 2008) and flowing water (Turner et al. 1994). Oyster reefs are, of course, occupied by many different organisms, and the effects of only a few of those species on oyster settlement have been investigated. Most notably, bacterial biofilms have been shown to increase oyster larvae settlement and Pech et al. 2002 have suggested that both oysters and bacterial biofilms produce the same or similar metabolites which serve to induce oyster larvae to settle. Barnacles have been shown to increase the settlement of oyster larvae on settlement plates (Butler 2011, Osman et al. 1989), and the reciprocal also appears to be true whereby oysters may similarly stimulate barnacle settlement (Bushek 1988). Most interspecific interaction studies involving fouling organisms have utilized artificial plates that differ from natural substrates in their species composition and degree of fouling cover (Sutherland 1987). In addition, oyster larvae have been shown to preferentially recruit to natural oyster shells over PVC substrate (Tamburri et al. 2008).

Many species of oysters are protandric hermaphrodites, i.e. they first mature as males, and in the following spawning season they may change sex and become females. Unfortunately, sexual dimorphism does not occur, so it is not possible to determine the sex of a given bivalve without opening it and examining the gonads (Coralie et al. 2020).

The period the larvae spend in the plankton holds numerous dangers. Oysters produce thousands of larvae though only two (one male, one female) are necessary to maintain the parent population size (Nor Idayu et al. 2015). The larvae depend on phytoplankton and/or bacteria for food and their delicate bodies are usually more vulnerable than are the adults to environmental changes such as temperature, salinity, and contaminants

(Robert et al. 2017). They also form the food for larger predatory organisms such as shrimps and small pelagic fish (Maathuis et al. 2020). Thus, the supply of healthy spat depends on suitable environmental conditions, including a sufficient supply of phytoplankton (Devakie & Ali 2000, Robert et al. 2017). The larvae have to be very specific about selecting a site for settling. The settling period is a time when the young oyster is very vulnerable because it has to change all of its living habits; the swimming structure, the velum, is cast off or resorbed, but the gills have not yet developed to become feeding structures (Gosling 2015). This means that getting enough food will be difficult and spat that have been able to build up reserves in the form of fatty tissue will be more successful than spat which have spent all their energy searching for a suitable substrate (Devakie & Ali 2000). Increasing the available substrate by deploying spat collectors may yield healthier spat. However, the site where these spat collectors are deployed must be carefully selected to be suitable also for the adult bivalves and to prevent pollution from other human activities (Preston et al. 2020, Smyth et al. 2020).

Pollution, particularly industrial pollution, can cause extensive mortalities in juvenile and adult oysters (Belhabib et al. 2015). Both industrial and domestic pollution can be problems for hatchery operations and must be avoided. Domestic pollution can increase organic and bacterial loads in water as well as contributing a wide range of potentially toxic materials (Mahu et al. 2022). Little is known of the combined effects of sub-lethal levels of the wide range of organic and organo-metallic compounds of man-made origin that may be present in such effluents.

Oysters in the larval, juvenile and adult stages are preyed upon by a wide variety of animals that can cause severe mortalities. In the natural environment plankton feeders probably consume large quantities of larvae (Kraeuter & Castagna 2001). Oysters are hosts to parasites that can cause mortalities, particularly in the adult stage. Shell boring worms, *Polydora* sp., and sponges burrow into the shells and weaken them, thus causing mortalities (Suja et al. 2020).

2.3 Oysters in context of global challenges to provide food in a sustainable way. The SDG Concept

The global human population strongly depends on the ocean as it provides as much as two thirds of the planet’s ecosystem services natural capital (Worm et al. 2006). However, ecosystem services often fall into the category of open access or public services, providing little incentive for beneficiaries to manage these services sustainably (Chee 2004). This has led to an increasing risk of habitat degradation, shifts in species distributions and loss of ecosystem function (Arkema et al. 2015, Hattam et al. 2015). Coasts are a focal point for human migration and economic activities and are exposed to increasing human induced pressures (Glaser & Glaeser 2014).

Rapid exploitation of living marine resources during recent decades has been undertaken in an unsustainable manner in several parts of the world leading to overfishing, degradation of fish stocks, habitats, ecosystems and biodiversity (Troell et al. 2023). The resulting economic loss is estimated at $83 billion per year for fisheries and over $6 billion per year from diseases in aquaculture (FAO 2023). This is further exacerbated by climate change, which is likely to have a severe effect on fishing and shellfish farming communities in many parts of the world causing loss of livelihoods, displacement and migration of populations because of floods, storms or changes in fisheries distributions (Barange et al. 2018).

From ancient times, fisheries and aquaculture have been a major source of food and a provider of employment, recreation, trade, culture and economic benefits to many people throughout the world (FAO 2023). These activities attain greater relevance along the coastal areas of many developing countries where there are significant obstacles for employment and where access to fisheries and aquaculture resources sometimes remains the only option open for earning a livelihood, improving income and the quality of lives (Barange et al. 2018, UNFSS 2016). Unfortunately, there is evidence that easier access to fishery resources has not always translated in the long term into better incomes and increased well-being of coastal communities (UNFSS 2016).

Although fisheries and aquaculture contribute to several goals, in 2015 the 2030 Agenda for Sustainable Development adopted, for the first time, a Global Goal on Oceans and Seas. The Sustainable Development Goal 14 (SDG 14) is exclusively dedicated to "conserve and sustainably use the oceans, seas and marine resources for sustainable development (FAO 2018). It has ten targets relating to marine pollution, protecting marine and coastal ecosystems, minimizing ocean acidification, sustainable management of fisheries and ending harmful fisheries subsidies, conserving coastal and marine areas, increasing economic benefits to Least Developed Countries (LDCs) (UNFSS 2016).

Achieving the trade related targets of SDG 14 requires the catalysis of policies, investment and innovations to restore the productive capacity of the oceans and increase economic benefits to developing countries (Farmery et al. 2021). Innovations that integrate best practices for harvesting, value addition in processing and distribution, can benefit greatly from opportunities offered around the concepts of Oceans economy/blue economy, eco labelling and certification, value chain analysis and seafood clusters (FAO 2018).

Meeting the 2030 global agenda for sustainable development will be challenging and will require partnership, innovation, and holistic and harmonized approaches and strategies at multiple scales. Shellfish aquaculture can be well-positioned to be part of the solutions but progress toward its contribution to achieving the SDGs is dependent on good governance at all levels (local, national, regional and international) of decision-making Troell et al. 2023).

As marine resource managers strive to find innovative solutions to halt fisheries decline, shellfish aquaculture has also gained increasing social acceptance in some regions as a sustainable solution for ecosystem restoration and enhancement (Beck et al. 2011, Jones 2017, Theuerkauf et al. 2021). Shellfish farming is an important sector contributing to human well-being and plays an increasingly important role in efforts to meet the SDGs (Hambrey 2017) while shellfish farming brings opportunities to contribute to most of the SDGs, there are many factors influencing what the outcomes for SDGs will be from different types of aquaculture systems in different situations (FAO 2018).

Shellfish farming holds huge potential to contribute positively to human and planetary well-being when outcomes are aligned with the Sustainable Development Goals. The potential for the sector to further contribute to these aligned goals using frameworks such as that developed for the UN SDGs seems large. The full potential of aquaculture to achieve targets of the SDGs may also only be realized if brought into broader food and natural resource systems decision-making (Troell et al. 2023).

2.4 Contribution of shellfish aquaculture to food production from the sea.

The world's population is projected to reach 9.7 billion by 2050 (United Nations, World Population Prospect 2019) and global demand for animal proteins may rise by as much as 88% (Cottrell et al. 2018, Searchinger et al. 2018), much of which will be consumed at levels exceeding guidelines for healthy eating. How to feed a growing population a healthy (nutritious) and sustainable diet is one of the greatest challenges facing humanity today (Willett et al. 2019) and the food system connects to the SDGs in multiple ways through resources, environments, economics, and people's well-being.

The importance of the seafood sector for nutrient and food security is increasingly being stressed for many countries with coasts and freshwater systems (Béné et al. 2016, Bennett et al. 2018). Recent reviews have drawn attention to the need to derive more proteins from aquatic sources by restoring fish stocks and increasing sustainable aquaculture development (Costello et al. 2019, Hicks et al. 2019, Willett et al. 2019).

Captured or cultured, from freshwater or marine ecosystems, aquatic foods play an important role in food security and nutrition for billions of people and support livelihoods, economies, and cultures all around the world (FAO 2023). Aquatic foods, and particularly the expansion of aquaculture, may become more important as the world seeks to create just food systems that support the health of people and the planet (Bennett et al. 2019, FAO 2023). Global per capita seafood consumption has increased from 9.0 kg in 1961 to 20.2 kg in 2020. Fish and other seafood provide about 3.3 billion people with almost 20% of their intake of animal protein (FAO 2022).

Seafood is the most traded food commodity in the world (by value), where a relatively small number of seafood species and countries dominate global trade (FAO 2022). Regardless of capture fisheries potential, the expectation that aquaculture will be responsible for the bulk of future seafood supply is very high. At the global level, already more than 80 million tonnes (Mt) of shellfish and fish and 30 Mt of seaweeds originate from around 400 farmed species, reared in highly diverse systems under diverse conditions (FAO 2020).

2.5 Use and threats to oyster populations

Recently, oyster populations are facing a range of challenges, including overfishing, habitat loss, and pollution (Allison et al. 2011). Overfishing has led to a decline in oyster populations, as they are a popular food source for humans and have been harvested in large quantities for thousands of years (Christo & Absher 2006). Habitat loss is also a significant issue, as coastal development, dredging, and other human activities have destroyed or altered many of the shallow-water habitats where oysters live and grow (Lenihan & Peterson 1998). Additionally, pollution is a major threat to oyster populations. Oysters are filter feeders, meaning they ingest nutrients and pollutants from the water as they feed. This makes them particularly vulnerable to the effects of pollution, which can accumulate in their tissues and harm their health (Doney et al.

2012). Pollution can also reduce the amount of oxygen in the water, which can suffocate oysters and other marine organisms (Baker & Mann 1992).

The exploitation of coastal living resources by harvesters has adverse impacts on the coastal ecosystems. Simple overexploitation may lead to the collapse of target populations (de Boer et al. 2000). Since most marine invertebrates have planktonic larvae, it has been assumed that even if a population is locally extinct, the absence of fishing pressure will lead to re-colonisation of such species, provided the environment remains in suitable condition (Hambrey 2017). However, this turns out not to be the case in all systems.

In recent years, climate change is also a growing concern for oyster populations. Rising temperatures and sea levels can alter their habitat and make them more vulnerable to disease and other stressors (Barros et al. 2013). These challenges facing oyster populations have significant implications for the health and sustainability of our oceans and coastal communities (Christo & Absher 2006).

In Mozambique, the exploitation of resources by intertidal harvesters often results in decrease in biomass and average size of exploited species (de Boer et al. 2000). A decrease in size may result in lower fertility of the population. It has been claimed that a low to moderate exploitation of sessile species would actually have a positive effect on biodiversity (Hockey & Bosman 1986) because it would create a mosaic of habitats, which could harbour more different species ("Intermediate disturbance hypothesis"). However, other studies have failed to find significant changes in biodiversity (de Boer et al. 2000, de Boer and Prins 2002). Previous studies on exploitation of rocky shores in South Africa have shown that only biomass of macrofauna is affected by exploitation. Other differences detected could not be unequivocally attributed to exploitation (Haupt et al. 2010; Griffiths et al. 2010).

The exploitation of oysters in Maputo Bay is an activity of great importance for the subsistence of inhabitants of the coastal zone. This activity has been increasing due to the population growth that has occurred in recent years as a consequence of migration to urban and coastal areas. Oysters are intensively exploited for food consumption or as ornamental objects for collectors, tourists and other communities (de Boer et al. 2000, Marcelino 2014, Gimo 2015, Balidy 2003). The fishing of oyster in Maputo Bay have been suffering a very marked reduction during the last decade, since the average weight of the individuals landed in 2019 was only 60% of that of the oysters landed in 2013 (Nafio 2019). In addition, other anthropogenic factors, such as marine pollution in the Bay (Scarlet 2005), may exacerbate their abundance and threaten the conservation status of the fishery and in particular the oysters. This situation generally leads to uncontrolled catches of marine resources, which may contribute to their decline in the wild, affecting the structure and function of communities and compromising their sustainability (de Boer et al. 2000).

The most common oyster species collected by local communities in Maputo Bay is *P. capensis* followed by *S. cucullata*. Oysters are part of the diet habits of these communities. The oysters are collected using knives, machetes, hoes and other instruments that physically damage the oyster banks. Therefore, the form of exploitation carried out has proved to be unsustainable for both oysters and the ecosystem (Nrepo 2011, Marcelino 2014, Gimo 2015).

There is a great difficulty in finding oysters of the species *P. capensis* and *S. cucullata* with a satisfactory commercial size (around 90 – 100 mm and 70 -80 mm respectively) as it is observed in several areas of Maputo Bay, particularly at the vicinity of the more populated areas near the city of Maputo, and Inhaca Island (Marcelino 2014, Gimo 2015). This occurs primarily in the community areas where the lack of alternatives is accentuated due to limited access to other livelihoods, aggravating the degradation of local ecosystems with the uncontrolled capture of aquatic organisms.

Due to lack of enforcement some of the resources collected along the coast seem to be "open access", despite the existence of laws regulating fisheries exploitation i.e. anybody can collect anything in any amounts at any time. This often leads to serious conflicts among different resource users, especially between large-scale commercial fishermen and small-scale, subsistence collectors. This problem has been referred to as "the tragedy of the commons" (Hardin 1968). The fact that nobody "owns" a resource means that nobody takes care of it. This eventually leads to overexploitation of resources, and possibly to civil unrest among resource users.

Implementation of resource management systems, including constant monitoring of resource availability and environmental state, is one way of maintaining sustainability of resource exploitation. However, economic pressures on the people who collect these resources often result in noncompliance with the management regulations. Aquaculture through low-technology and low-investment culture systems could be a viable alternative to produce commercial oysters.

CHAPTER 3

MATERIAL AND METHODS

3. Material and Methods

3.1 Study area

The fieldwork was mostly carried out in the intertidal areas of Inhaca Island (latitude 25 58’S - 26º07’S, longitude 32º56’E – 33º00’E), located in southern Mozambique at the eastern end of Maputo Bay, 32 km east of Maputo City (Fig. 2). It is separated from land by the short, narrow and deep Ponta Torres Strait which connects the Indian Ocean to Maputo Bay. The strait is kept open by strong oceanic wave action and tidal currents, but its shape seems to change even on short time scales. To the west of Ponta Torres Strait and south of Inhaca are vast tidal flats with a total area of 300 ha. Several narrow and shallow tidal channels appear in this area. The Island has a total area of approximately 42 km^2 and is part of the Ponta de Ouro Marine Park. It is located on the border between the shallow Bay of Maputo and the open waters of the Indian Ocean, in a transition zone from tropical to sub-tropical climate, which creates a rich diversity for both terrestrial and marine ecosystems (Bandeira & Paula, 2014). There are two distinct seasons: the hot and rainy season (from November to April) and the cold and dry season (from May to October) with an average air temperature of 23ºC, and the seawater temperature varying from 18ºC to 32ºC (de Boer and Prins 2002).

The eastern part of the Island is characterized by strong currents and waves while the western part is more protected (Emanuelsson & Isaksson 2016). The tides are semidiurnal and have maximum amplitudes of about 3.1 meters in high spring tides (Bandeira & Paula, 2014.). During low tide a large stretch of beach is exposed, making it an interesting site for the collection of many invertebrates, including oysters (Emanuelsson & Isaksson 2016, de Boer et al. 2000). A detailed description of the island’s general ecology, fauna and flora is given in Kalk (1995).

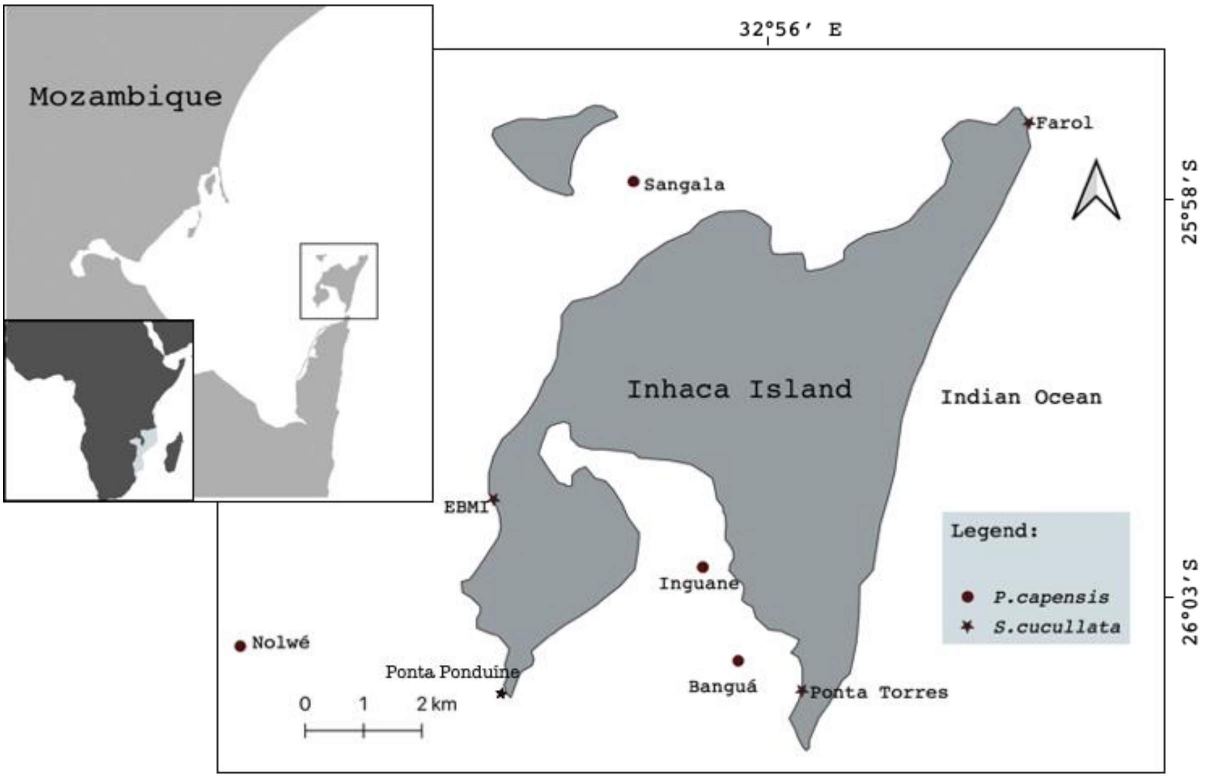

Figure 2: Map illustrating the study area

3.2. Applied Methodology

The thesis encompasses a variety of methodologies, including conventional field surveying techniques, a manipulative field experiment, laboratory analysis and assorted statistical concepts. An intense fieldwork effort using standard sample collection and measurement methods in marine biology were used. Specific and detailed descriptions of methodologies are given in the appended papers.

3.2.1 Oyster exploitation

The study was conducted in four seagrass banks with known occurrence of *P. capensis* and other four rocky shores habitats with known occurrence of *S. cucullata* in intertidal areas around Inhaca Island (Figure 2). At each selected sampling site, quantitative data were collected using transects and quadrates approach (0,5 x 0,5 m), systematically placed. For both species, density and catch per unit effort was quantified as well as the composition in size. Semi-structured interviews to local oyster collectors and sampling in the natural banks were used to select sites for field sampling as well as to collect data on socio-economic value of these species. The questionnaire was designed to determine how large and important the pearl fishery was in the past and where the most productive grounds were located, and to assess how many oysters were currently present at which sites.
For more details on sampling procedure, see (Paper **I Appendix**).

3.2.2 Reproductive status

Based on the results obtained in the first study (Paper **I),** two sampling sites were selected to study the reproductive status of oysters: site I (Bangua) for *P. capensis* and site II (Ponta Torres) for *S. cucullata.* The selection of these sites was based on the abundance of adult oysters and their accessibility. Sampling was conducted monthly during spring tide, over a period of 2 years.Fifty specimens of adult oysters from each species were randomly collected monthly in each selected site. All collected sample were processed and analysed in the Laboratory at the Department of Biological Sciences. The detailed methodology used in the lab are given in (**Paper II appendix**).

The animals were classified histologically, according to the type of germ cells present in their gonads, into males, females, hermaphrodites in which both oocytes and spermatozoa were found in the same individuals and in sexual resting in which no germ cells were found in the gonads, making sex determination impossible (Lenz & Boehs 2011).

The reproductive condition of the oysters was verified based on degree of development and numerical density of the gametes, being determined by the examination of the macroscopic appearance of the gonads and the microscopic examination of gonads tissue smears. Due to a lack of external dimorphism, the shell valves were parted, and a subjective estimation of gonad volume made. Gonad smears were examined at 100× magnification. Each specimen was classified based on the presence or absence of mature or immature germ cells according to the scale modified from (Guillou et al. 1990), (Table 1).

Table 2: Stages of gonadal development in oyster.

Stage	Characteristics
Indifferent	No gonad visible. This has two possible explanations. Adults with recovering gonads after spawning or immature juveniles
Developing I	Gonad tissues visible, but it is very difficult to distinguish sex
Developing II	Gonad tissues evident and sexes can be distinguished. Gametes are abundant, but the majority of the spermatozoids are hardly moving, and pedunculated oocytes are present
Ripe	Gonad with rapid moving spermatozoids or spherical oocytes
Spent	Gonads are empty and thin. Coexistence of cells being reabsorbed and mature cells

3.2.3 Oyster larvae development

Adult specimens of *S. cuccullata* were collected manually during low spring tide on the rocky shores of Ponta Torres (32° 57 'S, 26° 4' E) on Inhaca Island. The oysters were placed in a waterless plastic bucket and capped with Rachel mesh to minimize sunlight stress and brought to the Inhaca Marine Biology Station Laboratory (EBMI). At EBMI, the oysters were conditioned in seawater with continuous aeration prior to spawning and fertilization experiments. For more detailed description of methods for spawning and embryo development, see (Paper **III appendix**).

Twenty-seven culture tanks each containing 5 L of filtered (1µm) and UV-treated seawater were prepared about 24 hours after fertilization. Larvae were reared in the combinations of three temperatures (24, 30 and 34ºC) and three salinities (30, 35 and 40), each with three replicates. The D-larvae were directly transferred to experimental tanks in three replicates per combined temperature x salinity. Approximately 25,000 D-larvae were transferred to each culture tank at an initial density of 5 larvae mL-1. Water volume in each tank was maintained at 5 L. The salinities of 35 and 30 were obtained by diluting seawater (salinity 40) with distilled water. Moderate aeration was provided using air stones. Every 48 h, the bottom of the tanks was siphoned and 50 % of the water volume was changed to ensure good water quality. To avoid the elimination of live larvae as well as to select the largest diameter larvae, 60 and 40µm overlapping meshes were used (smaller mesh over the larger one). A density of 400 cells mL-1 of the microalgae *Isochrysis galbana* was provided daily to the D-larvae.

Embryos of *S. cucullata* were reared to evaluate the combined effects of temperature and salinity on the larval development. A factorial experimental design tested three temperatures (24, 30 and 34 ºC) and three salinities (30, 35 and 40 parts per thousand) over a seven-day period. Larval survival and growth were assessed regularly by sampling, counting and measuring larvae under an optical microscope equiped with a micrometric scale.

3.2.4 Oyster recruitment and fouling fauna

Settlement tiles (collectors) were deployed at four sites with known occurrence of adult oysters: two sites with seagrass meadows, Bangua and Sangala for *P. capensis*, and two locations, EBMI and Ponta Torres for *S. cucullata* (Figure 2).

The settlement collectors consisted of quadratic ceramic tiles measuring 269 cm^2, one side with a smooth surface texture and the other with a rough texture. Spat collectors were deployed in each site attached with iron poles at five-meter interval each along line transects up to 2 m depth (Figure 3). Prior to deployment in rocky habitats, the bottom was probed using an iron pole to ensure placement onto hard substrate.

The quantity and size of oyster spat and co-occurring fouling organisms on the collectors were quantified during two seasons: late summer (January-April) and late winter (July – October). In each season, 40 collectors were deployed at each of the four sites, in total 160 collectors. At intervals of 1, 2, 3 and 4 months, 10 tiles were collected from each site. The 10 collectors removed monthly at each site were used to evaluate the size and number of live and dead oysters together with the abundance and diversity of fouling fauna (Papers **IV and V**). Tiles collected after 1 month in both seasons were not analysed due to lack of macroscopically visible settlement. At the end of the experiment, the size of oysters (hinge length) was measured to assess the growth. For size measurement, only tiles collected after 3 and 4 months were considered (Paper **IV**).

In the laboratory, the ceramic tiles were stored in chest freezers until analysed and archived. Samples were examined at 20X using NIKON Stereoscope to identify and quantify spat. Spat abundance were validated when two consecutive counts agreed within 5%. The presence of other fouling organisms such as mussels, gastropod, barnacles and others were also counted.

Figure 3: Deployment scheme of the tiles in the field

3.3 Data Analysis

This thesis used a variety of statistical methods for both uni- and multivariate data. Among univariate methodologies, we used Students' t-tests (Paper **I**), a single factor analysis of variance (one way ANOVA; Papers **I** and **II**), Kruskall-Wallis non-parametric test (H) (Paper **I**), multifactorial analysis of variance (multi-factorial ANOVA models; Papers **IV** and **V**), a chi-square test (X^2) (Paper **II**) and simple correlations (Paper **II** and **V**).

Paper **I**: all monthly data recorded in this study was pooled by sampling site over the 3 years sampling period. For quantitative data prior to the analysis, variables (density, length and CPUE) were checked for homogeneity of variances using Cochran's C test and data were transformed as required. Paper **II**: data were grouped into size classes (4.5 mm SL) to detect variations in the sexual proportion as a function of specimen size. Sex ratio data were pooled for the whole study period. In order to identify seasonal trends in gonad development, each gonad stage (i.e., immature, developing, ripe/mature and spent) was reported by size in proportion to the total catch of oysters. Sizes at sexual maturity (SW_{50}) were estimated separately using the proportion of specimens in each 4.5 mm SL size classes with mature gonads and only oysters considered mature (the last developing stage) were used. Paper **IV**: to achieve homogeneity of variances by Cochran tests ($P<0.05$), all settlement data were log(X+1)-transformed, and normality assessed graphically using residual plots. Because overall densities varied strongly among locations, effects of season, duration, surface texture and interactions among those fixed factors were analyses separately for each location (Papers **IV** and **V**). Student-Newman-Keuls test (SNK) was used as *post hoc* test of means using procedures described in Underwood (1997). A simple correlation analysis was carried out to determine whether there is a relationship between the abundance of oysters and barnacles in the rocky habitat (Paper **V**).

CHAPTER 4

KEY RESULTS AND DISCUSSION

4. Key results and discussion

Knowledge about oyster population dynamics within a specific spatial area is vital for the effective planning and management of this important fishery resource. The management of fisheries is largely dependent on estimating reproduction and development, mortality, recruitment patterns, and assessing the dynamics and resilience of the current stocks (Kumari et al. 2006, Vivekanandan 2005). In this thesis, I identify historic trends in oyster exploitation, reproductive activities, recruitment and examine how fouling fauna affects oyster spat recruitment. These are all aspects of great significance for sustainable management of naturally occurring oysters as well as for the development of more productive aquaculture efforts.

My results suggest that the two oyster species have different levels of exploitation. The pearl oyster has been intensively exploited and this has contributed to the low density recorded in the sites where it occurs on the island (Paper **I**). The results obtained on the reproductive cycle indicate that both species show continuous reproduction throughout the year with high peaks in the warmer months of the year and very low peaks in the colder months (Paper **II**). These findings coincided with those obtained on larval settlement, which also showed the same pattern (Paper **IV**). Environmental factors such as temperature and salinity have been identified as crucial for the development of oyster larvae, especially for the rock oyster *S. cucullata* (Paper **III**).

The transition from pelagic to benthic life, larvae are likely to come into contact with many other invertebrates living in oyster habitats. Of these, fouling fauna are generally believed to reduce the settlement of interspecific larvae through competitive exclusion and predation. The combined roles of both positive and negative interactions between oyster larvae and fouling fauna require evaluation under field conditions. The results from this thesis highlight the need for clarification of these roles to more thoroughly understand the settlement behaviour and mortality sources of recruiting oyster larvae and consequently, to optimize oyster restoration efforts (**Paper V**).

4.1. Oyster exploitation

The results obtained in this study indicate that the most exploited oyster species on Inhaca Island is the pearl oyster *P. capensis*. The density recorded for this species (14 ± 6 ind. m^{-2}) was lower than for rocky shore oyster *S. cucullata* (61 ± 7 ind. m^{-2}). Intense collection by local inhabitant of Inhaca Island in intertidal areas of Inhaca Island during spring low tides could explain these differences. Among the locations where *P. capensis* occurs, Nolwe and Inguane had highest densities. These locations are areas which is difficult to access, and it are permanently submerged. The decrease in abundance and size of the pearl oyster *P. capensis* throughout the study period was evident, even in fishing grounds, which in the past yielded high densities of oysters according to the answer of the questionnaire made by harvesters. In contrast, there was no evidence of decreasing abundance and size of individuals of *S. cucullata* during the study period. This is probably due the lower level of exploitation of this species at Inhaca Island.

Oyster shell size frequencies varied from 21–30 mm size intervals to 81–90 mm for *P. capensis* (Figure 4A) while for *S. cucullata*, the size frequencies varied from 11–20 mm to 71–80 mm along the sampling sites (Figure 4B). Oysters of smaller sizes were recorded in the shallower sites such as the seagrass banks of Bangua and Sangala. For *S. cucullata*, individuals larger than 70 mm were recorded at Ponta Torres. Other sites such as Farol and EBMI recorded the highest number of oysters smaller than 40 mm. The dominance of small size oyster in easily accessible areas may be associated with the high harvesting pressure in these areas. During the field-sampling period, we noted harvesting pressure on the pearl oyster *P. capensis*. Additionally, in some shallower and more accessible areas, there is evidence of unselected oyster harvesting. In the absence of regulations of minimum oyster size, harvesters target all sizes, especially immature oysters, affecting natural populations to self-recruit, leading to overexploitation (Strayer 2006). The prevalence of smaller oysters in more accessible shallow waters poses a long-term threat to the population, as these individuals may never attain sexual maturity and reproduce (Rius et al. 2006). Throughout the world, unregulated harvesting of bivalves is known to adversely affect wild stocks (Haupt et al. 2010)

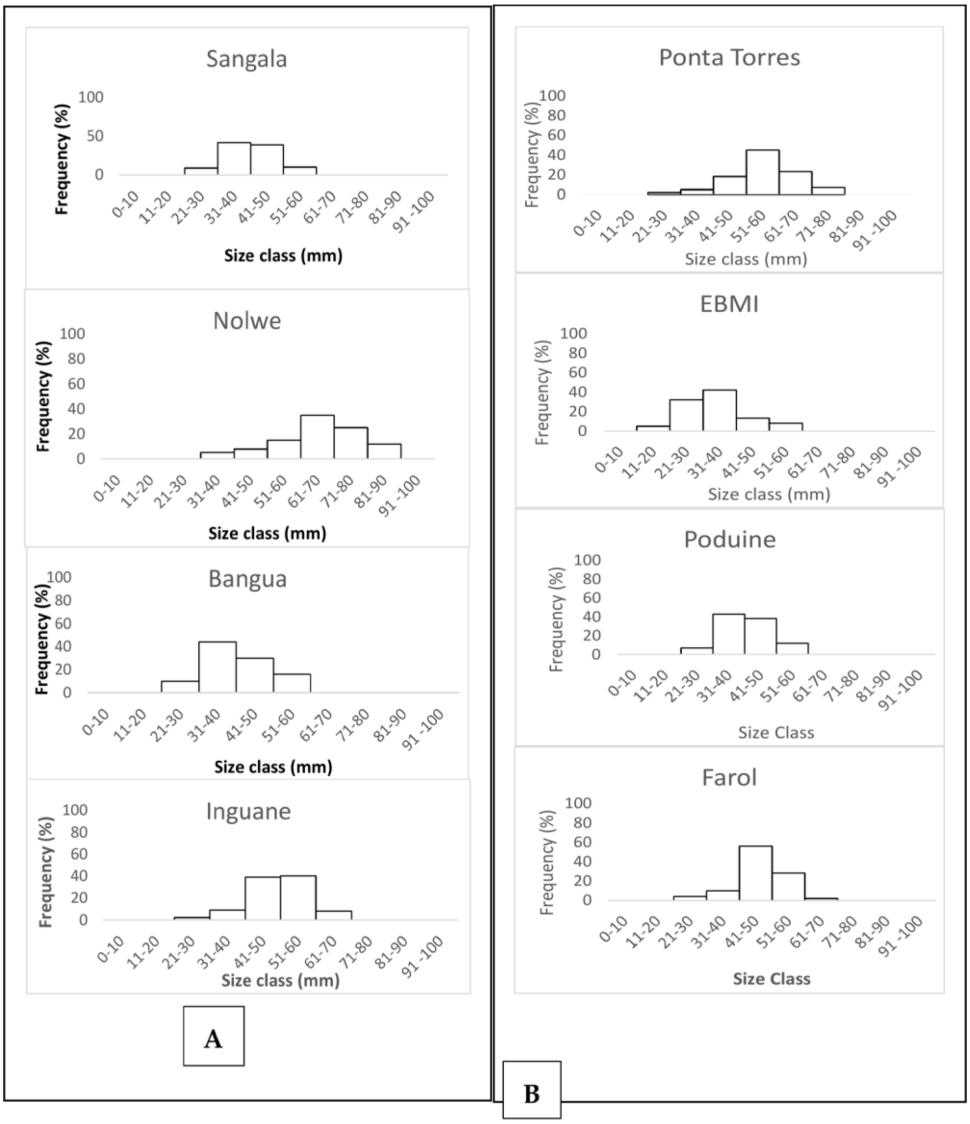

Figure 4: Length frequency distribution of the shells for *P. capensis* (**A**) and Height frequency distribution of the shells for the rocky shore oyster *S. cucullata* (**B**) from the sampling sites on Inhaca Island.

4.2 Reproductive status of the oysters

Reproduction is one of the most important physiological processes in the life cycle of any bivalve including oysters (Enríquez-Díaz et al. 2009). A more thorough understanding of these reproductive cycles allows for better determination of the optimal moments for harvesting, with the aim of obtaining oysters with a higher meat weight before spawning and seed collection. The results obtained in this study (Paper **II**), provides new and extensive information about several life-history characteristics, in particular reproductive aspects of two common and important oyster species in southern Mozambique. This information can help contribute to a more sustainable use of these valuable species in the area and may also bring some light to biological and ecological aspects of similar species in tropical and sub-tropical areas.

4.2.1 Sex ratio, condition index and reproductive condition of the oysters

There was high predominance of females over males for both pearls and rocky oysters during the study period. The sex ratio (Male:Female) was 1:1.5 for *P. capensis* and 1:1.6 for *S. cucullata*. The predominance of females over males and the occurrence of hermaphrodites in natural populations observed in this study is consistent with what has been found in many other studies on oysters' reproductive activities. The pearl oyster sex ratio showed a clear dominance change from male to females with increasing size classes. The majority of individuals <45 mm SL were males and females were more common from 55–60 mm to upper size of 90–95 mm. For rock oysters, most individuals <40 mm SL were males. Above this size, the proportion of females increased progressively, reaching 100% at the upper size classes.

Values of the condition index (CI) for both oyster species varied during the study period and over the year. CI showed a tendency to increase with rise of temperature and decrease in periods of low temperature. CI can be indicative of the reproductive stage and/or nutritional status of individuals (Aswani et al. 2004). A high CI in warmer seasons can be justified by the greater availability of food during this period. Many studies have attempted to explain the effects of environmental parameters on gonadal development and reproduction of different species of bivalve molluscs. Various factors such as temperature, salinity, and food availability in the environment affect the gametogenic cycle of bivalves (Kang et al. 2000).

For both species, the results indicate that a large proportion of the population spawns in summer with a peak from January to March and a minor peak from October to December. The remaining period of the year is characterized by a lower proportion of spawning individuals for both species (Figures 5 and 6). The reproductive cycle of the oysters is affected by the seasons correlating to seawater temperature. The recorded data of temperature and histological results in this study showed that oysters at the early development stage were first detected during the months when the temperature of seawater was close to 21°C. Animals in the late growing stage began to appear during October, when the average temperature rose above 24 ◦C. As the temperature continued to rise from November through March, oysters reached the mature and spawning stages. Overall, the histological analysis demonstrated that oyster gametogenesis is continuous during the year and tends to be more intense in the summer periods when seawater temperature rises and lower or absent during the winter when seawater temperature drops.

The results on the size at sexual maturity revealed that males of *P. capensis* mature earlier at 26.5 mm SL compared to females at 27 mm SL and males of *S. cucullata* also mature earlier at 28.3 mm SL compared to females at 32.8 mm SL, as is generally the case for most oyster species (Pouvreau 2000).

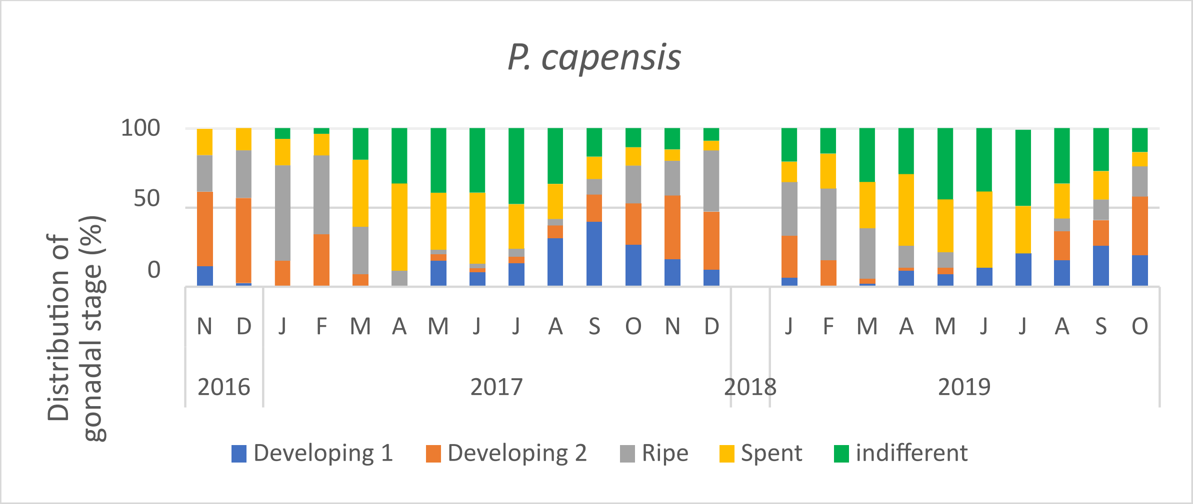

Figure 5: Monthly frequency distribution of gonadal development stages (males and females combined) of *P. capensis* in Inhaca Island.

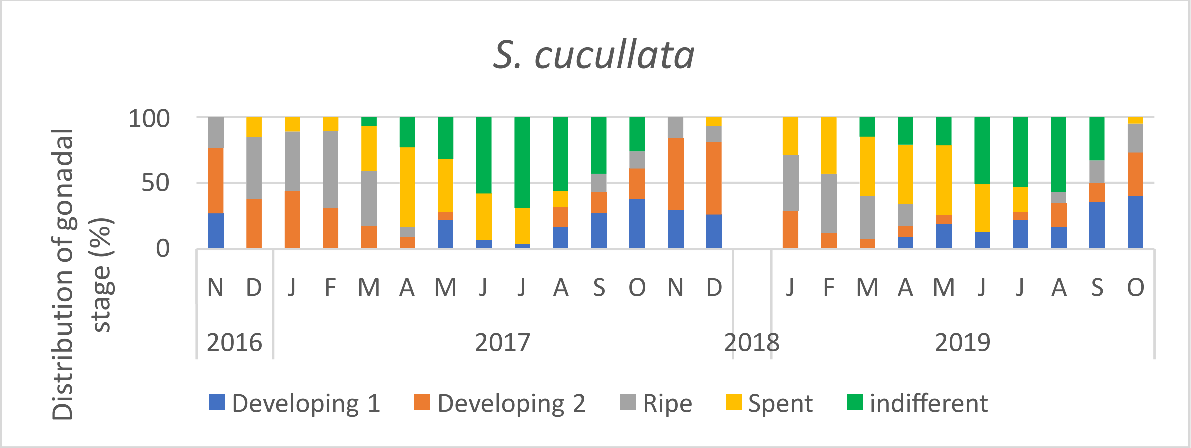

Figure 6: Monthly frequency distribution of gonadal development stages (males and females combined) of *S. cucullata* in Inhaca Island.

4.3 Oyster larvae development in laboratory conditions

The growth and mortalities of *S. cucullata* larvae after 7 days was affected by different combination of temperature and salinity. The results showed that *S. cucullata* larvae grew fastest at 30 °C temperature x 35 salinity. However, the highest survival of larvae was obtained at 34 °C temperature x 35 salinity (Figure 7 and 8). 30 °C is well above the average annual temperature of 25 ± 1 °C at Inhaca Island, where the brood stock was collected. According to Kalk (1995) the highest average is 27.5 °C and the lowest 21.7 °C. The mean salinity level at Inhaca is 33 ± 2 (de Boer et al. 2000) with a range from 32 to 42 (Pinto 1996). This confirms the findings of Kalyanasundaram & Ramamoorthi (1986) that *S. cucullata* larvae tolerate a wide of temperature and salinity variation though with different growth and survival trends.

In general, oyster larvae tolerate a wide salinity range between 25 and 40 (Lemos et al. 1994). However, this study showed that salinity affects the growth of *S. cuccullata* larvae, with the lowest performance at salinity 30. It may be possible that osmotic disturbances lead to energy allocations that ultimately impair other functions such as growth (Deaton 2008). Temperature is regarded as the most important environmental factor influencing bivalve culture (Robert et al. 1988, Helm et al. 2004). As shown in Paper **II**, temperature significantly affected gonad maturation and spawning for both rock and pearl oysters.

The lowest larvae survival in the present study (26.4 %) was achieved at salinity 40, suggesting this is a threshold salinity level for *S. cuccullata* larvae. All treatments at 24 °C resulted in significantly lower survival rates (Figure 8). This seems to be the lower temperature limit tolerated by the larval stages of this species. Another factor to consider is the abrupt submission of the larvae to low temperatures without any previous acclimation, which may have caused a thermal shock and contributed to a decrease in survival rates.

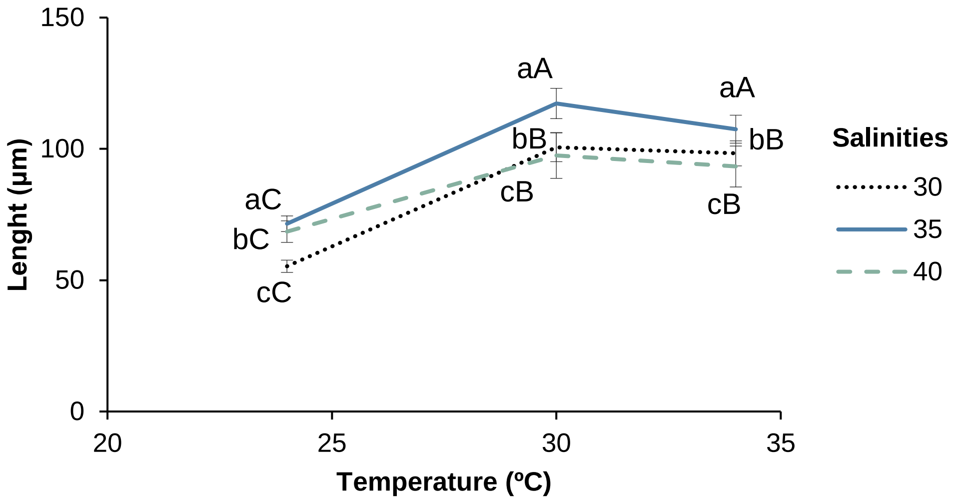

Figure 7: Means (± standard deviation) of length of larvae of the rock oyster, *S. cucullata* reared at combinations of temperature (24, 30 and 34) and salinity (30, 35 and 40) for seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity (p< 0.05).

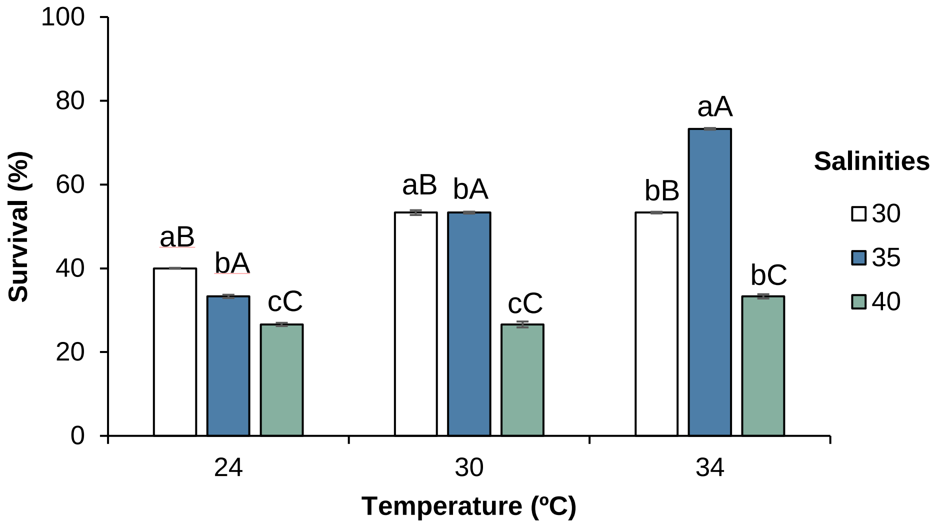

Figure 8: Percentage of survival of rock oyster, *S. cucullata* larvae reared at combinations of temperature (24, 30 and 34 ºC) and salinity (30, 35 and 40) after seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

4.4 Oyster settlement

Understanding temporal and spatial variation in oyster larvae settlement and oyster recruitment dynamics provides invaluable information on site selection and strategies employed for oyster culture in their natural environment (MacKay et al. 2016). In this study, I have studied seasonal patterns of *P. capensis* and *S. cucullata* spat recruitment and growth in different habitats at Inhaca Island. Overall, the results showed that *S. cucullata* recruited in larger densities (up to ≈100 per tile) than *P. capensis* (up to ≈3 per tile). As shown in Paper **I**, the density of adult oysters of *P. capensis* was lower in all sites where it was recorded. This could potentially explain the lower density of recruitment recorded for this species in this study.

Larvae of *S. cucullata* and *P. capensis* recruited more extensively on rough surfaces in all sites of the field experiment. Spat appear to prefer the rough surface of tile than the smooth. Surface roughness has previously been reported to improve settlement of a wide range of invertebrates including oyster larvae (Saucedo et al. 2005). In other species, such as the silver-lip pearl oyster *P. maxima (*Taylor et al 1998) reported that roughened texture of the PVC slats provides better tactile stimuli to crawling pediveliger or spat than smooth surface monofilament nylon in the pearl oyster.

There were large differences in oyster spat settlement among sites, particularly for *S. cucullata* (Figures 9 and 10). This illustrates the complexity of the recruitment process and the dependence on external environmental factors. Thus, the high settlement rate attained at EBMI in late summer compared to that recorded at Ponta Torres for *S. cucullata* could be related to different environmental condition in these sites. Collectors deployed at EBMI remained immersed most of the time during the entire sampling period. This may have favoured the recruitment of oysters where the level of exposure is lower and where the collectors were exposed only in spring and low tides period. Buttrago & Alvarado (2005) limited the encrusting organisms by exposing the collectors out of the water for at least 3 hours each week and suggested that studies should be done to determine the effectiveness of different periods of emersion of the collectors.

The analyses of the two temporal aspects of the experiment, season (summer vs. winter) and duration (2, 3 and 4 months of deployment) suggested that duration was more important than season. The largest recruitment was almost consistently observed after 4 months both in summer and winter. This indicates that larvae were available during both seasons, even though oyster settlement was higher in late summer than in late winter for both species. This pattern is fully consistent with what was observed in Paper **II** of which the increase in temperature during the summer season was a fundamental factor in the release of the gametes of *S. cucullata* and *P. capensis* at Inhaca Island. Increase in the frequency of mature oysters during the later summer season (which have higher temperature) may be related to the higher concentrations of organic matter reported in the water during this period (Paula et al. 1998). Pouvreau et al. 2000 also suggested that the suspended organic matter is necessary for gonadal maturation of the oyster since that gamete production requires considerable energy input (Paper **III**).

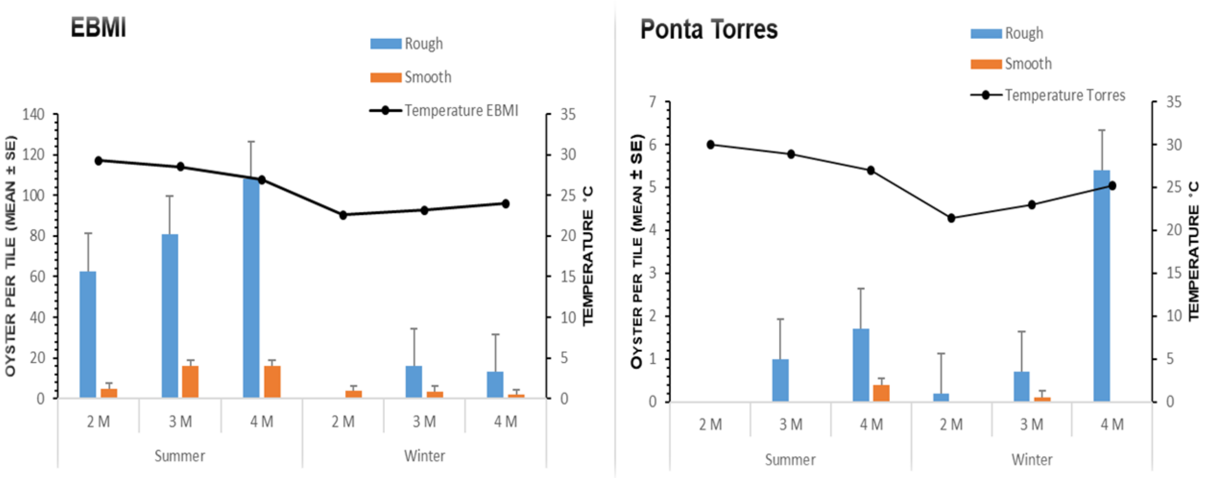

Figure 9: Number (mean±se) of settled *S. cuccullata* and measured temperature at EBMI and Ponta Torres in different seasons, durations and surface texture (note the different scales on y-axes).

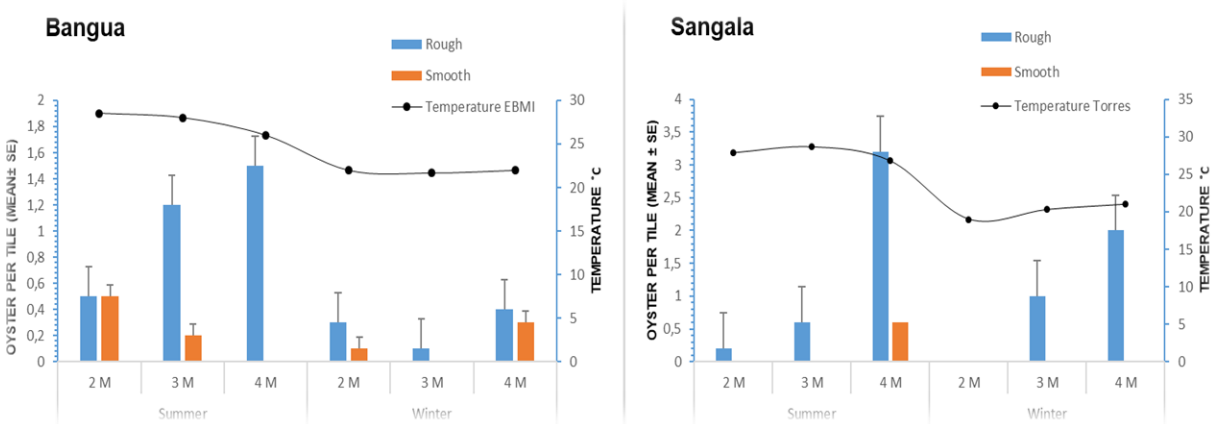

Figure 10: Number (mean±se) of settled *P. capensis* at Bangua and Sangala in different seasons, durations and surface texture (note the different scales on y-axes).

The sizes of oyster spat recorded after 3 months and at the end of experiment were quantified. *S. cucullata* spat attain a large size of 5.8 mm with an average of 3.6 mm. *P. capensis* spat attain a large size of 7.6 mm with average of 5.01 mm. For *P. capensis* there was a tendency towards more homogeneous size after 4 compared to 3 months of the experiment while for *S. cucullata* the relative variability in size was quite similar in both months of measurement. Overall, growth rates were 40-50% greater for *P. capensis* and both species tended to have increasing growth rates the longer the duration.

4.5 Fouling fauna on oyster collectors

Fouling fauna generally reduce the settlement of larvae of different species through competitive exclusion and predation. In Paper **V** fouling fauna was evaluated over 8 months period in two seasons on oyster collectors deployed in seagrass and rocky habitats (Paper **IV**). The results suggest that the amount and composition of fouling fauna varies among habitats, locations, seasons and surfaces and depend on the duration of the deployment. Barnacles dominate among the fouling on collectors. As observed for oysters spat settlement in Paper **IV**, collectors used in this study were almost fully covered by the third month, while the collectors never had any fouling organism at the end of the first month of immersion. In seagrass habitats, oysters and barnacles were generally less abundant, allowing other taxa to have higher relative abundance, while higher density of individuals was recorded in rocky habitat. (Figure 11). This is evidence of the importance of seagrass meadows as a repository of higher diversity. Seagrasses provide complex structural habitat and suitable environmental fauna assemblages conditions than rocky shore habitat (Vonk et al. 2010). Structurally complex habitats typically support dense, diverse faunal assemblages and can influence predator– prey dynamics (Lemire & Bourget 1996).

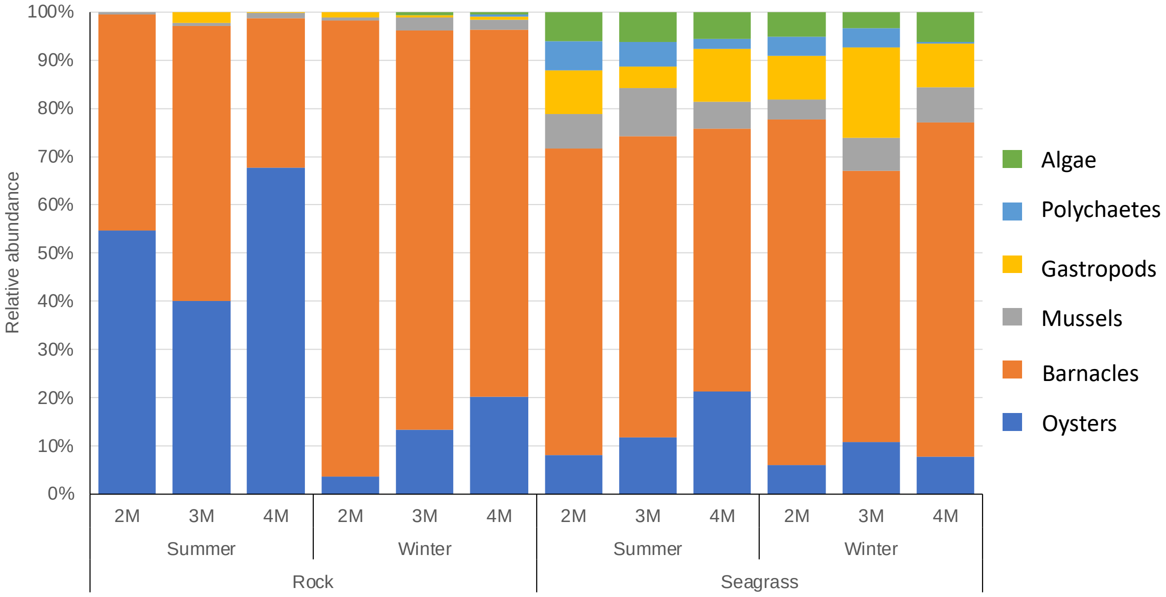

Figure 11: Relative abundance of fouling fauna on oyster collectors after 2, 3 and 4 months in rocky and seagrass habitats at Inhaca Island.

4.5.1 Abundance of fouling organisms

Due to complex and large variability in the total abundance of fouling organisms, the analyses of spatial and temporal variability revealed only significant variability among locations within habitats (See appended Table 1, Supplement S1 Paper **V**). In particular, there were large differences between the two rocky shore locations. Substantially more fouling was found on the less exposed EBMI than at Ponta Torres. The high fouling rate attained at EBMI in late summer compared to that recorded at Ponta Torres for oysters and barnacles could be related to different environmental condition in these sites. Coastal hydrodynamics can affect the dispersal of invertebrate larvae (Meyer 2021, MacKay et al. 2016, Adams et al. 2013).

More conclusive patterns were observed for individual taxonomical groups. For example, significantly more bivalves were found on rough than on smooth surfaces, and bivalves, gastropods and polychaetes all showed significantly larger abundances in seagrass than in rocky habitats. The estimated abundance of barnacles, on the other hand, was consistently larger in rocky habitats, but similarly to the oysters, the large variability among locations made it difficult to statistically detect a difference between habitats.

4.5.2 Abundance of oysters in relation to barnacles

To assess potential interactions between oysters and other fauna, bivariate correlations with the other dominant competitor for space, barnacles, were done for individual locations and seasons (Table 2). Mussels, gastropods and polychaetes occurred at very low densities and any influence on the oyster recruitment were therefore not tested. Three out of eight tests indicated significant relationships between abundances of barnacles and oysters. As might be predicted from competitive interactions, all of these instances were negative correlations. The negative correlation between oyster and barnacles recorded at EBMI could potentially be related to ecological interactions, such as competition or larval predation, but they could also be due to responses to external environmental factors, such as resistance to desiccation. Barnacles appear to be more resistant to desiccation (Franken 2015, Marques-Silva et al. 2006, Ruwa & Polk 1994) and there is evidence that they may displace young oysters (Bonicelli et al. 2023, Osman et al. 1989). At Ponta Torres, there was no evidence of a correlation between the number of oysters and barnacles. Surprisingly, in seagrass habitats, where there were generally lower number of oysters and barnacles, two tests showed similar negative correlations (Table). These relationships were, however, weaker than at EBMI (r^2=0.15-0.20). Nevertheless, the fact that all significant correlations were negative suggests that oysters and barnacles occasionally influence each other either by competition or possibly predation.

Table 3: Summary of correlation analyses between the density of barnacles and oysters in individual sites and seasons.

Habitat	Site	Season	R	T	p
Rock	EBMI	Summer	**-0.48**	**-2.88**	**0.01**
Rock	EBMI	Winter	-0.06	-0.31	0.76
Rock	Ponta Torres	Summer	0.04	0.20	0.85
Rock	Ponta Torres	Winter	0.25	1.38	0.18
Seagrass	Bangua	Summer	-0.05	-0.27	0.79
Seagrass	Bangua	Winter	**-0.47**	**-2.78**	**0.01**
Seagrass	Sangala	Summer	**-0.39**	**-2.25**	**0.03**
Seagrass	Sangala	Winter	0.17	0.90	0.38

CHAPTER 5

CONCLUDING REMARKS

5. Concluding remarks

This thesis contributes to the knowledge on aspects of population dynamics of two commercial important oyster species in the country. This information is necessary for possible future development of small and large scale aquaculture in Southern Mozambique. I believe that the results obtained demonstrate that the biological prerequisites are in place, to warrant further studies addressing additional challenges for developing sustainable oyster cultivation in these parts of Mozambique. Some of the most important findings in this study allowed to answer the questions posed at its early stages and demonstrating that:

- The two oyster species *Pinctada capensis* and *Saccostrea cucullata* are valuable resources for the communities living on the Inhaca Island and that the most exploited species is the pearl oyster *P. capensis*. The perception of harvesters regarding their harvesting habits and awareness about the state of the stocks have also been identified and constitute an important component to consider when implementing management and monitoring measures for the sustainable exploitation of oyster resources. The analysis contributes to improving knowledge on both *P. capensis* and *S. cucullata* fisheries and for an urgent call for the introduction of harvest management measures, especially for the most exploited species *P. capensis*. It is hoped that it will provide an incentive to value all the ecosystem services provided by oyster habitats at a local level.

- The results of the histological analysis demonstrated that oyster gametogenesis is continuous during the year but tends to be more intense in the summer when the seawater temperature rises and lower or absent during the winter when seawater temperature drops. This justifies the greater numbers of recruitment recorded in summer than in the winter. This information should also be accounted for when drawing up measures for the sustainable use of the resource (e.g. by establishing closed seasons to allow the oysters to reproduce and grow to commercial size).

- The optimal temperature and salinities for oyster *S. cucullata* larvae development range from 27°C to 33°C and from 32ppm to 35ppm respectively. Together with the optimal water salinity and temperature for larval rearing established here, this may contribute to the further development of the culture of *S. cucullata*. These findings related to the environmental variable thresholds for *S. cucullata* larvae are relevant for future studies on recruitment, as well as to evaluate the direct impact of global warming on this species.

- Spat collection of the rock oyster, *Saccostrea cucullata*, and the pearl oyster, *Pinctada capensis*, is possible using the simple and cheap ceramic tiles used in this study. Because we only evaluated one type of material, we cannot exclude the possibility that other materials may work. Higher densities of recruitment were recorded in *S. cucullata* than *P. capensis* and both species recruited at larger densities on the roughly textured side in the tiles used in this study. However, it is likely that a slightly roughened surface may be advisable for both species. Furthermore, deploying these types of tiles for as little as four months could be an efficient way to collect large numbers of *S. cucullata* as well as *P. capensis* spat in their respective habitats. Apart from the applied aspects, these results also improve our understanding of spatio-temporal patterns and processes affecting the biology of the two species of oysters.

- Apart from oyster recruitment, other fouling fauna recruited on oyster collectors. The effects of other fouling fauna on oyster abundance appear modest up to 4 months after tiles are placed. These results, provide valuable information on the multiple roles of the oyster spat collectors as a suitable substrate for a variety of other species of larvae. They serve as nursery habitats for important source of food for different species including humans but also as a baseline for a variety of manipulative studies in the southeastern Indian Ocean region.

- More reliable knowledge about levels of recruitment and habitat preferences in a local context is critical for future attempts to restore and manage natural populations as well as for developing local practices for aquaculture

- The results improve our general understanding of the spatio-temporal patterns and processes that affect the colonization of intertidal invertebrates in the southeastern Indian Ocean.

Overall, the results of this work provide information about spatial and temporal patterns and fundamental population ecological parameters of the rock oyster *Saccostrea cucullata* and the pearl oyster *Pinctada capensis*. Patterns of current use of natural oyster populations by local residents and methods for supporting production by enhancing recruitment have also been assessed in the Mozambican context. Used wisely, I believe that this knowledge can contribute to a much needed ecologically, socially and economically sustainable industry in the area.

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ANNEXES

Printed copies of articles published in scientific journals as part of this Doctoral Thesis

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Article

Catch per Unit Effort, Density and Size Distribution of the Oysters *Pinctada capensis* and *Saccostrea cucullata* (Class Bivalvea) on Inhaca Island, Southern Mozambique

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Abstract: Oysters are important resources for the daily diet, a source of economic income for many coastal communities and a delicacy for the tourism industry. In this study, the oysters (*Pinctada capensis* and *Saccostrea cucullata*) were investigated with the aim to assess the catch per unit effort, density and size composition. The study was conducted over a three-year period on Inhaca Island, Southern Mozambique. For both species, perception of fishery trends from collectors was carried out through habitat censuses and interviews. Transects, quadrats and daily fisheries catches approaches were used. Results show that *P. capensis* is the most exploited on the island. A total of 72.1% of respondents pointed that the oyster *P. capensis* is decreasing, due to excessive catching (75.4%) followed by natural death (24.6%), while 20.9% affirmed that the resource is stable and 7.0% are unaware about the resource trend. Oyster densities, sizes and catches per unit effort were higher in less accessible areas only for *P. capensis*. The present study provides valuable baseline information to recommend best practices to improve the exploitation, and access the need for introduction of aquaculture, towards the sustainable management and conservation of oysters, and ultimately to ameliorate people's livelihoods.

Keywords: intertidal resources; pearl oyster; rock oyster; coastal communities; Western Indian Ocean

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1. Introduction

Oysters are widely distributed around the world. Some oysters are key species for the ecology of coastal and estuarine areas [1]. They provide important ecological services such as improving water quality, helping stabilize the coastline and protecting erosion by building their reefs, and they are also an important product of fisheries and aquaculture in many countries [2].

In tropical regions, oysters occur in rivers and coastal areas, with most species assembled in narrow bands or dense banks at a tidal range where desiccation, fouling and predation are minimized [3]. Oysters are targeted by small-scale fisheries and their harvest is usually handmade by residents, mainly by women and children, at the spring low tide period [4,5].

Oysters contribute as a source of animal proteins and to the income of coastal communities. Not surprisingly, their increasing demand in recent years has promoted a global decline on stocks due to overfishing [6], pollution, habitat destruction and disease, especially in Africa where small-scale fishing is considerably unregulated [1,7].

Mozambique has a long coast with a wide diversity of habitats, providing a variety of resources, which include fisheries' resources, supporting the livelihood of more than half of the coastal population [8]. Among the fisheries' resources, the most collected bivalves are clams and oysters. The importance of each group varies depending on their abundance and the needs in different areas [9]. In southern Mozambique, the sand oyster or pearl oyster *Pinctada capensis* is found associated to seagrass beds, and the rock oyster *Saccostrea cucullata* is associated with rocky shores [8,10], and considered the most harvested bivalves with economical relevance [11].

On Inhaca Island, situated 32 km from Maputo city, the exploitation of *P. capensis* and *S. cucullata* in intertidal areas has been carried out for several generations and probably for some centuries [12]. Oyster harvesting is one of the main subsistence activities practiced by the population living on this small island. There is a great dependence of the population on the resource due to the lack of subsistence alternatives [13].

Besides this provisioning role to the population, oysters and oyster reefs usually provide other relevant ecosystem services, namely, water quality improvement, seashore stabilization, carbon burial, habitat provisioning for mobile fish and invertebrates, habitats for epibenthic fauna and diversification of the landscape [14]. Human exploitation can affect the distribution and abundance of bivalve populations, which can compromise the ecosystem and fisheries' management approaches [15].

Despite their biological and socioeconomic importance on Inhaca Island, oysters have not been studied to assess their abundance, size distribution and perceptions of the status of stocks by fishermen. Therefore, the present study investigated trends in *P. capensis* and *S. cuculata* abundance, size distribution and catch-per-unit effort over a 3-year period (January 2015–December 2016 and January–December 2019). This information is critical to improve knowledge of the fisheries and support management decisions regarding the sustainable exploration of these living resources.

2. Materials and Methods

2.1. Study Area

The study was carried out on Inhaca Island (latitude 26°07′ S, longitude 32°56′ E), southern Mozambique, located 32 km off Maputo City, Mozambique (Figure 1). The Island has a total area of approximately 42 km^2 and is part of the Ponta de Ouro Partial Marine Reserve. It is located on the border between the shallow Bay of Maputo and the open waters of the Indian Ocean, in a transition zone from tropical to sub-tropical climate, which creates a rich diversity of both terrestrial and marine ecosystems [16].

There are two distinct seasons: the hot and rainy season (from November to April) and the cold and dry season (from May to October) with an average air temperature of 23 °C, and the sea water temperature varying from 18 °C to 22 °C and 23 °C to 32 °C for the cold and hot season, respectively [12]. The eastern part of the Island is characterized by strong currents and waves while the western part is more protected [17]. The tides are semidiurnal and have maximum amplitudes of about 3.1 m in high spring tides [16]. During low tide, a large stretch of beach is exposed, making it an interesting site for the collection of many invertebrates, including oysters [10,17].

The sampling sites were defined along the eastern and western sides of the Island and comprised eight main study areas: 4 in the seagrass meadows (*P. capensis*) and 4 in rocky shore (*S. ucullate*), (Figure 1). Two sites, selected (Sangala & Inguane) for *P. capensis* sampling, were located along the intertidal accessible during low tides, while Nolwe and Banga are located in a bank reachable only by boat during low tides. For *S. cucullate*, both sites were on rocks along the intertidal areas with direct accessibility.

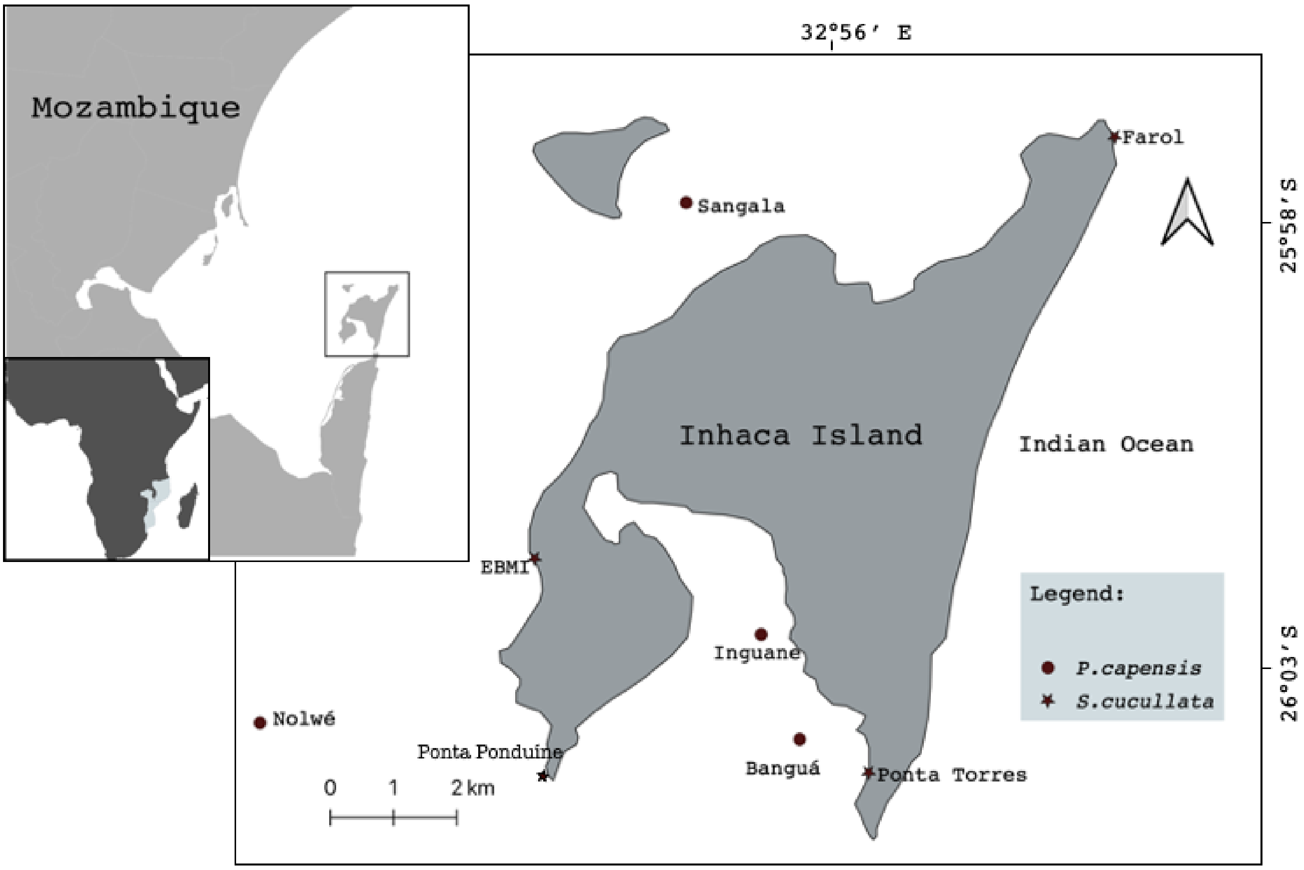

Figure 1. Map of study area showing sampling sites.

2.2. Data Collection

Data on oyster exploitation were collected using two different approaches in a subtropical setting on Inhaca Island. Semi-structured interviews with the local oyster harvesters were conducted using questionnaires to gather data on the socio-economic value of these species. Overall, 73 harvesters were interviewed in the course of the study: 63 harvesters regarding *P. capensis* while only 10 harvesters for *S. cucullata*. The lower number of harvesters for *S. cucullata* is representative with regard to the frequency in the area and reflects the low interest to exploit this species due to the difficult collecting method and being time consuming. Questions focused on obtaining data on harvesting effort (numbers of harvesters per site), catch per harvester and perception of the current state of the resource. Catch (kg) per harvester was obtained by weighing the total product collected daily.

Data obtained during the application of the questionnaires was used to select sites for field work. Sampling was conducted over a three-year period (January 2015–December 2016 and January–December 2019) within four seagrass banks for the pearl oyster *P. capensis*, and at four rocky shore habitats for *S. cucullala*. Samples were collected every spring tide (full and new moons) during the study period. Species abundance, composition in size as well as catch per unit effort were evaluated. In general, subsequent sampling was done at sites that were utilized for harvest. At each selected sampling site, quantitative data were collected using a systematic (0.5 × 0.5 m) quadrats-based approach, placed along randomized 10 m transects (parallel to the coastline as well from the artisanal daily catches). The rocky shore species *S. cucullata* was sampled in the selected locations of the Island by means of (0.5 × 0.5 m) quadrats using a digital camera and samples from the artisanal daily catches. Samples from the digital camera were processed using the Image J Program software version 1.43. Overall, 5 random transects were made for each pearl oyster occurrence site and 5 transects on each site of occurrence of rocky shore oysters.

In each sampling site for both species, biomass per collector (Kg/person/day), number of collectors and oyster sizes were recorded from the catches of the artisanal collectors.

The abundance for each oyster species was assessed determining the density (ind/m^2) by counting all individuals present in the quadrats allocated along the transect.

Shell size (dorsoventral measurement or shell length for *P. capensis* and dorsoventral measurement or shell height for *S. cucullata*) was measured with a caliper to 0.01 mm precision. Shell sizes were measured for all living individuals present in the quadrats and 30 individuals selected randomly from the catches of each collector interviewed. The mean shell size and size distribution frequency were compared between the sampling sites.

2.3. Data Analysis

For statistical analysis, all monthly data recorded in this study was pooled by sampling site over the 3 years sampling period.

Percentages and frequencies were used to analyze the harvester's perception level of the current state of the resources. For quantitative data prior to the analysis, variables (density, length and CPUE) were checked for homogeneity of variances using Cochran's C test and data were transformed as required. One way ANOVA was used to compare variations in density, length and CPUE between sites for *P. capensis* followed by Turkey's post hoc test [18] to assess the significance of difference in the variables (density, length and CPUE). The Kruskall-Wallis non-parametric test (H) was used to compare the *S. cucullata* densities, as data were not normally distributed after transformation [18]. The values of the mean shell size were also compared between the sampling sites. To compare the mean shell size among the sampling sites for *S. cucullata*, one-way ANOVA was used followed by Turkey's post hoc test. T-student tests were used to compare *P. capensis* mean shell size obtained from sampling and oyster harvesters. CPUE (Kg/Harvester/day) per site was estimated after the sum of all weighted catches divided by the number of harvesters. The results are represented as a mean (±standard deviation) and the significance level used for the tests was $p = 0.05$. Statistical analysis was performed using SPSS for windows version 20.

3. Results

3.1. Interviews

The results obtained in this study indicate that the most exploited oyster species on Inhaca Island is the seagrass pearl oyster *P. capensis*. According to the questionnaire, the exploitation of oysters on Inhaca Island is dominated by women (more than 90%). Age of collectors varied from 15 to 58 years old with an average age attaining 37 years. The activities of oyster harvesting and other invertebrates is mostly done during spring low tides.

Of the 73 respondents in the interviews, about 72.1% answered that the pearl oyster *P. capensis* is decreasing in number due to excessive catching (75.4%), followed by natural death (24.6%), while 20.9% meant that the resource is stable and about 7% were unaware about the resource trend (Figure 2A,B).

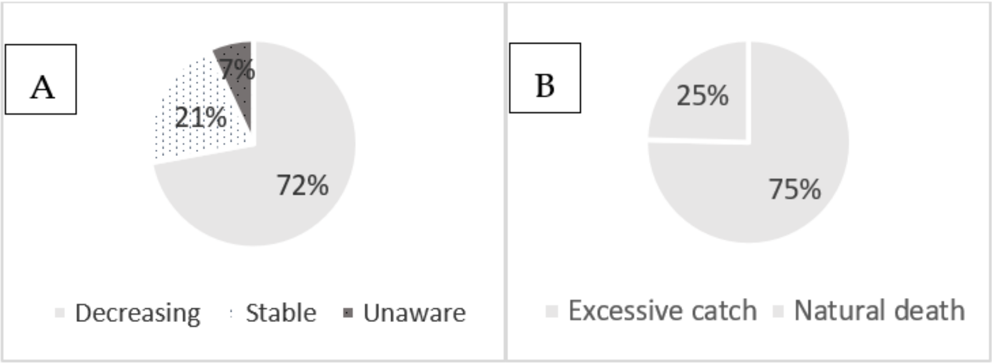

Figure 2. Perception of oyster harvesters about the trend (**A**) and cause of decreasing (**B**) of the resources on Inhaca Island.

3.2. Field Sampling

The densities recorded during the three year period of study for both species are presented in Table 1. Maximum density for pearl oyster *P. capensis* with 14 ± 6 ind/m^2 was recorded at Inguane and lower density occurred at Sangala with 4 ± 3 ind/m^2. The density of *S. cucullata* was higher than *P. capensis*, with higher values recorded at EBMI compared to other sites. There were statistically significant differences among sites in density for both oyster species ($p < 0.05$).

Table 1. Mean (±SD) density of the oyster *P. capensis* and *S. cucullata* recorded in each sampling sites on Inhaca Island. (Different letters in the same column indicate significant differences $p < 0.05$).

Pinctada capensis		***Saccostrea cucullata***	
Site	**Density (ind/m^2)**	**Site**	**Density (ind/m^2)**
Bangua	11 ± 2.8 ^{a}	EBMI	61 ± 7 ^{a}
Inguane	14 ± 6 ^{a}	Farol	50 ± 5 ^{b}
Nolwe	11 ± 3.5 ^{a}	Ponta Ponduine	35 ± 4 ^{c}
Sangala	4 ± 3 ^{b}	Ponta Torres	51 ± 8 ^{b}

Size distribution of the oysters for both species varied among different sites. Overall, for *P.capensis* oysters, length varied from 19 at Sangala to 88.8 mm at Nolwe (Table 2). Higher mean size length was recorded at Nolwe while the lower mean size was found at Bangue (Table 2).

Table 2. Mean (±SE), minimum and maximum shell length of pearl oyster *P. capensis* in the sampling sites on Inhaca Island. (Different letters in the same column indicate significant differences $p < 0.05$).

Site	**Mean ± SE (mm)**	**Minimum (mm)**	**Maximum (mm)**
Bangua (n = 364)	31.9 ± 9.9 ^{a}	21.5	56.6
Inguane (n = 466)	35.3 ± 8.3 ^{a}	19	70.2
Nolwe (n = 360)	59.8 ± 18.6 ^{b}	24.9	88.8
Sangala (n = 176)	32 ± 17.8 ^{a}	20.7	60

S. cucullata varied in length from 13 at EBMI to 79.4 mm at Ponta Torres (Table 3). The higher mean size for this species was recorded at Ponta Torres while the lower mean size was reported at EBMI (Table 3).

Table 3. Mean (±SE), minimum and maximum shell height of rocky shore oyster *S. cucullata* in the sampling sites on Inhaca Island. (Different letters in the same column indicate significant differences $p < 0.05$).

Site	**Mean ± SE (mm)**	**Minimum (mm)**	**Maximum (mm)**
EBMI (n = 105)	30.1 ± 6.2 ^{a}	13	51.3
Farol (n = 245)	30.6 ± 7 ^{a}	21.6	63
Ponta Punduine (n = 102)	33.2 ± 6.9 ^{a}	22.9	55.4
Ponta Torres (n = 106)	42.9 ± 8.8 ^{b}	24	79.4

Oysters of smaller sizes were recorded in the shallowest sites such as the seagrass banks of Bangua and Sangala (Figure 3). For *S. cucullate*, individuals larger than 70 mm were recorded at Ponta Torres. Other sites such as Farol and EBMI recorded the highest number of oysters smaller than 40 mm.

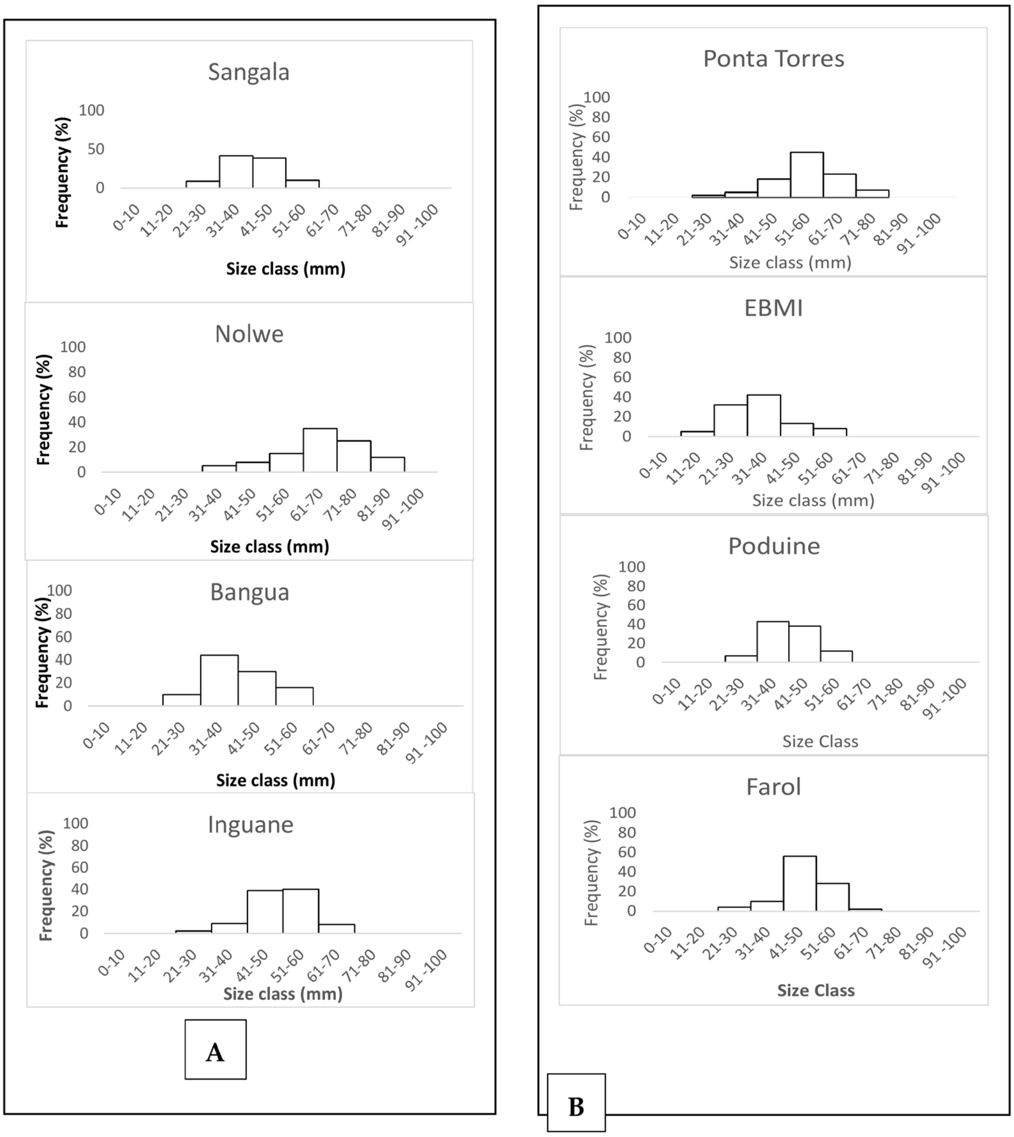

Figure 3. Length frequency distribution of the shells for *P. capensis* (**A**) and Height frequency distribution of the shells for the rocky shore oyster *S. cucullata* (**B**) from the sampling sites on Inhaca Island.

Oyster shell size frequencies varied from 21–30 mm size intervals to 81–90 mm for *P. capensis* (Figure 3A) while for *S. cucullate*, the size frequencies varied from 11–20 mm to 71–80 mm along the sampling sites (Figure 3B).

The modal size frequencies varied between sites. Nolwe presented the higher modal size at 61–70 mm, for Bangue and Sangala at 31–40 mm while Inguane was at a 51–60 mm size interval for *P. capensis* (Figure 3A). For *S. cucullata* oysters, higher modal size frequency was recorded at 51–60 mm at Ponta Torres, 31–40 mm at EBMI and Ponta Punduine, while for Farol, they were at a size interval of 41–50 mm (Figure 3B).

Overall, mean shell sizes of oysters harvested by collectors were higher than the oysters collected from the sampled quadrats. However, statistical analysis only showed significant differences between the sizes of *P. capensis* collected at the Nolwe site ($p < 0.05$) (Figure 4).

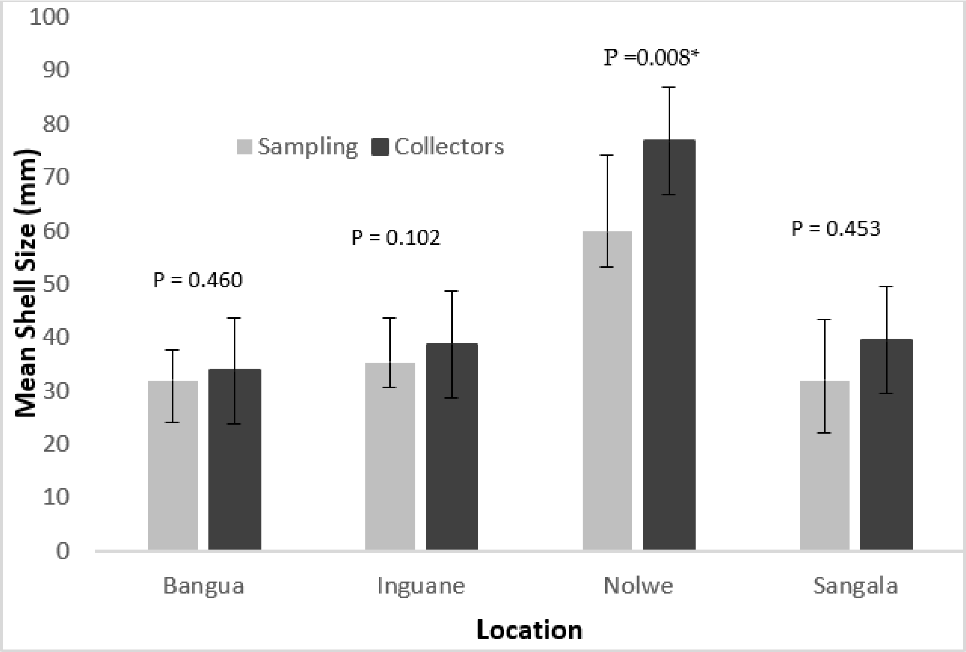

Figure 4. Mean shell size of the oyster *P. capensis* recorded from quadrats sampling and from the harvesters in the sampling sites on Inhaca Island. (* There was statistical significance difference).

In the shallower areas (Bangua, Inguane and Sangala), there were no significant differences between the oyster size harvested by collectors and that recorded in the field ($p > 0.05$) (Figure 4).

Capture per unit effort of *P. capensis* from the sampling sites on Inhaca Island are presented in Figure 5. The CPUE along the sites showed very low catches in 3 sites (Sangala, Inguane and Bangue 7–15 Kg/collector/day) comparatively to Nolwe where catches attained a 5–10 times higher number (75 kg/collector/day), Figure 4. Catch per unit effort (CPUE) among sites was significantly different (Anova I, $p < 0.05$).

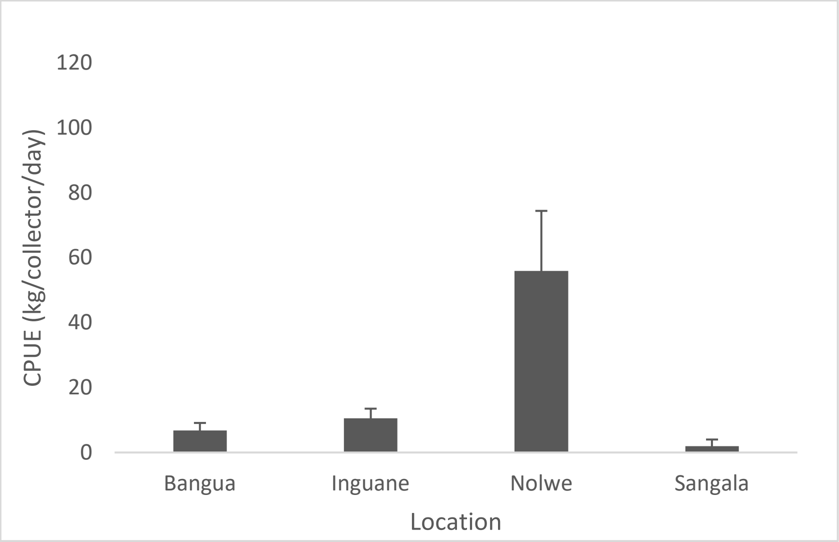

Figure 5. Capture per unit effort of *P. capensis* from the sampling sites recorded on Inhaca Island.

As there was a low preference for collecting the rocky shore oyster *S. cucullata*, it was not possible to determine catch per unit effort for each sampling sites for this species.

4. Discussion

This study is the first attempt to assess catch per unit effort, density and size composition of the pearl oyster *P. capensis* and rocky shore oyster *S. cucullata* occurring at the coastal areas of Inhaca Island, Mozambique.

The densities recorded in this study for *P. capensis* (4–14 oyster/m^2) were lower than those recorded in other regions of Mozambique such as Vilanculos and Inhassoro with 9–20 oyster/m^2 [19], Bazaruto Island with 21–260 oyster/m^2 [20] and in other geographic regions such as the Qatar Gulf of Arabia with 32–45 oyster/m^2 [21], EL Gimsha bay of Red Sea with 164 oysters/m^2 [22] and Venezuela with 16 to 50 oyster/m^2 [23]. Two explanations could be given for this. Firstly, intensive searching for this species in intertidal areas during spring tides, as reported previously for the area [12], increases the total stress load of the substrates and thereby affects the abundance. Secondly, both *P. capensis* and *S. cucullata* have specific environmental and physical habitat requirements such as the type of sediment, habitat composition and hydrodynamic condition of the site. These factors are crucial for the abundance and distribution of benthic invertebrates [24] and may not be optimal at the studied sites. Other factors that can influence the density of oysters are predation in their natural environment as well as mortality, larval dispersion [25] and lack of appropriate substrate for settlement [26].

The higher densities recorded in Nolwe and Inguane are explained by low harvesting pressure, due to their remote location and difficult access. The remaining areas are located near the village, where they are closer and more accessible by the communities living on Inhaca island. The decrease in abundance of the pearl oyster *P. capensis* throughout the study period was evident, even in fishing grounds, which in the past yielded high densities of oysters [12]. This suggests that the level of harvesting observed during the present study have negative implications for the oysters' stocks.

In contrast, there was no evidence of decreasing abundance and size of individuals of the rocky shore oyster *S. cucullata* during the study period. This is probably due the lower level of exploitation of this species on Inhaca.

Previous studies suggest that decrease in oyster mean shell size is also an indicator of stock decline and overfishing [12]. The average size of oysters recorded on Inhaca in this study for *P. capensis* (39.75 ± 5 mm) was lower than those recorded in the same area in 2000 where the size was 48.8 ± 4.3 mm [13] and from other regions of the country such as Vilanculos and Inhassoro, 55 ± 7.1 mm [20]; Bazaruto Island, 47.3 ± 3 mm [27]. Oysters over 40 ± 6.2 mm in size were lower in number in all sampled sites except for Nolwe bank. The low number of large size pearl oysters observed during this study is consistent with the results of the survey undertaken on Salomon Island, Australia [28]. On the other hand, there was a tendency for reduction in the percentage of oysters of larger size (>40 mm) during the study period, which resulted in the reduction of the average size in all sampled sites. The cause of this reduction may indicate that the stock is being intensively exploited. The decrease of mean size of oysters from difficult access sites to ease access sites suggests a negative impact of unregulated exploitation of oyster stocks. This finding is supported by evidence from South Africa where size of oyster specimens of protected zones was higher than oysters of unregulated zones in relation to human pressure degree [29]. A similar trend is reported for mussels whose size was smaller in open access zones than in protected sites [30]. Despite Inhaca Island being a marine protected area, the harvesting of oysters is open to the communities without restrictions for capture.

Data obtained from the questionnaire of the collectors suggests that the pearl oyster *P. capensis* is decreasing in the natural banks when compared to its yield in the past. According to [11,25], human exploitation is a factor that can disturb the distribution and affect the reproduction, growth and development of the bivalves. Pearl oysters of larger mean shell size were more abundant in the catch from the harvesters than those obtained from field sampling. This suggests selectivity of oyster harvesters preferring larger oysters and corroborates previous observations [12]. Despite lesser oysters ever reaching adult size, they reach the maturity stage very early, starting at 6 months (about 27 mm) and larvae production may not be affected [31–33]. Similarly, to other studies, there is evidence that pearl oyster population can be reduced to a point at which recovery is barely possible [28]. The yield is affected, since it is in the second year of the life cycle that pearl oysters considerably increase their body weight [34,35].

The variations of size frequency distribution among sites indicate a higher proportion of smaller oysters in shallower and accessible sites; it is hypothesized that in absence of size selection, indiscriminate harvesting is occurring. The consequent dominance of smaller individuals in shallower accessible sites constitutes a threat for the oyster population in the future [30].

Throughout the world, unregulated harvesting of bivalves is known to adversely affect wild stocks [29]. During the field-sampling period, we noted harvesting pressure on the pearl oyster *P. capensis*. Additionally, in some shallower and accessible areas, there is evidence of unselected oyster harvesting. In the absence of minimum oyster size, the exploitation by harvesters targets all sizes, especially immature oysters, affecting natural populations to self-recruit, and leading to overexploitation [36,37].

A community-based management approach of exploited oyster populations is considered as one of the most promising ways to link sustainability and economic growth [38]. Appropriate management strategies are required to overcome threats to pearls oysters on Inhaca Island with the support and involvement of the local community. Previous studies have demonstrated that size limits can be established as useful tools for harvest regulations, mainly in cases where management data was not available [37], such as in the case of Inhaca island. Maximum catch per person, closed harvesting seasons or areas are among other most-used measures for protection and sustainable management of oyster fisheries [39].

Another important management measure to implement would be to return the empty shells to the intertidal areas. It has been demonstrated that conspecific shells were preferred substrates for larval settlement and stock recovery [40].

Despite the multitude of stressors that can impact oyster populations and oyster reefs such as overharvesting, pollution and diseases [7], the anthropogenic pressure from catchers is likely the most relevant for the decline of abundance, individual size and catch per unit effort of *P. capensis* on Inhaca island. Our study involved the perception of harvesters regarding their harvesting habits and awareness about the condition of stocks. They are an important ecosystem component to consider when implementing management and monitoring measures for the sustainable exploitation of the oyster resources. The analysis presented here contributes to improved knowledge on both *P. capensis* and *S. cucullata* fisheries and for an urgent call for the introduction of harvesting management measures for the former species. Hopefully, it will provide an incentive to value the whole ecosystems' services provided by the oyster habitats at the local level.

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Informed Consent Statement: All interviewees, received a written statement explaining the propose of the study. The informed consent statement highlighted that participation in the study was voluntary and there were no known risks to participation beyond those encountered everyday life. It was also underlined that participants could decline or leave any question they did not wish to answer and their responses would remain confidential and anonymous.

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Gametogenic Cycle of the Oysters *Pinctada capensis* (Sowerby III, 1890) and *Saccostrea cucullata* (Born, 1778) (Class Bivalvia) in Inhaca Island, Southern Mozambique: A Subsidy for Bivalve Culture in the Region

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Abstract: This study describes reproductive aspects of the *Pinctada capensis* (pearl oyster) and *Saccostrea cucullata* (rocky shore oyster) in Inhaca Island, southern Mozambique (Western Indian Ocean). Adult oysters were collected monthly over two years within seagrass banks for *P. capensis* and rocky shore habitats for *S. cucullata*. The animals were evaluated using biometric and histological analyses of the gonads. Of the total population, females were predominant among larger individuals (>55 mm) and males were more dominant among smaller individuals (<55 mm) for both species. The sex ratio was (1 M–1.5 F) for *Pinctada capensis* and (1 M–1.6 F) for *Saccostrea cucullata*. Five gonad maturation stages were identified: indifferent, developing I, developing II, ripe and spent. The size at first maturity was mm and 26.2 mm for pearl oysters females and males, respectively, whereas for rocky shore oysters was 32.8 and 28.3 mm for females and males, respectively. We conclude that the reproduction of *S. cucullata* and *P. capensis* occurs mainly in summer, with a short resting period in winter, when many oysters are in the indifferent stage. These results provide valuable information to fisheries for management of both species in the area.

Keywords: Maputo Bay; reproductive biology; mollusca; pear oyster; rocky oyster; Western Indian Ocean

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1. Introduction

Oysters are key species for the ecology of coastal and estuarine areas [1]. They provide important ecological services such as improving water quality, helping to stabilize the coastline and protecting erosion by building their reefs, and they are also an important product of fisheries and aquaculture in many countries [2]. These invertebrates are widely distributed around the world. In tropical regions, oysters occur in rivers and coastal areas, and most species occur in narrow bands or form dense banks at a tidal range where desiccation, fouling and predation are minimized [3].

Although most of the biological aspects of tropical oysters are comparable to that of temperate oysters, the effects of environmental factors on reproductive activity are not well documented. The sex ratio in oysters may vary from species to species due to factors such as organ age and genetic differences, and the gametogenic cycle could be influenced by environmental parameters [4,5]. The development of the gametes is a process that consumes a lot of energy depending on the nutrients obtained in the recent feeding or the reserves accumulated during the period of reproductive rest [3].

The reproduction activity of oysters can occur at any time of the year, greatly influenced by the changes in water temperature and the availability of food [6]. Spawning is related to water temperature fluctuations (25 to 30 °C), reaching a peak when temperatures are very high [7]. Additionally, spawning cycles in bivalves are related to food availability, which is often measured as seasonal variations in chlorophyll *a* or suspended particulate matter (SPM) [8].

The most reliable method for evaluating the development of the reproductive cycle of oysters is based on the histology of the gonads [9]. Histological methods categorize the reproductive cycle of the oysters according to the characteristics of tissue cells observed at different stages of gonadal development [10]. However, this type of analysis tends to be subjective and should be used in conjunction with quantitative methods, such as the condition index (CI), for a better assessment of the reproductive aspects [7,10].

Studies of the reproductive cycle, spawning periods, and occurrence of larvae of oyster species are essential in understanding the population dynamic of wild stocks, a basic requirement for the management of natural banks and application of conservation and/or exploitation of commercial marine/food species [11]. Moreover, a better understanding of the reproductive cycle of the native oysters and its relation to the environment is needed for farming [12], as this will permit the development of more effective and efficient techniques for the maintenance of the oyster and breeding conditions in the laboratory and for the successful production of seed.

Among the oysters, the pearls oyster *Pinctada capensis* (G.B. Sowerby, 1890) inhabits tropical and sub-tropical waters. This species is abundant along the western coast of Africa, where it occurs associated with seagrasses beds in sheltered lagoons, channel, intertidal reef platforms and other habitats [13]. *Saccostrea cucullata* (Born, 1778) has a wide Indo-Pacific distribution, ranging longitudinally from East and South Africa [14], to the Pacific Islands, and latitudinal from Japan [15] to Australia [16] and New Zealand [17]. This species is abundant in the eastern African coast, where it forms a distinct band in the mid to upper balanoid zone. Spawning has been reported to be continuous except for the monsoon time in India, and continuous but peaked during the rainy season in East Africa [18].

In Mozambique, *P. capensis* is found in the Bazaruto Archipelago and Maputo Bay. It thrives in shallow and relatively clear waters, generally fixed in substrates within seagrass beds, at depths from 5 to 30 m [4]. *S. cucullata* is found in Pemba Bay, Palma and Mocímboa da praia (northern Mozambique), Bazaruto Archipelago [19], Xai-Xai beach as well as in Maputo Bay [20]. These species stand out due to the important role they play as a protein source for local communities as well as in the use of shells in art craft. In Inhaca Island, *P. capensis* is more important for the local community than *S. cucullata*, because its harvest is higher as it is collected at more accessible sites [21].

Information on the reproductive activity of *P. capensis* and *S. cucullata* is not available in Mozambique despite their biological and socioeconomic importance as a component of benthic fauna in Inhaca Island. This paper is part of research on the population dynamics of these oyster species in Maputo Bay aimed at providing information for possible use in the development of oyster culture in the region. Therefore, the present study aimed to report baseline information on their reproductive biology by investigating the reproductive activity, sex ratio and its relation to size and size at first maturity.

2. Materials and Methods

This study was carried out in Inhaca island (latitude 26°07′ S, longitude 32°56′ E), which is located 32 km in front of Maputo City, Mozambique (Figure 1). The Island has a total area of approximately 42 km^2 and is part of the Ponta de Ouro Marine Park. The area mediates the shallow Maputo Bay and the open waters of the Indian Ocean, a transition zone from a tropical to a sub-tropical climate with a rich diversity of both terrestrial and marine ecosystems [22].

There are two distinct seasons—the hot and rainy season (from November to April) and the cold and dry season (from May to October)—with an average air temperature of 23 °C and a sea water temperature varying from 18 °C (July) to 32 °C (January) [18].

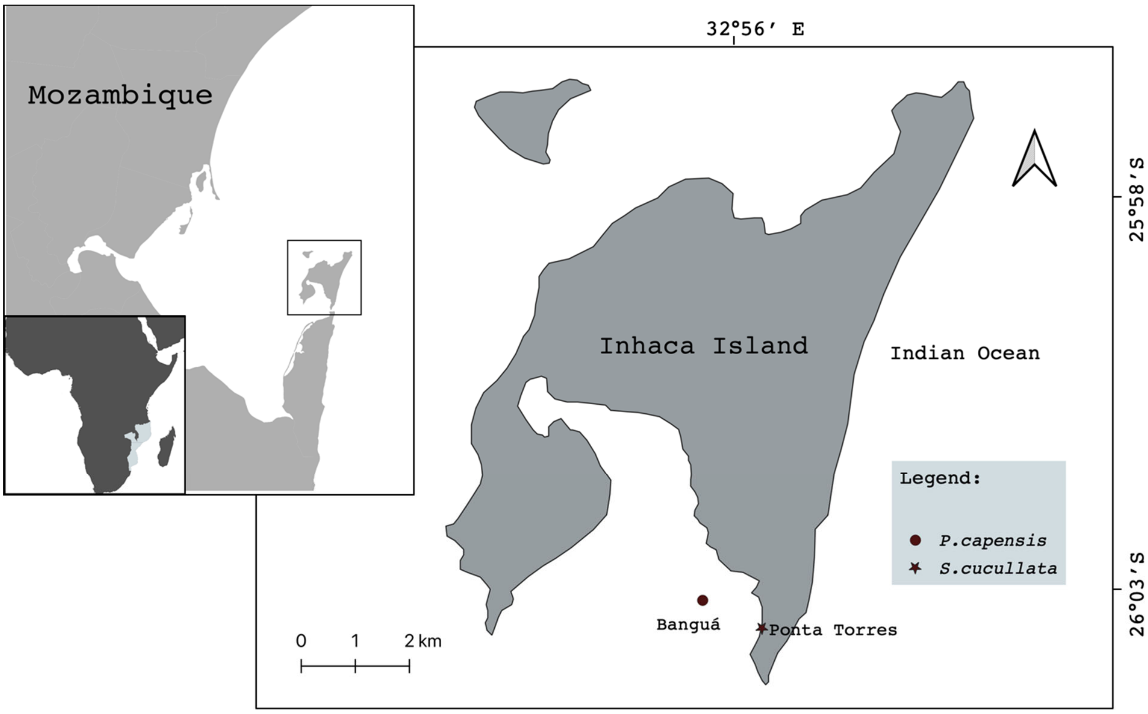

Figure 1. Map illustrating the study area (Inhaca Island).

The eastern part of the Island is characterized by strong currents and waves while the western part is more sheltered [23]. The tides are semidiurnal and have maximum amplitudes of approximately 3.1 m in high spring tides [22]. During low tide, a large stretch of beach is exposed, making it an interesting site for the collection of many invertebrates, including oysters [23,24].

Two sampling sites were established in Inhaca Island based on occurrence and accessibility: site I (Bangua) for *P. capensis* and site II (Ponta Torres) for *S. cucullata* (Figure 1). Sampling was conducted monthly during spring tide, over a period of 2 years (November 2016–December 2017 and January to October 2019) within seagrass banks for pearl oyster *P. capensis* exposed during low tides (around 3 m deep in high waters) and rocky shore habitats for *S. cucullata*. Data on seawater temperature throughout the year were obtained from existing records (*Asia Pacific Research Data Center Home*—http://apdrc.soest.hawaii.edu/data/data.php accessed on 27 March 2020). Fifty specimens of oysters from each species were randomly collected in the two defined sampling sites, they were transported to the Ecology Laboratory, Department of Biological Sciences at Eduardo Mondlane University and were kept in a freezer at 4 °C for further processing. In the Lab, the oysters were cleaned, and shell length (SL) and shell width (SWi) were measured using a digital caliper (precision of 0.001 mm), while total wet weight (TW), tissue weight (TiW) and shell weight were weighed using a digital scale (precision of 0.001 g) [25].

The animals were classified histologically, according to the type of germ cells present in their gonads, into males, females, hermaphrodites (in which both oocytes and spermatozoa were found in the same individuals) and in sexual resting (in which no germ cells were found in the gonads, making sex determination impossible), according to [26].

For all individuals, soft tissues were carefully separated from shells and washed in distilled water. Both soft tissue and shells were put in an oven at 80 °C for 24 h and then weighed. The condition index (*CI*) was calculated individually as the ratio of dry weight of soft tissues divided by the dry weight of shell × 100 using the following formula [27]

$$CI = 100 * \frac{Wsoft_{\ tissue}}{W_{total}}$$

The reproductive condition of the oysters was verified on the basis of degree of development and numerical density of the gametes, being determined by the examination of the macroscopic appearance of the gonads and the microscopic examination of gonads tissue smears. Due to a lack of external dimorphism, the shell valves were parted, and a subjective estimation of gonad volume made. Gonad smears were examined at 100× magnification. Each specimen was classified based on the presence or absence of mature or immature germ cells according to the scale modified from [28] (Table 1). The sex ratio (expressed as number of females per number of males F:M) was determined.

Table 1. Stages of gonadal development in oyster.

Stage	Characteristics
Indifferent	No gonad visible. This has two possible explanations—adults with recovering gonads after spawning or immature juveniles
Developing I	Gonad tissue visible, but it is very difficult to distinguish sex
Developing II	Gonad tissue are evident and sexes can be distinguished. Gametes are abundant, but the majority of the spermatozoids are hardly moving and pedunculated oocytes are present
Ripe	Gonad with rapid moving spermatozoids or spherical oocytes
Spent	Gonads are empty and thin. Coexistence of cells being reabsorbed and mature cells

To estimate the minimal size of first maturation, the relative frequencies of juvenile (immature) and adults (mature) were distributed by length classes (4.5 mm interval) of individuals in both sexes, analyzed separately. The minimum size at first maturation corresponds to the size at which 50% of individuals are sexually active [21].

The data were adjusted to a dose–response model to determine the size at which at least 50% of the individuals in the population can reproduce (L50). The data obtained were adjusted to the following logistical curve:

$$P = \frac{1}{1 + \exp[-r(L - L_{50})]}$$

where P is the proportion of mature specimens in each class of shell length (SL), r is the slope of the curve and L_{50} is the length of the first maturation [29].

Data Analysis

Data were grouped into size classes (4.5 mm SL) to detect variations in the sexual proportion as a function of specimen size. Sex ratio data were pooled for the whole study period. The chi-square test (X^2) was used to determine differences in sexual proportions. In order to identify seasonal trends in gonad development, each gonad stage (i.e., immature, developing, ripe/mature and spent) was reported by size in proportion to the total catch of oysters. Sizes at sexual maturity (SW_{50}) were estimated separately using the proportion of specimens in each 4.5 mm SL size classes with mature gonads and only oysters considered mature (the last developing stage) were used.

Data analysis was performed using SPSS software [30]. Normal distribution of the data was tested using the Shapiro–Wilks test. The variance homogeneity was evaluated using Cochran's test.

Monthly averages of CI were calculated for each species. ANOVA, followed by Tukey's post hoc test [31], was used to confirm monthly critical differences in the reproductive variables (CI). The results are presented as the means (±standard error) and the significance level used for the tests was 5%. In addition, simple Pearson correlation analyses were used to test whether monthly average CI could be related to variability in temperature.

3. Results

3.1. Sex Ratio

A total of 2400 individuals were collected during the study period, 1200 specimens of *P. capensis* and 1200 specimens of *S. cucullata.* From all *P. capensis* sampled, 374 (31.2%) specimens were males, 558 (46.5%) were females, 247 (20.6%) were indeterminate, while 21 (1.8%) had both sperm and eggs present in the gonad (Figure 2A). Bisexuality was not common for this species. Additionally, these specimens showed no evidence that both gonads were functional (i.e., intersexes). The sex ratio (male:females) for this species was 1:1.5. Statistically significant differences were found in the proportions of the overall sex ratio ($p < 0.05$). For *S. cucullata*, 405 (33.8%) were males, 643 (53.6%) were females, 70 (5.8%) undetermined and 82 (6.8%) had both sperm and eggs (Figure 2B). There was a greater predominance of females than males for the overall samples during the study period. The sex ratio (M:F) was 1:1.6 (1 M–1.6 F). Significant differences were found in the proportions of the sex ratio ($p < 0.001$).

Figure 2. Proportion of males, females, undetermined sex and hermaphrodites of *Pinctada capensis* (**A**) and *Saccostrea cucullata* (**B**).

The pear oyster sex ratio showed a clear dominance change from male to females with increasing size classes. The majority of individuals <45 mm SL were males and female were more common from 55–60 mm to upper size of 90–95 mm (Figure 3A). For rocky shore oysters, the majority of individuals <40 mm SL were males. Above this size, the proportion of females increased progressively, reaching 100% at the upper size classes (Figure 3B).

(**A**) (**B**)

Figure 3. Size frequency distribution of males and females of the species *Pinctada capensis* (**A**) and *Saccostrea cucullata* (**B**) from Inhaca Island.

3.2. Temperature and the Condition Index

Water temperature in this study area ranged between 21.5 and 23.6 °C in winter and between 24.4 and 27.8 °C in summer. The highest value (27.9 °C) was recorded in January 2019, whereas the lowest value (21.5 °C) was recorded in July 2017 (Figure 4).

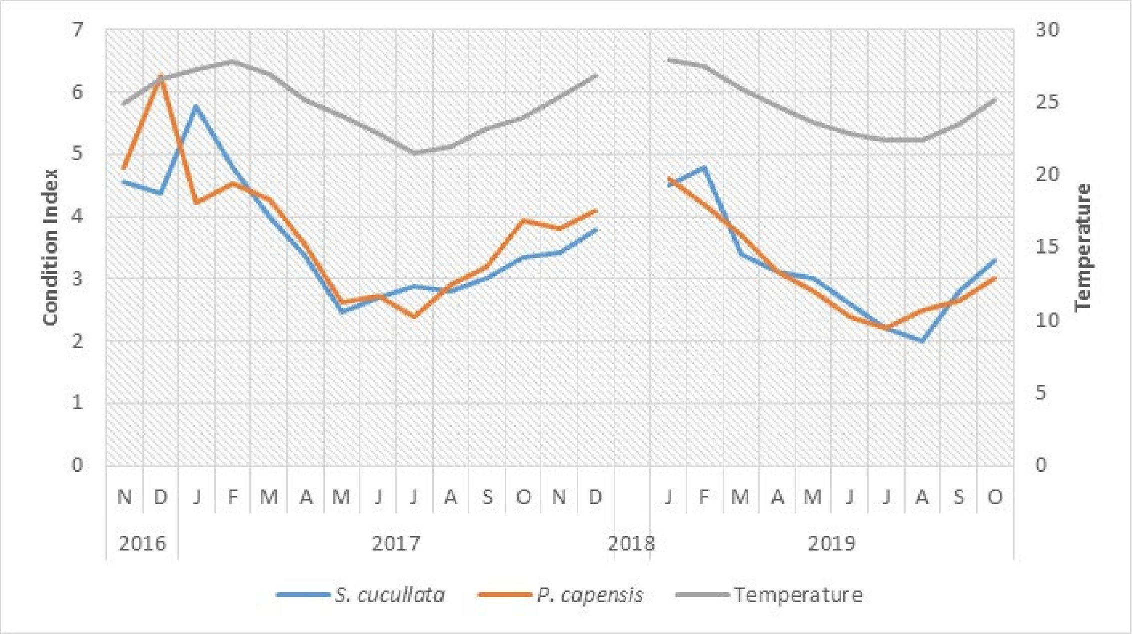

Figure 4. Monthly changes in temperature and the condition index of *Pinctada capensis* and *Saccostrea cucullata* from Inhaca Island (sample size per month is 50).

Changes on condition index (CI) of the oyster *P. capensis* and *S. cucullata* are illustrated in Figure 4. Condition index varied over the study period for both species. The major peaks occurred in December 2016 and January 2017 and 2019 while a minor peak occurred in July 2017 and 2019 for pear oyster. For the rocky shore oyster, a major peak was recorded in January 2017 and February 2019 and the minor peak were recorded in May 2017 and August 2019. Statistical analysis showed significant change in CI between the months over the study period for *P. capensis* (ANOVA, F = 7.12 $p < 0.05$) and for *S. cucullata* (ANOVA, F = 6.31 $p < 0.05$). Additional correlation analyses, showed that for both species, there was a strong correlation between monthly average temperature and average CI (Figure 5). Temperature explained 72% ($p < 0.001$) and 63% ($p < 0.001$) of the variability in CI for *S. cucculata* and *P. capensis* respectively. This indicates that, despite some variability in temperature and CI among years, the condition of both species is tightly linked to season and temperature.

Overall, results obtained in this study indicate that a large proportion of the population spawns in summer season with a peak from January to March and a minor peak from October to December. The remaining period of the year is characterized by lower proportions of population spawns for both species (Figures 6 and 7). During the winter season from May to July, more s are in the indifferent stages; and from August, the oysters begin to develop their gonad into developing stage 1. In the early warm season, when the water temperature starts to increase, more oysters of both species are in developing stage 2.

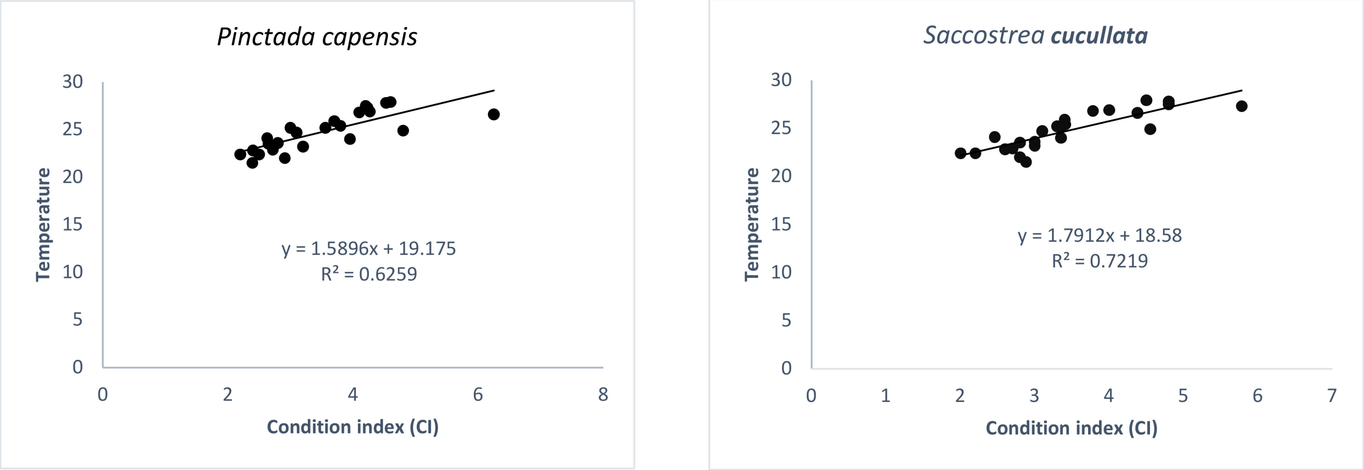

Figure 5. Correlation between temperature and the condition index (CI) of *Saccostrea cucullata* and *Pinctada capensis* from Inhaca Island.

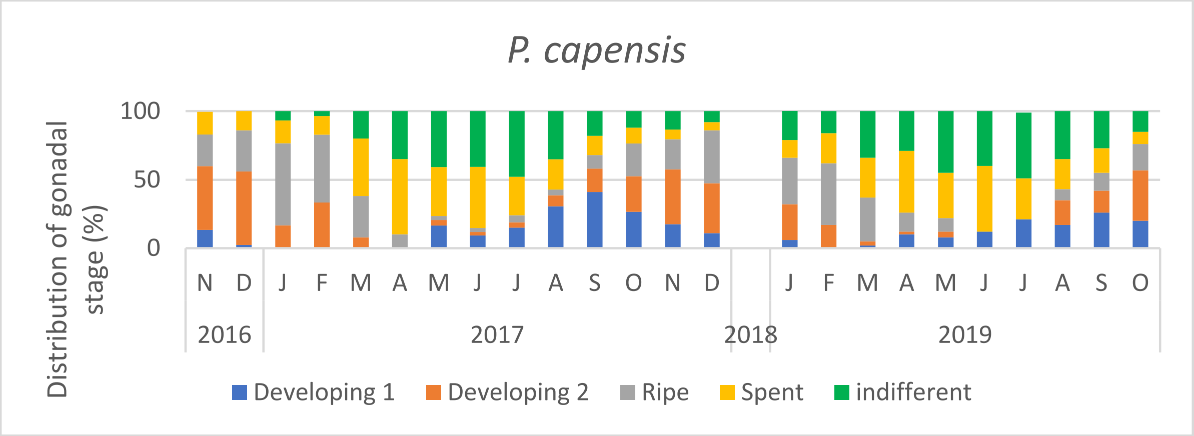

Figure 6. Monthly frequency distribution of gonadal development stages (males and females combined) of *Pinctada capensis* in Inhaca Island.

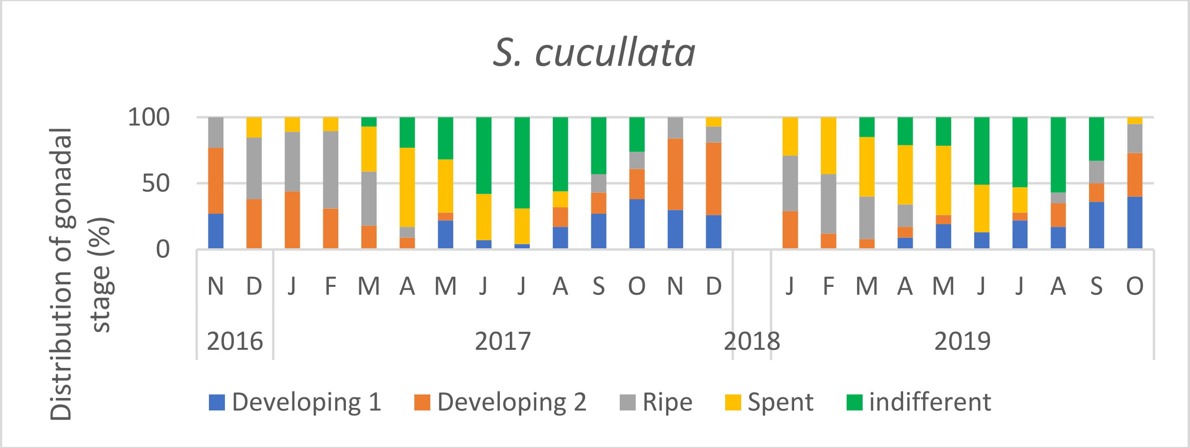

Figure 7. Monthly frequency distribution of gonadal development stages (males and females combined) of *Saccostrea cucullata* in Inhaca Island.

3.3. Minimum Size at First Maturity

The relationship between the percentage of mature *P. capensis* and total shell length (SL) for females and males is presented in Figure 8. Size at first maturation was 27 mm SL and 26.52 mm SL for female and males, respectively. For *S. cucullata*, the minimum size at first maturation was estimated as a size of 32.8 mm SL and 28.3 mm SL for females and males, respectively (Figure 9).

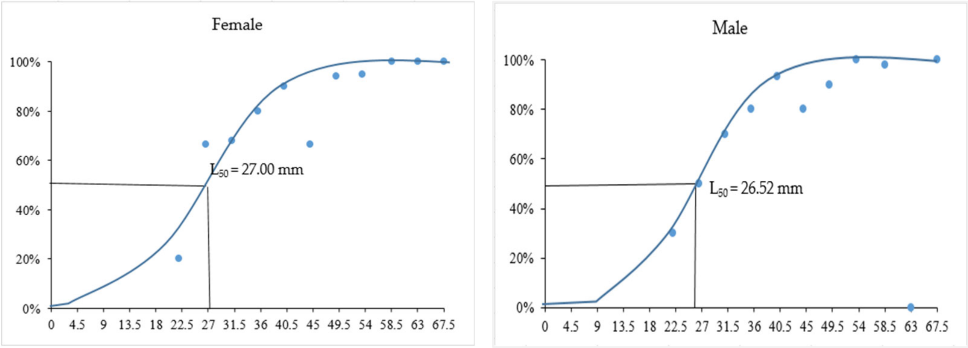

Figure 8. Percentage of mature individuals and the total shell length (SL) for females and males of *Pinctada capensis* from Inhaca Island.

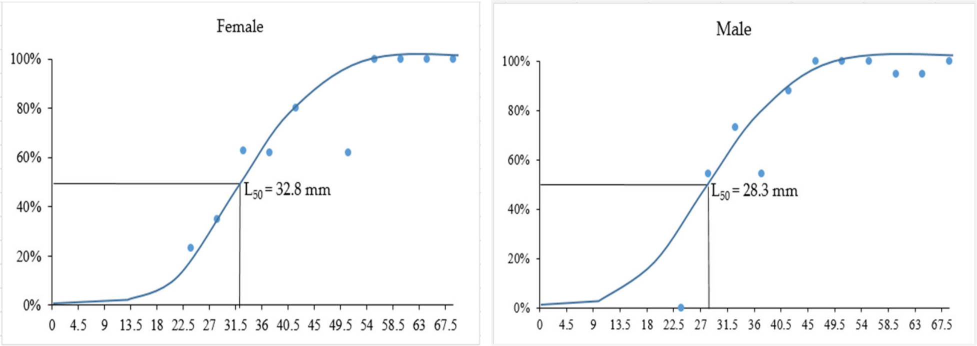

Figure 9. Percentage of mature individuals and the total shell length (SL) for females and males of *Saccostrea cucullata* from Inhaca Island.

4. Discussion

This study provides new and extensive information about several life-history characteristics, in particular reproductive aspects of two common and important oyster species, pear oyster *Pinctada capensis* and rocky shore oyster *Saccostrea cucullata*, in Inhaca Island in southern Mozambique. This information can help contribute to a more sustainable use of these valuable species in the area but may also be put in the perspective of similar species in tropical and sub-tropical areas.

For example, in the sex ratios observed for both oyster species in this study, with a predominance of females over males and the occurrence of hermaphrodites in natural populations is consistent with what has been found in many other studies on oysters. This includes a study [32] on *Crassostrea gigas* from Korea, a study [33] on *Crassostrea rhiziphorae* from Guaratuba Bay, a study [34] on *Crassostrea brasiliana* from Guaratuba Bay, Brazil, a study [35] on *Pinctada radiata* from northern Karkennah Island, Tunisia, a study [36] on *P. radiate* and *P. margaritifera* from southwestern and a a study [37] on *Saccostrea cucullata* from Maharashtra, India.

Similarly, females were larger than males, being more common in upper size classes of shell length. The authors of [38] found that 39% of oysters of the species *P. imbricata* less than 71 mm shell height were females but this ratio for oysters bigger than 70 mm was reversed (66% females). This coincides with our results in which females outnumbered males for pear oyster above 55 mm and rocky shore oyster above 50 mm in shell length, respectively. The authors of [2] used diocious and hermaphroditic *C. gigas* individuals to determine the genetic control of the coexistence of protandric sex change and observed different ratios of females. The ratio according to the authors was related to the age of the animal: a significant proportion of the oysters matured first as males and changed to females in later years (i.e., prontandrous hermaphroditism). Several authors suggested that these sexual changes are known to be potentially related to food availability. The authors of [8] suggested that good conditions will favor females, whereas bad conditions, or stress, favor males.

The presence of gonads with both male and female gametes may indicate a pathological finding. In this case, the animals were termed "intersex" (i.e., both reproductive systems were not functional) by [32], a phenomenon that, according to the authors, was induced by aquatic pollutants and chemical endocrine disruptors. These authors also suggested that the increase in intersex individuals within a population could serve as a bioindicator of environmental quality.

The lower proportion of males compared with females for both *P. capensis* and *S. cucullata* poses no risk to population maintenance within the environment because, according to [25], males may release gametes more frequently than females due to faster gonadal recovery.

In the present study, values of the condition index (CI) for both oyster species varied during the study period and over the year. This index, showed a tendency of increase with rise of temperature and decrease in period of low temperature. Values of the Condition Index (CI) can be indicative of the reproductive period or nutritional status of individuals [39]. In the summer (October–March), the increase in temperature increase the metabolic rate of many invertebrates including oysters and consequently increasing their consumption of microalgae and suspended particulate matter, contributing to the storage of energy in form of glycogen, lipids and proteins that will be used for reproduction [40].

A high condition index in warmer seasons can be justified by the greater availability of food that is obtained in this period. The authors of [41], in a study on the seasonal cycle of planktonic communities on the Island of Inhaca, found that the concentration of nutrients is higher in the warmer months (summer), a time when rain brings a greater flow of nutrients through the rivers flowing into Maputo Bay. Following the peak of nutrients, the authors of [41] also registered maximum values of chlorophyll-a in March, with a tendency to decrease in the colder months. Many studies have attempted to explain the effect of environmental parameters on gonadal development and reproduction of different species of bivalve mollusks. Various factors such as temperature, salinity, and food availability in the environment can affect the gametogenic cycle of bivalves [42].

Seasonal spawning activity observed in this study also correlates with observations of recruitment of other species in other tropical and sub-tropical regions which peaks in the summer, from February to April [43]. The reproductive activity of the oysters is affected by the seasons and, therefore, by increases in seawater temperature. The observations of temperature and histological data showed that oysters at the early development stage first were found during the months when the temperature of seawater was close to 21 °C. Animals in the late growing stage began to appear during October, when the average temperature rose above 24 °C. As the temperature continued to rise from November through March, oysters reached the mature and spawning stages. These events occurred at the beginning and the end of this period, respectively. This information is consistent with previous findings that the periods of gametogenesis of the same species may vary among geographical areas [44]. Studies performed in different locations showed that the time required for gametogenesis and for maturation of the sexual cells increase with latitude [45].

The results here obtained on the size at sexual maturity revealed that males of *P. capensis* mature earlier at 26.5 mm SL compared to females at 27 mm SL and males of *S. cucullata* also mature earlier at 28.3 mm SL compared to females at 32.8 mm SL, as is generally the case for most oyster species [8]. Early maturity in males may confer advantages in competition in egg fertilization from highly fecund females. This might be the biological factor to ensure that all eggs are fertilized because of the protandrous situation experienced by these species.

The results of the histological analysis demonstrated that oyster gametogenesis is not continuous during the year and tends to be more intense in the summer periods when seawater temperature rises and lower or absent during the winter when seawater temperature drops. Here, we provided valuable baseline information in a wide plan to decrease exploitation and introduce aquaculture practices, towards the sustainable management and conservation of *P. capensis* and *S. cucullata*, and ultimately to ameliorate people's livelihoods.

Author Contributions: M.M. and A.M. conceptualized this study; M.M. and M.R. designed the methodology; M.M., M.R. and T.T. collected the field data; M.M. and T.T. analyzed the data; A.M. and M.L. contributed to supervision; M.M. and M.R. contributed to original draft preparation; C.A., M.L. and A.M. contributed to writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data from this research are kept under lock and key in the Department of Biological Science Research database. Data will be available for re-use in future studies.

Conflicts of Interest: The authors declare no conflict of interest. All parts involved support the publication of the results.

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III

Original Article

Combined effects of salinity and temperature on survival and growth during the early life cycle of the rock oyster *Saccostrea cuccullata* (Born, 1778)

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Abstract

Temperature and salinity are among the critical factors affecting the survival and growth of bivalve larvae. The combined effects of temperature and salinity on the embryonic and larval development of the rock oyster *Saccostrea cuccullata* (von Born, 1778) in culture conditions were investigated in a laboratory study on Inhaca Island, Mozambique. A factorial experimental design tested three temperatures (24, 30 and 34 °C) and three salinities (30, 35 and 40 parts per thousand) over a seven-day period. Larval survival and growth (in height and length) were assessed by regular sampling by counting and measurement of larvae under an optical microscope equipped with a micrometric scale. Significantly higher larval survival was observed at the combination of 30 °C and 35 salinity. However, the mid-range temperature (30 °C) and highest salinity (40) resulted in faster growth of the larvae. The lowest temperature (24 °C) negatively affected growth regardless of salinity level and survival decreased linearly with increasing salinities. The present results will aid in the understanding of the environmental factors behind the natural recruitment of spat of *S. cuccullata* and contribute to the optimization of rearing protocols for the larval culture of this oyster species.

Keywords: temperature, salinity, rock oyster, larvae, growth, survival

Introduction

The rock oyster *Saccostrea cuccullata* (Born, 1778) is a widely distributed species in the Indo-Pacific and Eastern Atlantic Oceans and has been introduced into the Eastern Mediterranean Sea (Çevik *et al.*, 2001). *S. cuccullata* is an intertidal species mainly found in brackish water environments between 0 and 5 m depth, where it is usually attached to rocks, roots and trunks of mangrove trees and on pier piles (Dye *et al.*, 1994; Carpenter *et al.*, 1997; Poutiers, 1998). The rock oysters are a popular food consumed throughout its distribution area, but the overexploitation of their natural beds is reducing the catches in different countries, including South Africa (Dye, 1989), Kenya (Tack, 1999), Mozambique (Everett, 2008) and India (Tenjing, 2020). In Bangladesh, the decline of the rock oyster beds is leading to the collapse of breakwater reefs (Chowdhury, 2019). It is widely acknowledged that shellfish reefs provide relevant ecosystem services such as coastal protection, nursery grounds to enhance fisheries production, contributing to blue carbon capture, and pollutant removal and detoxification (Nagelkerken *et al.*, 2015; Waltham *et al.*, 2020).

S. cucculata is also an interesting species for aquaculture purposes due to its high commercial value, tolerance to extreme environmental conditions and fast growth. Most recent research and development efforts on *S. cuccullata* have focused on improving rock oyster natural populations through the development of improved substrates for spat collection and juvenile growth (Racuyal *et al.*, 2016; Chowdhury, 2019).

However, the culture requirements for *S. cuccullata* during their early life stages are poorly known, which seems to be a major bottleneck for the development of the culture of this species. Major advances thus far on the larviculture of *S. cuccullata* were the production of embryos (Awati and Rai, 1931), and the description of the embryological development and suitable temperature and salinity conditions for the production of straight-hinge veliger larvae (Kalyanasundaram and Ramamoorthi, 1986). Sukumar and Joseph (1988a, 1988b) provided a general insight on the natural breeding cycle of *S. cuccullata*, including gonadal maturation and the description of the factors influencing the spawning cycle. Recently Thanormjit *et al.* (2020) characterized the gametes and early development of *S. cuccullata*.

Temperature and salinity are major environmental factors that affect the survival and growth of bivalve molluscs during early life stages (Robert *et al.*, 1988). Considering the natural occurrence of *S. cucculata* in the intertidal zone, where environmental parameters are likely to reach extreme values, the current research tested a range of water salinity and temperature combinations to evaluate the most suitable conditions for larval culture of the rock oyster.

Materials and methods

Breeding and experimental animals

Adult specimens of *S. cuccullata* were collected manually with a concave iron tool during low spring tide on the rocky shores of Ponta Torres (32° 57 ‘ S, 26° 4’ E) on the island of Inhaca (Maputo, Mozambique). Only specimens larger than 20 mm were selected (Nascimento and Pereira, 1980; Dang *et al.*, 2010). During collection, water temperature (26.5 ± 1.0 °C) and salinity (35.5) were measured. The oysters were placed in a waterless plastic bucket and capped with Rachel mesh to minimize sunlight stress and brought to the Inhaca Marine Biology Station (EBMI). At EBMI, the oyster shells were cleaned using a nylon brush and a knife, washed with distilled water and transferred to a 50 L aquarium with natural seawater at constant temperature (25 ± 2 °C) and salinity (40). Aeration was continuous and illumination was based on natural light and photoperiod.

Ten oysters were randomly selected and their valves were opened through the sectioning of the adductor muscle (Legat *et al.*, 2017; Santos *et al.*, 2020; Thanormjit *et al.*, 2020). The oysters were then washed with filtered (1 µm) and UV-treated seawater to remove microorganisms lodged in the inner layer of the valves. The sex of oysters was identified by gonad biopsy using an optical microscope (Olympus model CK40; magnification of 40 x).

The gonadal tissue was cut with a scalpel and the gametes were collected using a Pasteur pipette. They were then transferred to a 100 mL beaker (male gametes) and a 1 L container (female gametes) filled with filtered (1 µm) and UV-treated seawater (Absher *et al.*, 2000). The viability of the gametes was examined under the microscope, i.e., the sperm cells were mobile and the shape of the oocytes became spherical 20 min after being exposed to water. In vitro fertilization was performed adding 2.5 mL of the sperm solution in 1 L of oocyte suspension. Through microscopic observations, the necessary adjustments were made to establish a ratio of about 3 to 5 spermatozoa for each female gamete to avoid polyspermy. Of the 10 sacrificed adult oysters, only four females and two males were used to obtain the required number of gametes for use in the experiment. Each female contained on average 5 to 10 million eggs and males about 2 million sperm. For fertilization, gametes (spermatozoa and oocytes) were evaluated through microscopic examination according to Helm (2004): mature gametes were considered all ovules normally pear-shaped when removed for the first time and round-shaped in contact with sea water within 20 minutes; the sperm was considered mature when it was motile. Embryos of approximately 40-45 µm were transferred to a 10 L tank filled with 5 L of filtered (1 µm) and UV-treated seawater with moderate aeration at the density of 204 embryos mL^{-1}, estimated using an optical microscope by counting the embryos present in 1 ml. The temperature was set at 28 °C with the use of thermostats (Dophin 50W). The embryos remained under these conditions until the appearance of D-larvae 24h after fertilization. Since the time required to reach the D-larvae stage was previously unknown, the stages of embryonic and larval development were observed every five minutes.

Experimental design

Twenty-seven culture tanks each containing 5 L of filtered (1 µm) and UV-treated seawater were prepared about 24 hours after fertilization. Larvae were reared in the combinations of three temperatures (24, 30 and 34°C) and three salinities (30, 35 and 40), each with three replicates. The D-larvae were directly transferred to those experimental treatments in three replicates per combined temperature x salinity. Approximately 25,000 D-larvae were transferred to each culture tank at an initial density of 5 larvae mL^{-1}. Water volume in each tank was maintained at 5 L. The salinities of 35 and 30 were obtained by diluting seawater (salinity 40) with distilled water. Moderate aeration was provided using air stones. Every 48 h, tank bottoms were siphoned (50 % water volume exchange) to ensure good water quality. To avoid the elimination of live larvae as well as to select the largest diameter larvae, 60 and 40 µm overlapping meshes were used (smaller mesh over the larger one). A density of 400 cells mL^{-1} of the microalgae *Isochrysis galbana* was provided daily to the D-larvae. A reduction of 50 % on the number of microalgae was provided in the days when no water was exchanged. Microalgal density was estimated daily with a Neubauer chamber. Growth was measured daily by sampling 30 larvae according to Hillerbrand *et al.* (1999). An optical microscope equipped with an ocular micrometric scale (Olympus CK40) was used to measure height (maximum distance between the dorsal and ventral regions) and length (maximum distance between the anterior and the posterior regions) (Hu *et al.*, 1993). The mean (± SD) initial length and height of the larvae were 50.28 ± 0.30 µm and 51.30 ± 0.25 µm, respectively. Survival was estimated as the number of larvae alive at the end of the experiment in relation to the initial number. Transparent larvae, which presumably indicated the absence of tissues, were considered dead (Ponis *et al.*, 2003). The trial lasted seven days. The data obtained on length, height and survival of oyster larvae were initially tested for normality and homogeneity of variances by the Kolmogorov-Smirnov (distance) test and the Spearman's test, respectively. The data presented a normal distribution and homogeneous variances ($p<0.05$), therefore they were later submitted to parametric tests. A two-way analysis of variance (ANOVA) was used to verify the influence of temperature and salinity on larval growth and survival. If there were significant differences, the Tuckey test was applied for detailed comparison of the differences (Zar, 2010). A significance level of 5 % ($\alpha=0.05$) was adopted. Statistical treatment was carried out with the aid of the GraphPad Prism V. 8.01.

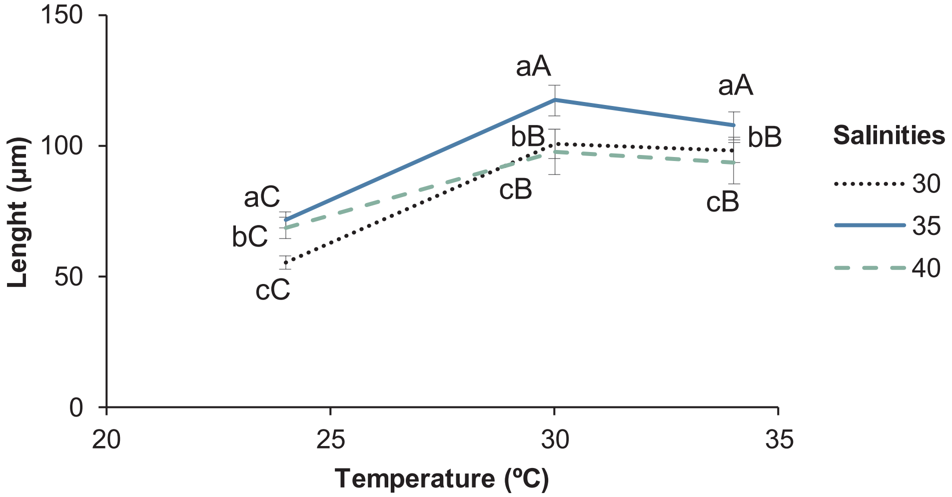

Figure 1. Means (± standard deviation) of length of larvae of the rock oyster, *Saccostrea cucculata*, reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) for seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

Results

Five minutes after the sperm solution was added to the oocyte suspension, sperm cells were observed surrounding the oocytes. The polar bodies were visible after 20 minutes. The different stages of embryonic development (2, 4, 8, 16 cells, the morula and blastula stages) were all observed within 2 h after fertilization. Trocophora larvae were observed 16 h after fertilization and were characterized by the presence of cilia and circular movements. The D-veliger stage appeared 24 h after fertilization and was characterized by the formation of the D-shaped larval shell.

At the end of the 7 day-long trial, means (± SD) of length (Fig. 1) and height (Fig. 2) of larvae after exposure to the different combinations of salinity and temperature were significantly different with ANOVA $F_{(4,18)} = 66.47$, $p < 0.0001$ for length and $F_{(4,18)} = 380.5$, $p < 0.0001$ for height. The largest larvae (117.30 ± 0.36 µm length and 125.08 ± 0.38 µm height) were observed at 30 °C x 35, while the smaller ones (53.30 ± 0.86 µm length and 54.69 ± 0.40 µm height) were those from treatment 24 °C x 30. The differences in survival rates among treatments were also significant (Fig. 3) with ANOVA $F_{(4,18)} = 1309$, $p < 0.0001$. The mean (± SD) survival ranged from 26.4 ± 0.4 % in treatment 24 °C x 30 to 35 salinity to 73.2 ± 0.2% in treatment 34 °C x 35 salinity. The highest survival was observed at 34 °C x 35 salinity. All treatments at 24 °C resulted in significantly lower survival rates.

Discussion

The present study suggests that *S. cuccullata* larvae can grow well at 30 °C temperature x 35 salinity to obtain largest larvae, however to obtain higher survival of larvae, they must be grown at 34 °C temperature x 35 salinity. The temperature of 30 °C is well above the average annual temperature of 25 ± 1 °C for the brood stock collection area at Inhaca Island, where (according to Kalk, 1995) the highest average is 27.5 °C and the lowest 21.7 °C. The mean salinity levels at Inhaca are 33 ± 2 (de Boer *et al.*, 2000) with a range from 32 to 42 (Pinto, 1996). This confirms the findings of Kalyanasundaram and Ramamoorthi (1986) that *S. cuccullata* larvae tolerate a wide temperature and salinity variation though with different growth and survival trends.

Temperature and salinity are among the main environmental factors affecting the growth (Robert *et al.*, 1988; His *et al.*, 1989; Manoj Nair and Appukuttan, 2003) and survival of bivalve larvae (Yuan *et al.*, 2016; Verween *et al.*, 2007; Manoj Nair and Appukuttan, 2003; Robert *et al.*, 1988). In this study, embryos and larvae reached all developmental stages defined by Kalyanasundaram and Ramamoorthi (1986). However, due to the distinct culture conditions of the treatments, the life stages were reached at different times.

The highest larval growth was observed at salinity 35, coinciding partly with the results obtained by Kalyanasundaram and Ramamoorthi (1986) which

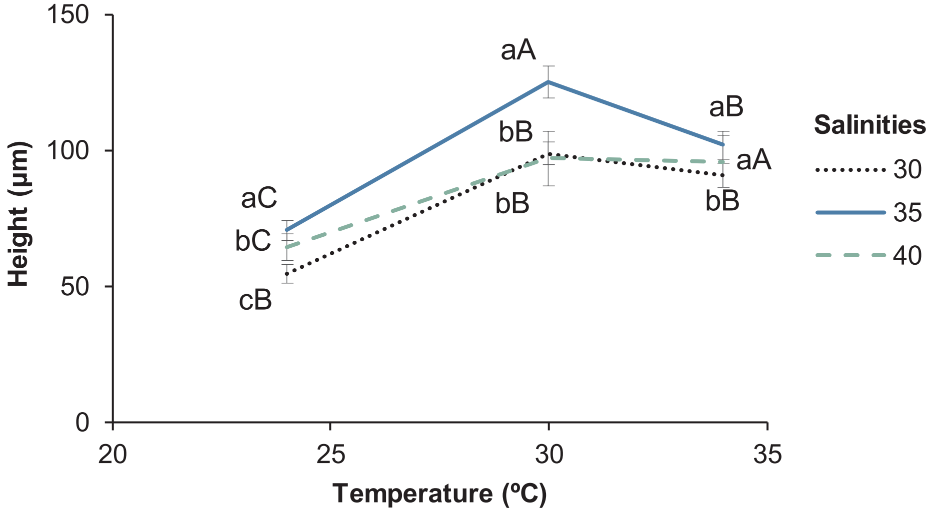

Figure 2. Means (± standard deviation) of height of larvae of the rock oyster, *Saccostrea cuccullata*, reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) for seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

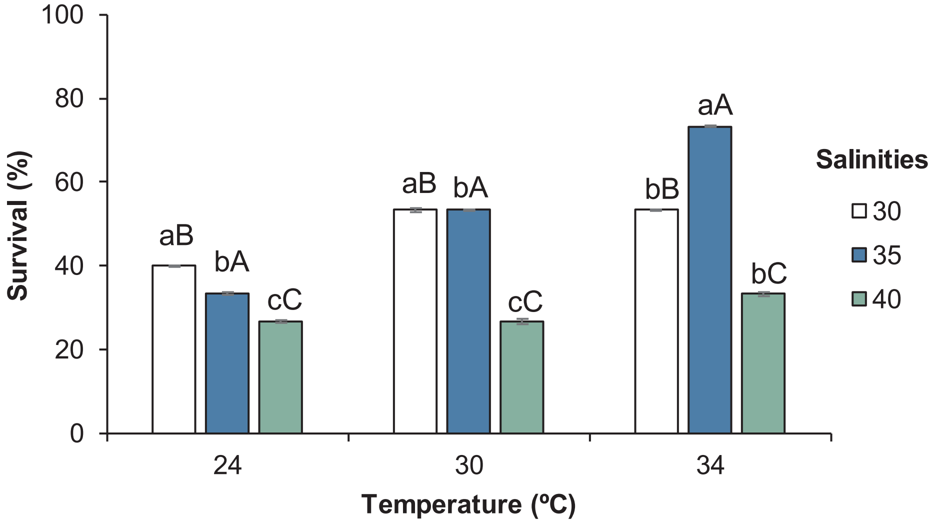

Figure 3. Percentage of survival of rock oyster, *Saccostrea cuccullata* larvae reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) after seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

reported good larval development of *S. cuccullata* at salinities from 20 to 35. In contrast, Sudrajat (1990) determined an optimum salinity of 25 for embryonic development of *S. cuccullata*. In general, oyster larvae tolerate a wide salinity range between 25 and 40 (Lemos *et al*., 1994). However, this study showed that salinity affects the growth of *S. cuccullata* larvae, being the lowest at salinity 30. It may be possible that osmotic disturbances lead to energy allocations that ultimately impair other functions such as growth (Deaton, 2008). Temperature is regarded as the most important environmental factor influencing bivalve culture (Robert *et al*., 1988; Helm *et al*., 2004). Temperature increments accelerate the growth of oyster larvae (Doround *et al*., 1999). The highest growth recorded here at 30 °C supports previous observations by Kalyanasundaram and Ramamoorthi (1986) that larval growth of *S. cuccullata* is higher at 30 °C. Yukihira *et al*. (2000) reported that the influence of temperature on the metabolism and physiological processes of oysters creates an optimal temperature range for each species providing a maximum rate of growth as well as survival. In fact, a similar trend was observed in the present study, where the maximum temperature tested (34 °C) resulted in reduced larval growth.

The greatest survival (73 %) obtained at the combination of salinity 35 x 34 °C, was higher than the one from the microalgae feeding experiments by Martínez-Fernández and Southgate (2007) in *Pinctada margaritifera* with 70.5 %. It was also greater than the 50 % larval survival obtained by Mafambissa (2009) for *Crassostrea rhizophorae*. Other oyster species such as *Crassostrea gigas* have shown higher larval survival rates of up to 87 % (Ponis *et al*., 2003).

The results from this study are in partial agreement with Nell and Holliday (1988) who reported higher larval survival of *S. commercialis* in salinities ranging between 23 and 39. However, the current results differ from those obtained by Coeroli *et al*. (1984) where they reported higher survival of *Saccostrea echinata* larvae at salinities ranging from 25 to 30 and temperatures from 25 to 29 °C. Heral and Deslous-Paoli (1990) demonstrated that *C. gigas* larvae tolerate higher salinities (from 45 to 50) with significant mortality above 50. These differences reflect the genetic variability among different species of oysters and their high adaptability to different environmental conditions. The lowest larvae survival in the present study (26.4 %) was achieved at salinity 40, suggesting this is a threshold salinity level for *S. cucculata* larvae.

All treatments at 24 °C resulted in significantly lower survival rates. This seems to be the lower temperature limit tolerated by the larval stages of this species. Another factor to consider is the abrupt submission of the larvae to low temperatures without any previous acclimation, which may have caused a thermal shock and contributed to a decrease in survival rates. Opposite to what was previously reported by Kent *et al*. (1999), the fertilization method used here was very effective as high survival of larvae was observed in treatments where appropriate environmental conditions were provided. Together with the optimal water salinity and temperature for larval rearing established here, this may contribute to the further development of the culture of *S. cuccullata*. These findings related to the environmental variable thresholds for *S. cuccullata* larvae are relevant for future studies on recruitment, as well as to evaluate the direct impact of global warming on this species.

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Experimental spat collection of the pearl and rocky shore oysters on ceramic tile substrates

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ABSTRACT

Temporal and spatial variation in oyster larvae settlement is a crucial factor determining the abundance and success of adult populations in their natural environment. The overall understanding of oyster recruitment provides information on site selection and strategies employed for obtaining spats for oyster culture. In this study, spat of *Pinctada capensis* and *Saccostrea cucullata* were collected from four selected sites in a sub-tropical setting at Inhaca Island, Southern Mozambique during an experimental period of 8 months to assess the effect of different factors (season, duration and surface) on spat settlement and abundance. Ceramic tiles were deployed as artificial substrates from the surface to 5 m depth. They were replaced monthly. Overall, settlement on ceramic tiles was higher for *S. cucullata* than for pearl oyster *P. capensis*. There was a tendency for higher numbers of spat settling during the summer than winter for both species. For both species larger numbers of recruits were found on tiles deployed for four months than for two months and on the roughly textured surfaces compared to smooth. The results have also indicated that the ceramic tiles could be used as substrate for rocky shore oyster *S. cucullata* settlement in the late summer.

1. Introduction

The life-history of bivalves involves a reproductive phase, in which gametes or larvae are released into the water, disperse and then settle within hours, days or weeks depending on species and larval feeding-mode (Dame, 2016). Natural populations of bivalves can be sustainably harvested, on the condition that there is a supply of larvae and that enough larvae settle, metamorphose, survive and are recruited into the adult, reproductive part of the population (e.g. Underwood and Fairweather, 1989; Chávez-Villalba et al., 2022). Settlement of marine bivalves is a complex process influenced by the physical and chemical characteristics of available substrates and environmental factors such as water temperature, light intensity and currents (Dame, 2016; Ruwa and Polk, 1994). Although many bivalve larvae can actively select or reject a particular surface for settlement, it is clear that the process of recruitment is also largely determined by external factors that are beyond the control of the individual larva, such as currents and availability of suitable habitat. (Anderson and Underwood, 1994).

Oysters are a diverse (paraphyletic) group of bivalves, which includes the families Osteridae (true oysters) and Pteriidae (pearl oysters). Historically these families are valuable resources to coastal communities as both nutritious and accessible food, but also for decoration and as a sign of social status (Michaelis, 2020). Because stocks of oysters are limited and often threatened, efforts to enhance natural populations or to cultivate are a growing business in developing as well as industrialised countries in different parts of the world (FAO, 2014; Zhu et al., 2019). Oyster spat, i.e., settled and metamorphosed juvenile oysters, represent the main supply for oyster culture. These can sometimes be purchased from specific hatcheries that produce spat but are often extracted directly from natural banks or obtained using artificial collectors introduced in the natural environment for their recruitment (Martínez-Fernández et al., 2003). Within bivalve aquaculture, spat collection with artificial substrates is quite widespread and many traditional oyster producers, as in some regions of Italy, France, China and Japan, still depend to some degree on capturing spat from artificial substrates (Funo et al., 2019). The collection of wild spat is critical to the success of the culture of many commercial bivalves. Attachment to substrate by bivalve larvae is not a completely random event and larval metamorphosis requires the availability of a suitable substrate to occur (Martínez-Fernández et al., 2003; Zhu et al., 2019).

The production of spat in hatcheries is a more delicate and laborious process. It requires appropriate facilities, good water quality, production

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of microalgae for live feed, good maintenance of the systems and a highly qualified workforce. Hatchery production is justified when the capture in the natural environment is insufficient to meet production targets, when the species to be cultivated is of high value, or when it is an exotic species, as is the case of the Pacific oyster *Crassostrea gigas* (Martínez-Fernández et al., 2003). Due to the difficulty and high cost of spat production in hatcheries, the culture of native oysters in many coastal areas in developing countries depends on spat recruited from the natural environment using artificial collectors (Castilho-Westphal et al., 2015).

Oyster farming can represent opportunities for income and employment, contributing to the improvement of community living conditions and for preservation of oyster populations in the wild. Production and growth of oysters from early to adult life stages have been carried out in several countries for the purpose of replenishing natural stocks in the wild (Zhu et al., 2019). In oyster cultures, a variety of materials have been used for collection of oyster spat. These include bivalve shells, hood, granite, concrete, fiberglass, rope, PET bottles and PVC material (Taylor et al., 1998). Many collectors have been considered inadequate because they are expensive, difficult to build or made of non-durable material, or due to difficulties in removing young oysters from the material. A suitable material for spat collectors must be economically accessible, biologically acceptable and environmentally favourable (Soniat and Burton, 2005). In general, rough surface substrates seem to be more attractive for larval settlement than smooth surfaces. Similarly, poorly illuminated surfaces are generally preferred to clear or illuminated surfaces (Taylor et al., 1998; Doroudi and Southgate, 2002).

For benthic invertebrates in general, it has been shown that specific chemicals associated with collectors can induce settlement and metamorphosis of oyster larvae (Crusot et al., 2021; Taylor et al., 1998). For example, biofouling on the surface of collectors by certain bacterial colonies positively influenced larvae settlement. Furthermore, encouraging the growth of diatomaceous films on the collectors' surface increased the settlement and early growth of some bivalve species (O'Foighil et al., 1990; Fogarty et al., 1991). A species-specific understanding of how the design and surface properties of collectors influence settlement and post settlement survival is important for optimisation of spat collection in the field or production in hatcheries.

In coastal areas of Southern Mozambique, such as on the Inhaca Island, two species of oysters are common: the pearl oyster *Pinctada capensis* (Sowerby III, 1890) and the rocky shore oyster *Saccostrea cucullata* (Born, 1778). Reproduction in these species is greatly influenced by changes in water temperature and can occur at any time of the year. Results obtained on Inhaca Island indicate that a large proportion of the population in both species spawns in summer with a peak from January to March and a minor peak from October to December. A lower proportion of the population spawns throughout the remainder of the year in both species (Mafambissa et al., 2023). While the pearl oyster is commonly exploited for local consumption, the rocky shore oyster is valued and sold in other localities of Southwestern Africa. In this region, however, it is very difficult to exploit naturally growing oysters due to their very strong attachment to rocky surfaces. Development of suitable artificial collectors could provide opportunities to also utilize *S. cucullata* for human consumption. The pearl oyster populations, at Inhaca Island in particular, have suffered great reductions in abundance and size (Mafambissa et al., 2022). Despite the high commercial value of the oysters occurring at Inhaca Island (De Boer et al., 2000), studies to understand oyster settlement and recruitment in Mozambique are non-existent. More reliable knowledge about levels of recruitment and habitat preferences in a local context is critical for future attempts to restore and manage natural populations as well as for developing local practices for aquaculture.

To address these knowledge gaps, we have studied seasonal patterns of spat recruitment and growth in different habitats at Inhaca Island. The purpose of the study was to evaluate the potential of using artificial collectors for settlement of the two native oyster species *P. capensis* and *S. cucullata* for future culture of these species that can reduce pressure of collection in the field. More specifically, we assess the effect of season, site and surface of tile on the recruitment potential of these oysters in the selected areas (two areas for each species) at Inhaca Island.

2. Materials and methods

2.1. Study area

The study was carried out in the vicinity of the Marine Biology Station (EBMI) on Inhaca Island (latitude 26º02'S, longitude 32º56'E), located 32 km east of Maputo City, Mozambique (Fig. 1). The island has a total area of approximately 42 km^2 and is part of the Ponta de Ouro Marine Park. It is located on the border between the shallow Bay of Maputo and the open waters of the Indian Ocean in a sub-tropical climate, which creates a rich diversity for both terrestrial and marine ecosystems (Kalk, 1995).

There are two distinct seasons: the hot and rainy season (from November to April) and the cold and dry season (from May to October) with an average air temperature of 23 °C, and the sea water temperature varying from 18 °C to 32 °C (De Boer and Prins 2002). The eastern part of the island is characterized by strong currents and waves while the western part is more protected (Emanuelsson and Isaksson, 2016). The tides are semidiurnal and have maximum amplitudes of about 3.1 m in high spring tides (De Boer et al., 2000; Kalk, 1995). During low tide, a large stretch of beach is exposed making it an interesting site for the collection of many invertebrates, including oysters (Emanuelsson and Isaksson, 2016).

2.2. Experimental design

Settlement tiles were deployed at four sites with known occurrence of adult oysters (Mafambissa et al., 2022). Two sites with seagrass meadows, Bangua and Sangala, were selected due to the occurrence of the pearl oyster *Pinctada capensis* and another two sites, EBMI and Ponta Torres, were selected due to the occurrence of the rocky shore oyster *Saccostrea cucullata* (Fig. 1).

The settlement collectors consisted of quadratic ceramic tiles measuring 269 cm^2, one side with a smooth surface texture and the other with a rough texture. Collectors were deployed in the seagrass meadows attached with iron poles at five-meter intervals along line transects up to 2 m deep (Fig. 2). The rocky shores at one site (EBMI) were frequently covered by a thin layer of mobile sediments (1–2 cm). Therefore, prior to deployment, the bottom was probed using an iron pole to ensure placement onto hard substrate.

Measurements of number and size of spat were performed in two seasons, starting in the late summer (January-April) and late winter (July – October). In each season, 40 collectors were deployed at each of the four sites, totalling 160 tiles. At intervals of 1, 2, 3 and 4 months, 10 tiles were collected from each site. The 10 collectors removed monthly at each site were used to evaluate the number of oysters settled and mortality. Tiles collected after 1 month in summer and winter were not processed due to lack of macroscopically visible settlement. At the end of the experiment, the size of oysters (hinge length) was measured to assess growth. For size measurement, only tiles collected after 3 and 4 months were considered. In the laboratory, the ceramic tiles were stored in chest freezers until processed and archived. Samples were examined at 20X using a NIKON Stereoscope to identify and quantify spat. Spat abundance was validated when two consecutive counts agreed within 5%. The presence of other fouling organisms such as mussels, gastropod, barnacles and others was also counted.

2.3. Data analysis

Settlement patterns were analysed using multifactorial analyses of

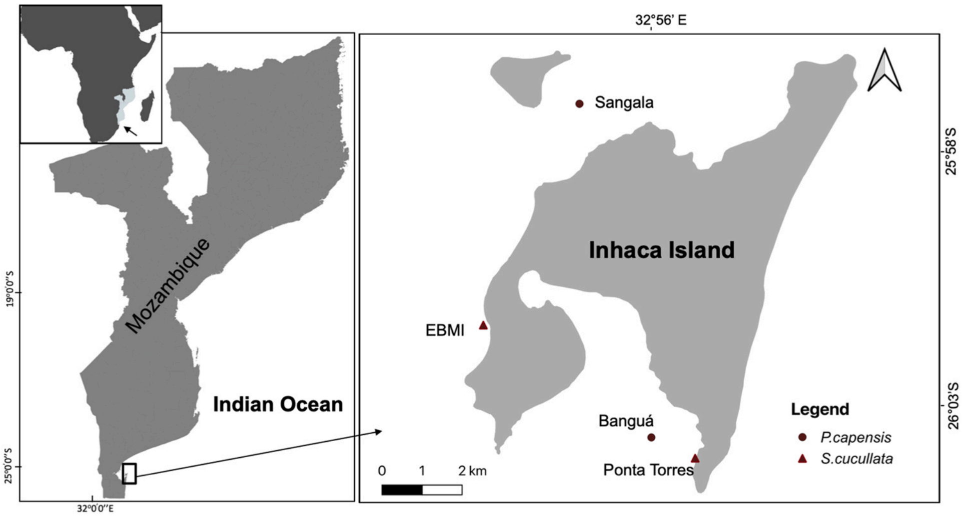

Fig. 1. Map illustrating the study area and sampling sites.

Fig. 2. Deployment scheme of the tiles in the field.

variance (ANOVA) and Student-Newman-Keuls test (SNK) as *post hoc* test of means using procedures described in Underwood (1997). Because overall densities varied strongly among locations, effects of season duration, surface texture and interactions among those fixed factors were analysed separately for each location. To achieve homogeneity of variances by Cochran tests ($P<0.05$), all settlement data were log (X+1)-transformed and normality was assessed graphically using residual plots. All analyses were performed using R (R Core Team, 2022) and the package GAD (Sandrini-Neto and Camargo, 2023).

3. Results

3.1. Settlement

3.1.1. *Saccostrea cucullata*

Settlement of *S. cucullata* was highly variable between the two sites

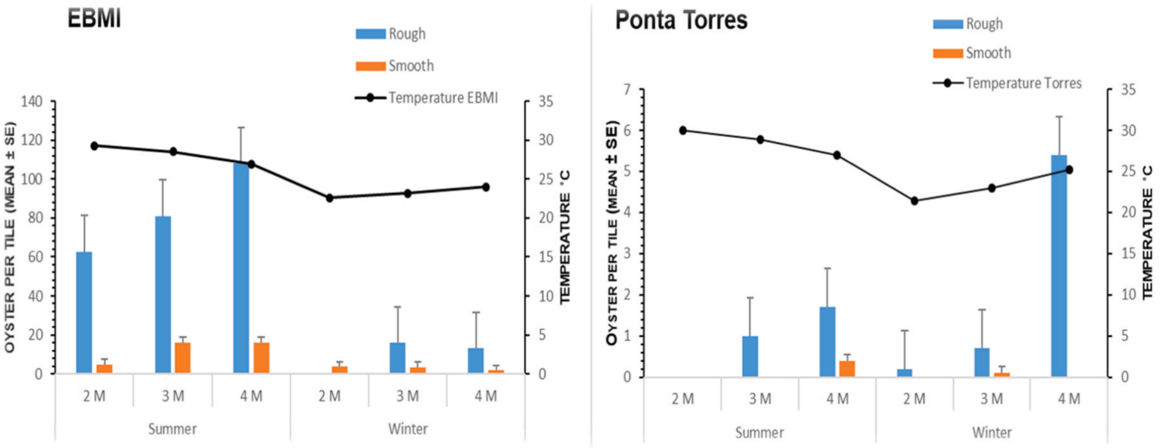

Fig. 3. Number (mean±se) of settled *S. cucculata* and measured temperature at EBMI and Ponta Torres in different seasons, durations and surface texture (note the different scales on y-axes).

EBMI and Ponta Torres (Fig. 3). At all sampling dates, the number of oysters per tiles was 1–2 orders of magnitude larger at EBMI (largest density ≈ 110 per tile after 4 months on rough tiles in the summer) than at Ponta Torres (largest density ≈ 5.5 per tile after 4 months on rough tiles in the winter).

Despite large differences in overall settlement/recruitment between the two sites, the responses to experimental factors were qualitatively similar (Table 1). Larvae of *S. cucculata* settled more extensively on rough surfaces in both sites. At EBMI seasonal differences were larger and therefore the differences between rough and smooth surfaces were larger in the summer than in winter (Fig. 3). Thus, at EBMI there are interactive effects between season and surface which are not observed at Ponta Torres where the effect of surface is relatively consistent among seasons (Table 1). Furthermore, there was a significant effect of duration at both sites meaning that tiles deployed for 4 months always had higher abundances than those collected after 2 months. The difference in duration varied among sites and surfaces, and the mean abundance after 4 months was ≈10 times higher than after 2 months.

Pinctada capensis. Both average settlement and difference between sites was generally smaller for *P. capensis* than that of *S. cuccullata* (Fig. 4). The largest average densities (2–3 per tile) were observed after 4 months on rough surfaces in the summer at both sites. Similarly to *S. cucculata*, analyses of variance revealed significantly larger settlement on rough surfaces than on smooth (Table 2).

As reported previously for *S. cucullata* larvae, the responses to experimental factors were also qualitatively similar for *P. capensis* larvae (Table 2). Larvae settled more on rough surfaces in both sites. At Sangala there were interactive effects between duration and surface which were not observed at Bangua where the effect of surface was relatively consistent among seasons (Table 2). There was also a difference in the number of larvae of *P. capensis* settled after 2, 3 and 4 months in the summer at both sites but only at Sangala in the winter. (Fig. 4).

3.2. *Size*

The sizes of oyster spat recorded after 3 months and at the end of experiment are shown in Table 3. The largest size of spat attained for *S. cucullata* at the end of the experiment was 5.8 mm with an average of 3.6 mm. The largest size recorded for *P. capensis* was 7.6 mm with average of 5.01 mm. The relative variability in size, measured as coefficient of variation (CV = standard deviation / mean), was generally 20 – 30%. For *P. capensis* there was a tendency towards more homogeneous size after 4 compared to 3 months of the experiment while for *S. cucullata* the relative variability in size was quite similar in both months of measurement. Overall, growth rates were 40–50% greater for *P. capensis* and both species tended to have increasing growth rates the longer the duration.

4. Discussion

This study provides new, valuable information about the recruitment, growth and potential benefits of collection of the native rock oyster *Saccostrea cucullata* and the pearl oyster *Pinctada capensis* on inexpensive and readily accessible artificial tiles. The major findings showed that (1) *S. cucullata* recruited in larger densities (up to ≈100 per tile) than *P. capensis* (up to ≈3 per tile), (2) both species recruited at larger densities on the roughly textured side in the tiles than on the smooth, (3) despite settlement of both species in both seasons, spat recruited in greater numbers in the summer than in the winter, (4) substantially larger number of recruits were found on tiles deployed for four months than for two months, and (5) growth rates were ≈50% greater for *P. capensis* than for *S. cucullata* during the first four months. Overall, this work confirms that deploying these types of tiles for as little as four months could be an efficient way to collect large numbers of *S. cucullata* as well as *P. capensis* spatin their respective habitats. Apart from the applied aspects, these results also improve our understanding of spatio-temporal patterns and processes affecting the two species of oysters.

First, even though we deployed tiles of standardised size, shape and texture, there were large differences among sites, particularly for *S. cucullata* (Fig. 3). This illustrates the complexity of the recruitment process and the dependence on external environmental factors. Thus, the high settlement rate attained at EBMI in late summer compared to that recorded at Ponta Torres for *S. cucullata* could be related to different environmental conditions in these sites. Collectors deployed at EBMI remained immersed throughout most of the sampling period. This may have favoured the recruitment of oysters where wave-exposure is less intense and where the collectors were exposed only in spring and periods of low tide. Buitrago and Alvarado (2005) limited the encrusting organisms by exposing the collectors out of the water for at least 3 hours each week and suggested that studies should be done to determine the effectiveness of different periods of emersion of the collectors. On the other hand, lower settlement rate was recorded in summer than in winter at Ponta Torres on rough surfaces and at 4 months. However, it is also possible that differences among seasons and sites were caused by more complex mechanisms involving interspecific competition. For example, competition could arise from barnacles, which were more abundant at Ponta Torres, particularly in the late summer when there are normally peaks of spawning of invertebrates including native oyster species. Some invertebrate species such as barnacles are more tolerant to desiccation as reported by (Foster, 1971) and there is evidence that they may displace young oysters (Bonicelli et al., 2023; Osman et al., 1989). These characteristics could confer an advantage over oyster spats at Ponta Torres in summer. Moreover, studies conducted reveal that barnacles are strong competitors of the oysters, mainly in the initial colonization phase. There is evidence that barnacles can also remove the

Table 1

Results of multifactorial ANOVA and *post hoc* Student-Newman-Keuls (SNK) test for settlement of *Saccostrea cucullata*.

Source	df	EBMI			Ponta Torres		
		MS	F	p	MS	F	p
Season	1	71.86	36.39	**0.00**	0.21	0.70	0.41
Duration	2	8.34	4.22	**0.02**	1.57	5.27	**0.01**
Surface	1	62.82	31.81	**0.00**	3.22	10.84	**0.00**
Season*Duration	2	0.14	0.07	0.93	0.20	0.67	0.52
Season*Surface	1	16.98	8.60	**0.00**	0.01	0.02	0.89
Duration*Surface	2	0.07	0.04	0.96	0.77	2.61	0.08
Season*Duration*Surface	2	6.23	3.15	0.05	0.57	1.92	0.15
Residuals	108	1.97			0.30		
Post hoc		**EBMI**			**Ponta Torres**		
SNK of Season		Winter<Summer			ns		
SNK of Duration		M2=M3=M4			M2<M3=M4		
SNK of Surface		Smooth<Rough			Smooth<Rough		
SNK of Season*Surface		Summer: Smooth<Rough Winter: Smooth=Rough					

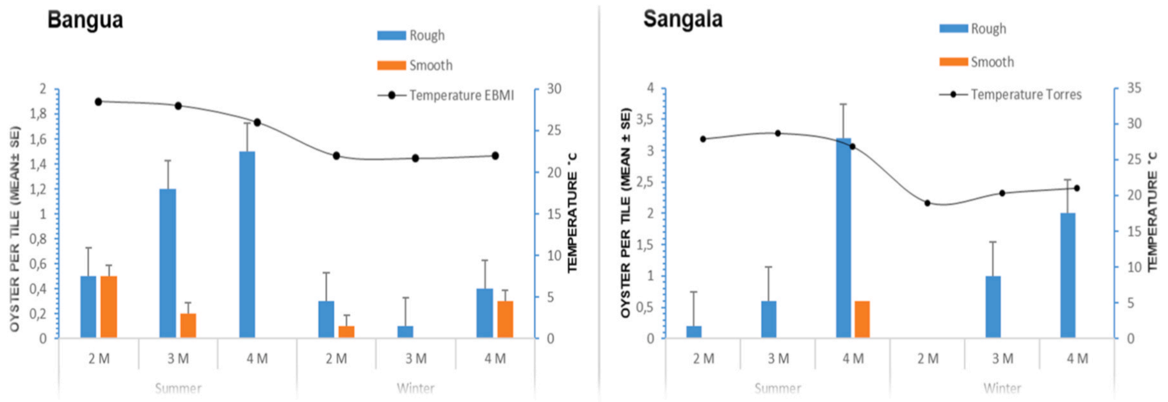

Fig. 4. Number (mean±se) of settled *P. capensis* at Bangua and Sangala in different seasons, durations and surface texture (note the different scales on y-axes).

Table 2
Results of multifactorial ANOVA and *post hoc* Student-Newman-Keuls test for settlement of *Pinctada capensis*.

Source	Df	Bangua MS	F	p	Sangala MS	F	p
Season	1	0.454	2.14	0.15	0.152	0.81	0.37
Duration	2	0.207	0.98	0.38	1.167	6.18	**0.00**
Surface	1	2.850	13.47	**0.00**	3.135	16.59	**0.00**
Season*Duration	2	0.080	0.38	0.69	0.202	1.07	0.35
Season*Surface	1	0.256	1.21	0.27	0.004	0.02	0.88
Duration*Surface	2	0.106	0.50	0.61	0.62	3.31	**0.04**
Season*Duration*Surface	2	0.107	0.50	0.61	0.0181	0.10	0.91
Residuals	108	0.212			0.189		
Post hoc		**Bangua**			**Sangala**		
SNK of Duration		ns			M2<M3=M4		
SNK of Surface		Smooth<Rough			Smooth<Rough		
SNK of Duration*Surface		ns			Rough: M2<M3=M4 Smooth: M2=M3=M4		

Table 3
Size and growth of *S. cucullata* and *P. capensis* recorded after 3 and 4 months of collector deployment. To assess the variability independently of average size, relative dispersion (i.e. coefficient of variation) was used.

Species	Duration	Average (mm)	CV	Min (mm)	Max (mm)	Growth (mm month^{-1})	n
S. cuccullata	3 months	2.44	0.23	1.4	4.0	0.8	120
	4 months	3.60	0.23	1.9	5.8	0.9	134
P. capensis	3 months	3.68	0.31	2.2	6.2	1.2	32
	4 months	5.01	0.21	3.5	7.6	1.3	43

oyster spats from the substrate (Ruwa and Polk, 1994; Ross, 2001). The strength and direction of prevailing winds may influence the extent of successful settlement between seasons. The south-western coast of Inhaca Island is subject to south-easterly (longshore) winds in summer and north-easterly (offshore) winds in winter (Sigauque et al., 2021). The nature of the Ponta Torres, a coast with numerous craters and pools, results in a complex pattern of coastal currents, many of which can be localised. This situation, largely influenced by the winds, can result in variable larval settlement patterns, which can differ considerably between the different groups of organisms.

Second, despite unpredictable variability among sites, indicating dominance of physical factors and possible modification due to ecological interactions, the results also showed that larvae recruited more often on rough surfaces than on smooth surfaces. Thus, spat appear to prefer the rough surface of tile than the smooth. Roughness surface has previously been reported to affect settlement of a wide range of invertebrates including oyster larvae (Saucedo et al., 2005). In other species, such as the silver-lip pearl oyster *P. maxima*, (Taylor et al., 1998) reported that roughened texture of the PVC slats provides better tactile stimuli to crawling pediveliger or spat than smooth surface monofilament nylon in the pearl oyster. This phenomenon may be explained by several existing models: (1) surface roughness is thought to provide a better tactile stimulus to settling larvae or crawling spat and to aid the physical retention of the larvae (Anderson and Underwood, 1994); (2) larvae can also settle preferentially in microsites of low shear created by bumps and crevices on a surface (Bushek, 1988); (3) crevices and pits in rough surface may provide protection from predators for small organisms (Hadfield, 2011; Ishengoma et al., 2011). Finally, the rough surface has a greater area and potentially greater microhabitat than smooth surfaces (Anderson and Underwood, 1994). Whatever the exact mechanism, it is striking that for both species and at all four sites, the overall recruitment was larger on rough than on smooth surfaces. This indicates that active choice or possibly behavioural constraints interact with the physical environment to create the observed patterns of recruitment.

Additionally, the analyses of the two temporal aspects of our experiment, season (summer vs. winter) and duration (2, 3 and 4 months of deployment) often suggested that duration was more important than season. The largest recruitment was almost consistently observed after 4 months both in summer and winter. This indicates that larvae were available during both seasons even though oyster settlement was higher in late summer than in late winter for both species. This pattern is fully consistent with what was observed by Mafambissa et al. (2023) who concluded that the increase in temperature during the summer was a fundamental factor in the release of the gametes of *S. cucullata* and *P. capensis* at Inhaca Island. Increase in the frequency of mature oysters during the late summer (which have higher temperature) may be related to the higher concentrations of organic matter reported in the water during this period (Paula et al., 1998). Pouvreau et al. (2000) also suggested that the suspended organic matter is necessary for gonadal maturation of the oyster since that gamete production requires considerable energy input (Marcelino et al., 2023). These results are similar with those obtained at all sampling sites for both oyster species, given that concentrations of particulate organic matter and Chlorophyll-a were significantly higher in the late summer.

We conclude that spat collection of the rock oyster, *Saccostrea cuculllata*, and the pearl oyster, *Pinctada capensis*, is possible using the ceramic tiles deployed here. Because we only evaluated one type of material, we cannot exclude the possibility that other materials may work. However, it is likely that a slightly roughened surface may be advisable for both species. Clearly, many additional aspects determine whether this would be a practically feasible and economically viable activity for one or both species (i.e. adult growth and survival, logistics, material- and production costs and sales prices). To assess these aspects and to develop viable business models is beyond the scope of this study. Nevertheless, we believe that this study demonstrates that the biological prerequisites are in place to warrant further studies addressing additional challenges for developing sustainable oyster cultivation in these parts of Mozambique.

CRediT authorship contribution statement

Mizeque Julio Mafambissa: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mats Lindegarth:** Writing – review & editing, Supervision, Software, Formal analysis, Conceptualization. **Adriano Macia:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Susanne Lindegarth:** Supervision, Project administration, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest. All parts involved support the publication of the results

Data availability

Data will be made available on request.

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Research article

Spatial and temporal variability of fouling communities on oyster spat collectors at Inhaca Island Southern Mozambique: Exploring the influence on recruitment of the oysters *Pinctada capensis* and *Saccostrea cucullata*

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ABSTRACT

In the transition from pelagic larva to benthic adult, larvae likely encounter a diverse assemblage of resident invertebrates in their habitat, which may also compete for space during post-settlement periods. Fouling fauna in rocky and seagrass habitats on Inhaca Island, southern Mozambique, was evaluated over 4 months in each of two seasons on oyster collectors fixed at 2 cm above the bottom. As expected, two species of oysters recruited to tiles: the rock oyster *Saccostrea cucullata* in rocky habitats and the pearl oyster, *Pinctada capensis* in seagrass habitats. The composition and density of other fouling fauna varied among habitats, location, seasons and surfaces and depending on the duration of the deployment. In seagrass habitats, oysters and barnacles were generally less abundant, allowing other taxa to have higher relative abundance, while higher density of individuals was recorded in rocky habitat. Barnacles dominate among fouling fauna on collectors in both habitats. Despite evidence consistent with negative interactions between oysters and barnacles, the effects of other fouling fauna on oyster abundance appear modest up to 4 months after tiles are placed. Overall, the results help improve our general understanding of the environmental processes that affect the colonisation of intertidal invertebrates, particularly in the southwestern Indian Ocean.

1. Introduction

One of the most striking characteristics of intertidal tropical habitats is the variations in fouling community structure. Spatial and temporal variations of larvae recruitment generally result from a number of factors, both physical and biological interaction [1]. The physical factors that affect the zonation and distribution of marine organisms are related to the regular cycles of inundation and exposure caused by tides [2,3]. Biological interactions such as competition for space and food, grazing and predation may also affect the zonation patterns of marine invertebrates [4]. Several studies have indicated that these interactions are complex and vary considerably from one area to another, giving rise to both small- and large-scale heterogeneity [5,6].

Recruitment may also vary throughout the year and, in many cases, showing a seasonal pattern [7], which is reflected in the

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composition and abundance of epibenthic fauna in each season [8,9]. Variation is ultimately explained by the geographical distribution of the species [10,11]. [5] Defined this variation as the sum of pre and post-settlement events. The pre-settlement events depend on sea water processes and cause differential larval transport and as a consequences affecting larval supply due to larval advection and mortality [4,5]. Post settlement events are largely influenced by the mortality of established benthic adult, affecting early post-larval supply, or realized recruitment [12].

Larval recruitment is very complex and most of the processes affecting it are poorly understood, especially in the case of interspecific interactions [13]. Oysters of the genus Crassostrea and Saccostrea are the most studied group of benthic invertebrates, and it has been reported that various factors such as the presence of conspecifics [8], biofilms [14], substrate heterogeneity and sedimentation influence larval settlement [15].

Several artificial collectors have been used in studies on oyster's settlement in the wild; however, other fouling species compete with the oysters for the space, negatively influencing their settlement. These organisms settle in the structures of the collectors and/or on the oysters [16]. Research indicates that larval recruitment is higher on substrates where there has been previous settlements. Furthermore, some authors argue that the presence of specific microorganisms tends to increase the preference for a given substrate over the presence of adults of the same species [17].

Fouling fauna associated with oysters in artificial collectors includes representatives of different taxonomic groups such as porifera, hydrozoans, polychaetes, molluscs, crustaceans [18–16]. Oysters and other fouling species could compete for the same space and food [19].

The fauna and flora of the intertidal seagrass and rocky shore habitats, which is flooded by the tide every spring high tide and neap tides on Inhaca Island, is dominated by crabs, gastropods, algae, filter feeder as barnacles and bivalves [3]. For example, the barnacles [20] are the dominant component of the fouling fauna on high intertidal rocky habitats in the Island while gastropods and bivalves are dominant in lower intertidal habitats.

Two commercially important oyster species occur on Inhaca Island: the pearls oyster *Pinctada capensis* and the rocky oyster *Saccostrea cucullata* [21]. The oysters *P. capensis* is mainly exploited for consumption, by local inhabitants of the Inhaca Island and around Maputo City [3–21], while *S. cucullata* is exploited for sale in South Africa and Maputo City [13–22]. Populations of pearl oysters, in recent years, have suffered reductions in size and abundance on this Island [21]. As a result of decline in stocks and economic value, interest in promoting culture of some oyster's species such as *P. capensis* and *S. cucullata* is growing on Inhaca Island and eastern Africa region [19]. Using tiles as substrates, we aimed to determine the spatial and temporal variability of fouling fauna on artificial collectors used for recruitment of the two commercial important oyster's species at Inhaca Island southern Mozambique. More specifically we tested how the recruitment of two species of oysters and other fouling organisms is affected by differences in surface roughness, duration and season. Thus, providing valuable information on the multiple role of the oyster spat collectors as suitable substrate for a variety of other species of larvae, serving also as nursery habitats for important source of food for different species including humans but also can serve as a baseline for a variety of manipulative studies in the southwestern Indian Ocean region.

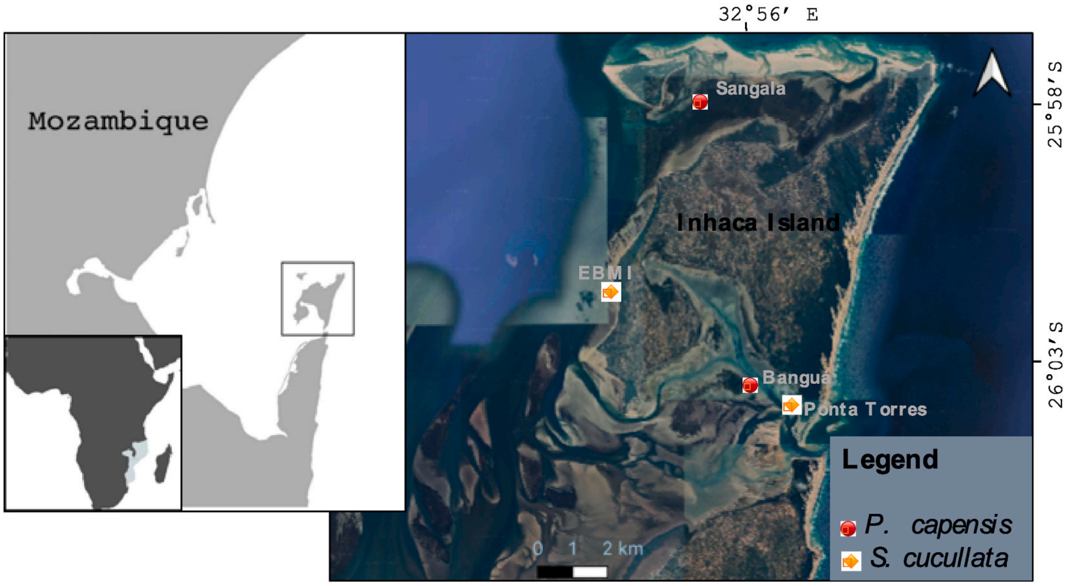

Fig. 1. Map of study area showing the locations of spat collection at Inhaca Island.

2. Material and methods

2.1. Study area

The field study was performed in the eastern part of Inhaca island, located (latitude 26°07′S, longitude 32°56′E) at a distance of 32 from Maputo City, Mozambique (Fig. 1). The total area of the Island is approximately 42 km^2 and it is part of the Maputo National Park. The Island constitutes the border between the shallow Bay of Maputo and the open waters of the Indian Ocean, in a transition zone from tropical to sub-tropical climate, which creates a rich diversity for both terrestrial and marine ecosystems [20].

The climate of Inhaca is characterized by two seasons, hot and rainy (from November–April) and warm and dry (from May–October) with an average sea water temperature of 22 °C and 29 °C for cold and hot season respectively [22]. The eastern part of the Island is characterized by strong currents and waves while the western part is more protected [23]. The tides of Inhaca Island are semi-diurnal with a mean spring and neap tidal range of 3.1 m and 0.7 m respectively [3]. During low spring tides, large extension of the intertidal area is exposed in the eastern part of the island, and used by locals for collection of a variety of invertebrates including oysters [23–3].

2.2. Study design

A series of settlement tiles were deployed at four defined locations with known occurrence of adult oysters [21]. Two locations with seagrass meadows, Bangua and Sangala, were selected for collection of the pearl oyster, *Pinctada capensis*, and another two locations, EBMI and Ponta Torres, were selected for the rocky shore oyster *Saccostrea cucullata* (Fig. 1).

The settlement collectors consisted of square ceramic tiles with an area of 269 cm^2, and one smooth and one rough textures sides. Collectors were deployed in the seagrass meadows attached with iron poles at 5-m intervals along line transects 2 cm above the bottom at depths of 4–5 cm above MWL. The rocky shores at one location (EBMI) were frequently covered by a thin layer of mobile sediments (1–2 cm). Therefore, prior to deployment in rocky habitats, the bottom was probed using an iron pole to ensure placement onto hard substrate. The tiles were attached by drilling 5 cm holes in the rock (Fig. 2).

Sample collection were performed in two seasons: in late summer (January–April 2021) and in late winter (July–October 2021). In each season, 40 collectors were deployed at each of the four locations, totalling 160 tiles. At intervals of 1, 2, 3 and 4 months, 10 tiles were collected from each location. Collectors removed monthly at each location were used to assess composition and abundance of fouling fauna. Due to lack of macroscopically visible settlement organisms tiles collected after 1 month in both seasons, were not processed. Sample collected from the second month until the end of the study were subsequently processed. All mobile fauna was identified and counted in the study location and the sessile fauna was transported to laboratory and stored in chest freezer for later analysis.

In the laboratory, samples were examined using 20× magnification, in a NIKON Stereoscope to identify and quantify fouling fauna. Sessile fauna abundance was validated after two consecutive counts confirmed the same results.

2.3. Data analysis

Recruitment patterns were analysed separately for each of six response variables with a five-factor analysis of variance (ANOVA) [24], habitat, season, duration, and surface texture (all fixed an categorical) and locations within each of the two habitats (random and categorical), and all possible interactions among factors. The response variables were oysters, other fouling organisms (excluding oysters), barnacles, mussels, gastropods and polychaetes. To achieve homogeneity of variances by Cochran tests ($P < 0,05$), all recruitment data were log(X+1)-transformed and normality was assessed graphically using residual plots. On the other hand, a simple correlation analysis was carried out to determine whether there is a relationship between the abundance of oysters and barnacles in the

Fig. 2. Image showing the layout of the tiles at the study location.

rocky habitat. All analyses were performed using R [25] and the package GAD [26].

3. Results

3.1. Overall composition and abundance of fouling communities

Fouling communities analysed from the field oyster spat collectors at Inhaca Island, include functional groups such as: barnacles, oysters, gastropods, mussels, polychaetes and algae. Of the organisms collected the barnacles and oysters were the dominant groups. The mobile organisms observed on the oyster collector at all four research locations were mainly composed of gastropods (Fig. 3).

The collectors used were almost fully covered by the third month, while the collectors never had fouling benthic fauna at the end of the first month of immersion. Visual inspections suggest that the diversity and evenness of fouling fauna was higher in seagrass habitats in summer as well as in winter compared to that recorded in collectors deployed in rock shore habitats (Fig. 3). Because some groups were only identified to a coarse taxonomic resolution, these patterns were not evaluated with rigorous statistical analyses.

In terms of relative abundance, at the end of the field study, the results of this study show that in rocky shore habitats in summer, more than 50 % of the individuals recruiting to the tiles were oysters followed by barnacles (>40 %), gastropods and mussels (<3 %; Fig. 3). In the winter, barnacles dominated with more than 80 % of total individual and ≈10 % were oysters. Similarly, for seagrass habitats the most abundant groups of organism were oysters and barnacles in the collectors. In contrast, to what was observed in rocky habitats, barnacles were more abundant than oysters in the seagrass habitat in summer and the number of individual were more than 50 % and oyster were more than 12 %.

3.2. Abundance of oysters

The main aim of this paper was to analyse the composition and abundance of fouling organisms and how these interact with oysters. Nevertheless, it is clear that overall the collectors successfully attracted oyster spat both in rocky and seagrass habitats both in summer and winter (Fig. 4).

The largest number of oysters were collected in rocky habitats (1–60 tile^{-1} compared to 0.5–1 tile^{-1} in seagrass habitats). The number of spat increased significantly from two to four months of deployment but there was also a strong and significant variability among locations (Fig. 4, Table 1 and Supplement S1).

3.3. Abundance of fouling organisms

Due to complex and large variability in the total abundance of other fouling organisms (i.e. excluding oysters), the analyses of spatial and temporal variability revealed only significant variability among locations within habitats (Table 1, Supplement S1). In particular, there were large differences between the two rocky locations. Substantially more fouling was found on the less exposed EBMI than at Ponta Torres (Fig. 4). While none of the fixed factors revealed any statistically significant effects, the estimated differences would potentially be of ecological importance if they are in fact representative. For example, the average abundance of fouling organisms was more than six times larger in rocky as compared to seagrass habitats and the difference between the largest and the smallest abundance was on average 2.5 times for the different durations (Fig. 4). The fact that none of these differences were detected as statistically significant indicate that conclusions about effects on fouling abundance aggregated among all taxa may not be so informative.

More conclusive patterns were observed for individual taxonomical groups (Fig. 5, Table 1). For example, significantly more mussels were found on rough than on smooth surfaces, and mussels, gastropods and polychaetes all showed significantly larger

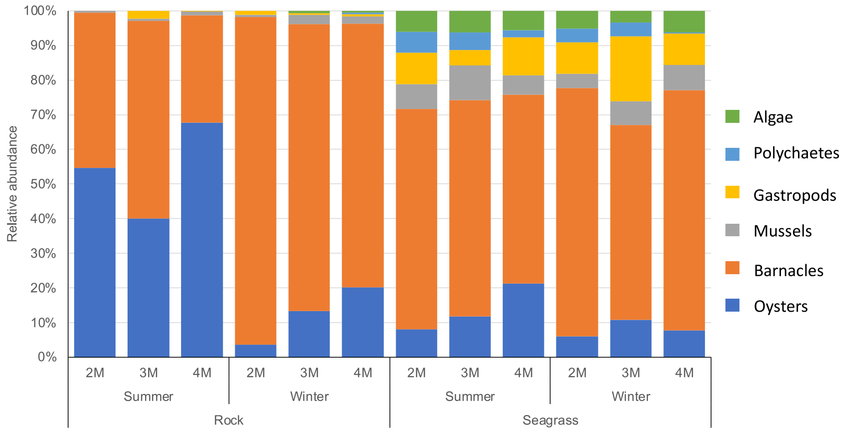

Fig. 3. Relative abundance of fouling fauna on oyster collectors after 2, 3 and 4 months in rocky and seagrass habitats at Inhaca Island.

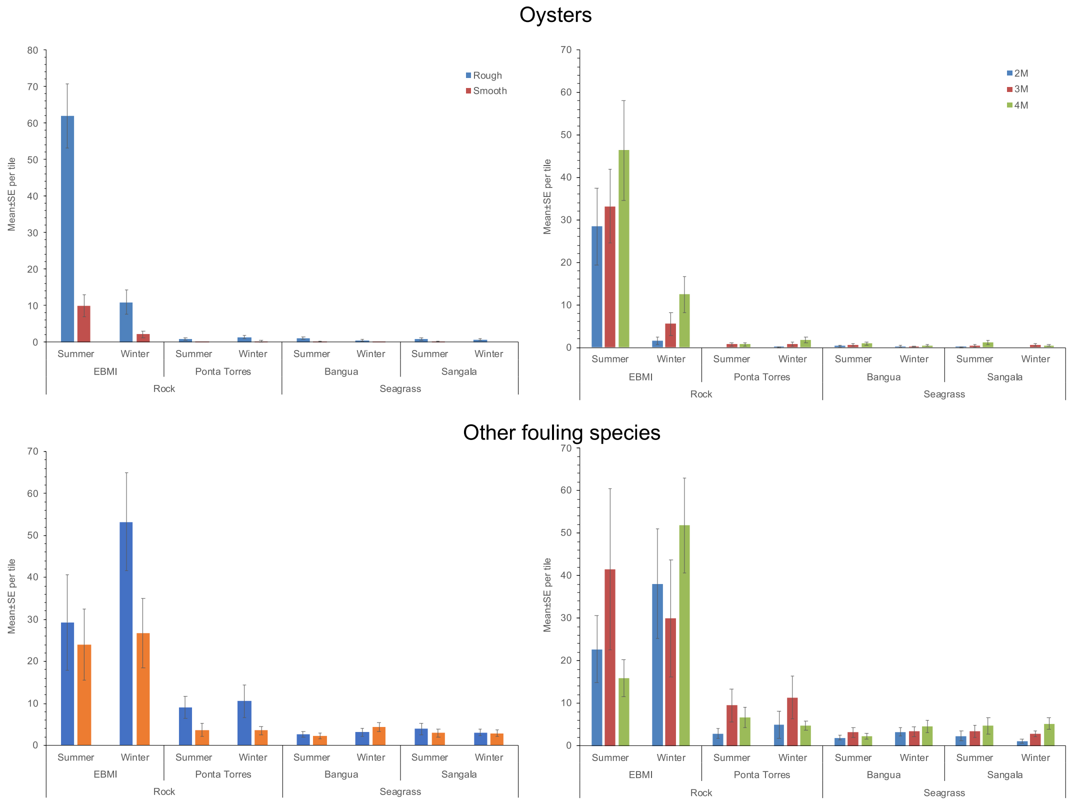

Fig. 4. Abundance of oysters (upper panels) and other fouling fauna (lower panels) on the rough and smooth side of oyster collectors (left panel) and after 2, 3 and 4 months (right panel) in rocky and seagrass habitats at Inhaca Island.

Table 1

Summary of significance tests of multifactorial analyses of variance for total abundance and conspicuous groups. ***, **, * and '.' represent significance levels of <0.001, <0.01, <0.05 and < 0.1 respectively. Details on degrees of freedom, F-ratios and appropriate error-terms are given in Supplement S1.

Source	Oysters	Other fouling	Barnacles	Mussels	Gastropods	Polychaetes
Surface, =Su	Ns	ns	ns	**	ns	ns
Season, =S	Ns	ns	.	ns	ns	ns
Duration, = D	***	ns	ns	ns	ns	ns
Habitat, =H	Ns	ns	ns	***	*	*
Su*S	Ns	ns	ns	ns	ns	.
Su*D	Ns	ns	ns	ns	ns	ns
Su*H	Ns	ns	ns	ns	ns	ns
S*D	Ns	ns	ns	.	ns	ns
S*H	Ns	ns	ns	ns	ns	ns
D*H	Ns	ns	ns	ns	ns	ns
Location, = L(H)	***	***	***	ns	ns	ns
Su*L(H)	***	ns	ns	ns	ns	ns
S*L(H)	***	ns	ns	*	ns	ns
D*L(H)	Ns	ns	ns	ns	ns	ns
Su*S*D	Ns	ns	ns	ns	ns	*
Su*S*H	Ns	ns	ns	ns	ns	ns
Su*D*H	Ns	ns	ns	ns	ns	ns
S*D*H	Ns	ns	ns	ns	ns	ns
Su*S*L(H)	**	ns	ns	.	ns	ns
S*D*L(H)	Ns	ns	ns	ns	ns	ns
Su*S*D*H	Ns	ns	ns	ns	ns	*
Su*D*L(H)	Ns	ns	ns	ns	ns	ns
Su*S*D*L(H)	.	ns	ns	*	ns	ns

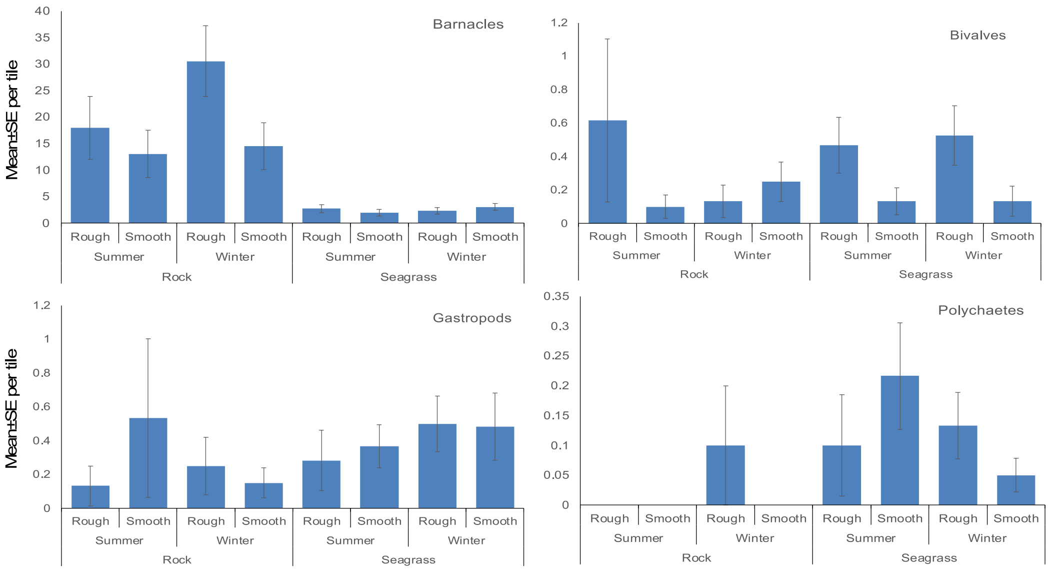

Fig. 5. Abundance of the most common fouling organisms on oyster collecting tiles in different habitats, seasons and surface roughness. (Gastropods were counted in the field and bivalves include mussels).

abundances in seagrass than in rocky habitats. The estimated abundance of barnacles, on the other hand, was consistently larger in rocky habitats, but similarly to the oysters, the large variability among locations made it difficult to statistically detect a difference between habitats. No other significant pattern was observed for the mobile gastropods, while the sessile mussels and polychaetes showed additional complex interactive patterns involving seasons, surfaces, duration, habitats and locations (Table 1).

3.4. Abundance of oysters in relation to barnacles

In order to assess potential interactions between oysters and other fauna, bivariate correlations with the other dominant competitor for space, barnacles, were done for individual locations and seasons (Table 2). Mussels, gastropods and polychaetes occurred at very low densities and any influence on the oyster recruitment were therefore not tested. Using pooled data for the three durations within sites and seasons, three out of eight tests, indicated significant relationships between abundances of barnacles and oysters. As might be predicted from competitive interactions, all of these instances were negative correlations. On rocky habitat at EBMI, a negative correlation was observed in the summer indicating that the number of oysters decrease when the number of barnacles increase (Fig. 6). Furthermore, it is clear that only a small fraction, ≈24 % of the variability in oyster spat can be accounted for by this relationship ($r^2 = 0.24$). At Ponta Torres, no evidence of correlation between the number of oysters and barnacles was observed. Surprisingly, in seagrass habitats, where there were generally lower number of oysters and barnacles, two tests showed similar negative correlations (Fig. 6). These relationships were, however, weaker than at EBMI ($r^2 = 0.15$–0.20). Nevertheless, the fact that all significant correlations were negative gives some support to the hypothesis that oysters and barnacles occasionally influence each other either by competition or possibly predation. Note, however, that these correlative patterns are based on data where tiles deployed for 2, 3 and 4 months were pooled (i.e. $N = 30$, $r_{crit} = 0.36$). This was done to increase statistical power and to minimize the number of tests, but it also means that assumptions about independence may be violated. Thus, to assess the robustness of these results we also performed correlation analyses for each duration separately (i.e. $N = 10$, $r_{crit} = 0.63$). As expected, this resulted only in fewer significant correlations despite the fact that many of the observed correlation coefficients were more extreme (Supplement S2). Nevertheless, the fact that 18 out of 21 coefficients clearly suggest that the number of recruiting oysters and barnacles are negatively correlated to an extent that is not random

Table 2
Summary of correlation analyses between the density of barnacles and oysters in individual locations and seasons.

Habitat	Location	Season	R	T	p
Rock	EBMI	Summer	**−0.48**	**−2.88**	**0.01**
Rock	EBMI	Winter	−0.06	−0.31	0.76
Rock	Ponta Torres	Summer	0.04	0.20	0.85
Rock	Ponta Torres	Winter	0.25	1.38	0.18
Seagrass	Bangua	Summer	−0.05	−0.27	0.79
Seagrass	Bangua	Winter	**−0.47**	**−2.78**	**0.01**
Seagrass	Sangala	Summer	**−0.39**	**−2.25**	**0.03**
Seagrass	Sangala	Winter	0.17	0.90	0.38

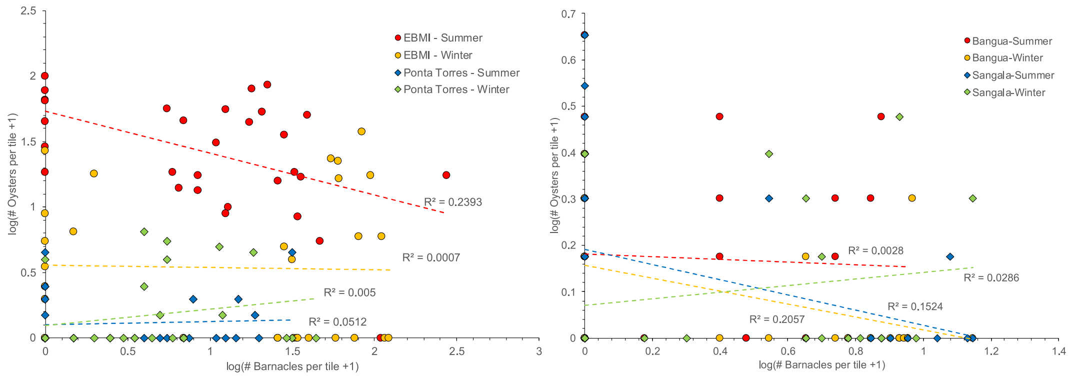

Fig. 6. Correlation between oysters and barnacles in rocky (left) and seagrass (right) habitats in summer and winter at Inhaca Island.

($p < 0.01$ using a two-tailed binomial test). Despite this prevailing pattern, we cannot fully exclude the possibility that the negative correlations are caused by differences in spatio-temporal patterns of settlement between oysters and barnacles rather than direct interactions.

4. Discussion

This study demonstrates that apart from collecting oysters, the easily accessible artificial tiles used in this study, also attracts a variety of fouling fauna when deployed in rocky and seagrass habitats. The results show that the amount and composition of fouling varies among habitats, locations, seasons and surfaces and depending on the duration of the deployment. The fact that there are prevailing negative correlations between the number of oysters and barnacles on our tiles, indicate that other fauna may a mild negative influence on the recruitment of oysters. This pattern may or may not indicate direct interactions, but in the aquaculture context the most important aspect of these findings is that it may influence the collection of these species and future use of collectors for commercial purposes or ecological studies in Maputo Bay and particularly on Inhaca Island.

Larvae of fouling fauna recruited in higher numbers on rough surfaces than smooth surfaces.

This result has also been previously reported to occur for recruitment of a variety of invertebrates and oyster larvae [27]. Surface roughness is referred to provide a better tactile stimulus to setting larvae or crawling juvenile and to aid the physical retention of the larvae, larvae settle preferentially in microsites of low shear created by bumps and crevices on a surface, crevices and pits in rough surface may provide protection from predators for small organisms [28,29].

Rough surface has been associated to greater area and potentially greater microhabitat than do smooth surface [8]. It is important to highlight that this result was consistent at all four sites, with overall recruitment larger on rough than on smooth surfaces, an indication that active choice and behavioural and physical interaction may be responsible for this pattern of fouling settlement reported in this study.

In this study, six broad taxonomic groups reached at least 95 % of the total abundance in seagrass habitats, but only 2 (oysters and barnacles) showed this pattern in rocky habitats. This pattern could of course reflect that oysters and barnacles were generally less abundant in the seagrass habitat, allowing other taxa to have higher relative abundance According to Ref. [30] seagrasses meadows provide structural complexity habitat and environmental conditions suitable for settlement of a variety of faunal assemblage than rocky shore habitat. Structurally complex habitats also influence predator– prey dynamics [31] reducing predator foraging efficiency by reducing prey encounter rate [13, 32–33.

Despite the standardised size, shape and texture of the tiles used, there were large differences among locations, particularly for oysters and barnacles (Figs. 4 and 5). This demonstrate that the larval recruitment process is complex and depends on external environmental factors. However, the relative abundance of species depended on the type of habitat and the season; for example, gastropods, mussels, algae and polychaetes were more abundant in collectors deployed in seagrass habitats than in collectors deployed in rocky shore habitats. The high fouling rates of oysters and barnacles recorded at EBMI in late summer compared to that reported at Ponta Torres may be associated to differences on environmental condition in these locations. Coastal hydrodynamics can affect the dispersal of invertebrate larvae [34–36]. The nature of the Ponta Torres, a coast with numerous craters and pools, results in a complex pattern of coastal currents, many of which can be localised. This can result in variable larval settlement patterns, which can differ considerably between the different groups of fouling organisms.

The results obtained does not reveal any marked seasonal patterns for fouling species. These findings are consistent with previous studies, which have shown that seasonal patterns of fouling fauna in tropical habitats are not particularly evident compared to those of temperate regions [4,37,38]. Similarly, in terms of spatial patterns, our findings are consistent with research undertaken at Inhaca Island [20,22–39], which suggest that barnacles and bivalves are the dominant group of invertebrates on intertidal rocky habitat, whereas gastropods and polychaete dominate the invertebrates of seagrass habitats.

The negative correlation between oyster and barnacles in summer found at EBMI could potentially be related to ecological interactions, such as competition or larval predation, but they could also be due to responses to external environmental factors, such as resistance to desiccation. Barnacles appear to be more resistant to desiccation [4,40,41] and there is evidence that they may displace young oysters [42,43]. Mussels, tube-dwelling polychaetes, barnacles and algae may compete with oyster seeds for space and/or food [44,45]. However in other environmental situations, fouling organisms may attract oyster larvae [43] and our results suggest a negative correlation between the density of barnacles and that of oysters at EBMI. This could be related to difference in recruitment intensity of fouling organisms between seasons. Although barnacle recruitment occurs in both season, the largest recruitment was recorded in late winter in rock habitats. The prevailing winds at that time on Inhaca Island may influence the success of larval settlement. The south-western coast is subject to south-easterly (longshore) winds in summer and north easterly (offshore) winds in winter [46]. Previous studies also reveals that prevailing winds were the major factor resulting in differences in barnacle settlement between rocky shores [46,47].

We conclude that easily accessible and inexpensive tiles can be used to collect oysters in both rocky (*Saccostrea cucullata*) and seagrass habitats (*Pinctada capensis*) at Inhaca Island. Thus, this method can enhance the availability, and offer better opportunities for practical handling of valuable resources, which may otherwise scarce (e.g. *P. capensis*) or impossible to use for local communities due to extremely strong attachment in natural habitats (e.g. *S. cucullata*). The increasing proportion of oysters, compared to other types of fouling organisms, from two to four months also indicate that the yield of oysters may increase through time. Overall, despite some evidence consistent with negative interactions between oysters and barnacles, the effects of other fouling organisms appear modest. This does not exclude the possibility that other types of fouling fauna should be minimised. Therefore, in this context these results may contribute to improve our general understanding of the spatio-temporal patterns and processes that affect the colonisation of intertidal invertebrates, particularly in the south Eastern Indian Ocean.

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Data availability

Data from this research are kept under lock and key in the Department of Biological Science Research database. Data will be made available on request.

CRediT authorship contribution statement

Mizeque Mafambissa: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mats Lindegarth:** Writing – review & editing, Supervision, Software, Formal analysis, Conceptualization. **Adriano Macia:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. All parts involved support the publication of the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35420.

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