Universidade de Aveiro
2021

Sara de Ornelas Nobre Carraça

Aquacultura comunitária de moluscos bivalves no Norte de Moçambique: otimização da produção e dos métodos de preservação

Community-based aquaculture of bivalve molluscs in the North of Mozambique: optimization of production and preservation methods

Universidade de Aveiro
2021

Sara de Ornelas Nobre Carraça

Aquacultura comunitária de moluscos bivalves no Norte de Moçambique: otimização da produção e dos métodos de preservação

Community-based aquaculture of bivalve molluscs in the North of Mozambique: optimization of production and preservation methods

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor Rui Jorge Miranda Rocha, professor auxiliar convidado do Departamento de Biologia da Universidade de Aveiro e diretor de operações e de inovação e desenvolvimento da Riasearch, e coorientação da Doutora Andreia do Carmo Martins Rodrigues, Investigadora do Departamento de Biologia e do Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro.

Dedico este trabalho à minha mãe, por ser o meu grande exemplo de perseverança, resiliência e dedicação.

A todas as pessoas da comunidade costeira do Norte de Moçambique que idealmente fruirão dos resultados obtidos nesta dissertação.

o júri

Presidente	Professora Doutora Maria Helena Abreu Silva Professora Auxiliar da Universidade de Aveiro
Vogal - Arguente	Doutora Domitília da Conceição Coutinha Matias Investigadora Auxiliar do IPMA - Instituto Português do Mar e da Atmosfera
Vogal - Orientador	Doutor Rui Jorge Miranda Rocha Professor Auxiliar Convidado da Universidade de Aveiro

agradecimentos

Aos meus pais, por todo o apoio crucial no desenvolvimento desta dissertação.

Ao meu orientador, Doutor Rui Rocha, por ter possibilitado e acompanhado as diversas vertentes inerentes à realização deste trabalho, assim como por toda a disponibilidade demonstrada no decorrer do mesmo.

À minha coorientadora, Doutora Andreia Rodrigues, por toda a ajuda essencial prestada durante as várias etapas desta dissertação e no esclarecimento de dúvidas mesmo fora do horário convencional.

À Doutora Ana Luísa Silva, pelo apoio imprescindível na quantificação dos microplásticos e por toda a sua contribuição para a melhoria e correção desta dissertação.

À Professora Especialista Teresa Baptista, à Mestre Ana Correia e ao Mestre Pedro Pires, por toda a disponibilidade prestada durante as análises microbiológicas e todo o tratamento dos dados associado.

A todos os colaboradores da Associação do Meio Ambiente (AMA) de Pemba, Moçambique que permitiram o contacto e a comunicação com as comunidades costeiras no Norte de Moçambique, assim como o desenvolvimento dos trabalhos práticos que estiveram na base da presente dissertação.

Ao Davide Silva, pela ajuda no tratamento e análise dos dados.

Aos meus amigos do coração, pelo incansável apoio e incentivo.

palavras-chave

Cabo Delgado, *Saccostrea cucullata, Modiolus philippinarum*, sazonalidade, métodos de preservação artesanais, mercúrio, microplásticos, reservas energéticas

resumo

O crescimento populacional em Cabo Delgado, a província mais a Norte de Moçambique, tem gerado um aumento da exploração dos recursos marinhos pelas comunidades costeiras. Como tal, tem-se verificado um declínio na abundância dos recursos pesqueiros e a degradação dos ecossistemas costeiros. Para possibilitar o restauro dos habitats marinhos deteriorados têm sido criadas áreas marinhas protegidas geridas pelas comunidades locais (LMMA). Devido à exploração limitada dos recursos dentro destas áreas, a implementação de projetos de aquacultura comunitária surge como uma medida compensatória promissora. Neste estudo foi analisado o potencial da ostra *Saccostrea cucullata* e do mexilhão *Modiolus philippinarum* para integrarem produções de aquacultura comunitária em duas aldeias de Cabo Delgado onde as LMMA já foram criadas, Mecúfi e Metuge. Para garantir a qualidade anual de ostras e mexilhões futuramente produzidos, foram estudadas variações sazonais (estação seca vs. chuvosa) na sua contaminação por um poluente persistente (mercúrio) e um poluente emergente (microplásticos) e nas suas reservas nutricionais (lípidos, açúcares e proteínas). Verificou-se que ambos os bivalves se encontravam adequados ao consumo humano durante as épocas seca e chuvosa. Contudo, as ostras e os mexilhões apresentaram valores nutricionais mais altos e níveis de poluentes mais baixos (mercúrio e microplásticos) durante a época seca. Adicionalmente, testaram-se diferentes métodos tradicionais de preservação de alimentos para identificar a metodologia mais eficiente na prevenção e retardamento da deterioração dos bivalves e da perda das suas qualidades nutricionais. A salga foi o método artesanal que mais eficazmente preveniu a proliferação de microrganismos e que melhor conservou o conteúdo proteico das ostras e dos mexilhões. Por último, implementou-se um projeto de aquacultura comunitária de *M. philippinarum* em Mecúfi e em Metuge em conjunto com as mulheres das aldeias. Após um período de seis meses, os mexilhões cultivados em Metuge apresentaram um maior incremento do tamanho da concha e do peso, assim como uma menor contaminação por microplásticos e um maior conteúdo nutricional que os mexilhões cultivados em Mecúfi, o que indica que um projeto de aquacultura comunitária de *M. philippinarum* seria melhor sucedido se implementado em Metuge. Contudo, para permitir a implementação de uma produção de *M. philippinarum* através de aquacultura comunitária em Mecúfi, sugere-se que seja selecionado um local mais propício ao desenvolvimento do mexilhão nas redondezas da aldeia.

keywords

Cabo Delgado, *Saccostrea cucullata, Modiolus philippinarum,* seasonality, artisanal preservation methods, mercury, microplastics, energetic reserves

abstract

Population growth in Cabo Delgado, the northern province of Mozambique, has pushed coastal communities to increase marine resources exploitation to ensure their livelihoods. This has led to a decline of marine resources stocks and to a degradation of coastal ecosystems. To help restoring marine habitats, Locally Managed Marine Areas (LMMA) have been established in Cabo Delgado coastal villages and community-based aquaculture projects have been pointed out as one of the promising measures compensating for the limited resources exploitation inside LMMA. In this study we analysed two bivalve species, *Saccostrea cucullata* oysters and *Modiolus philippinarum* mussels and their potential for community-based aquaculture in two villages of Cabo Delgado where LMMA have been established, Mecúfi and Metuge. To assess the suitability for human consumption of future aquaculture-produced oysters and mussels throughout the whole year, we studied oysters and mussels' seasonal variations (dry vs. wet season) of a persistent (mercury) and emerging pollutants (microplastics) contamination as well as their nutritional reserves (lipids, sugars and proteins). We verified that both oysters and mussels were proper for human consumption during dry and wet seasons. However, oysters and mussels had higher nutrient contents and less pollutants (mercury and microplastics) contamination during dry season. Additionally, we tested distinct traditional preservation methods to identify the technique that best prevented bivalves' spoilage and loss of their nutrient qualities. Dry salting was the artisanal preservation method that more efficiently prevented microbial growth and that best kept protein nutritional qualities of oysters and mussels. Finally, we implemented a community-based aquaculture of *M. philippinarum* in Mecúfi and Metuge with women villagers. After a six-month period, we verified that mussels grown in Metuge had higher shell and weight increment, less microplastics contamination and higher nutritional content than mussels grown in Mecúfi. So, a community-based aquaculture of *M. philippinarum* mussels might be better succeeded if installed in Metuge. Nevertheless, we suggest that a different location nearby the village of Mecúfi should be selected to develop a community-based aquaculture of *M. philippinarum.*

Index

1. General introduction

Population in Mozambique has been growing in the last decades and projections for 2050 predict that this trend will continue (INE, 2020a). In the last four decades, Mozambique population more than doubled, increasing from 12.1 million people in 1980 to 27.9 million people in 2017 (INE, 2020b). In Cabo Delgado, the northern province of Mozambique, the trend has been the same. Here, the population reached more than 2 million people in 2017 (INE, 2020c), representing 8.1% of the total population living in Mozambique in that year. Population growth implied a higher food requirement which in a rural and poor region like Cabo Delgado (INE, 2020c) is not easy to ensure. To fight hunger, coastal communities in Cabo Delgado had increased marine resources exploitation using unsustainable practices, leading to stocks depletion and damage of coastal marine ecosystems (Rosendo *et al.,* 2020).

Aquaculture emerges as a solution that enables coastal communities to ensure their livelihoods by keeping exploring and consuming marine resources and at the same time allowing the recovery of depleted coastal ecosystems resources (Ateweberhan *et al.,* 2014; Rosendo *et al.,* 2020). Community-based aquaculture practices have expanded in recent years throughout tropical and subtropical coastal developing countries (Ateweberhan *et al.,* 2014; Gaertner *et al.,* 2020). Community-based aquaculture is managed by a community group using extensive and low-technological methods (Ananth *et al.,* 2014), ensuring that coastal communities have the rights of fishing resources they explore and at the same time they are part of this sustainable marine resource management (Rosendo *et al.,* 2020). The fact that the production methods are low-technological and extensive simplifies the implementation of community-based aquaculture in different locations and reduces negative impacts on coastal ecosystems (Ananth *et al.,* 2014; Ateweberhan *et al.,* 2014). Moreover, local materials are preferentially used to build structures required for community-based aquaculture and native species are favourably cultivated.

Bivalves are explored and consumed by Cabo Delgado coastal communities (Fernando *et al.,* 2012), and they are nutritious foods advisable to include in a healthy diet (Cruz-Romero *et al.,* 2008). Bivalve community-based aquaculture alleviates the pressure on wild stocks and provides a source of protein to people integrating the lowest socioeconomic status (women and children) in Cabo Delgado. In fact, the staple diet of women and children in Cabo Delgado is rich in starches like *xima* (maize meal) and cassava, which provides them low protein and mineral levels (Lusambili *et al.,* 2020). Besides, men are usually fed first than women and children due to socio-cultural beliefs (Lusambili *et al.,* 2020). So, a community-based aquaculture of bivalves leaded by women in the North of Mozambique would provide them a reliable source of income and a protein-rich food that would enable the diversification of women and their children livelihoods. Here we studied the rock oyster *Saccostrea cucullata* and the brown mussel *Modiolus philippinarum*. The rock oyster *S. cucullata* is broadly distributed in the Indo-Pacific and subtropics, occurring from East Africa to the Pacific Islands

(Kalyanasundaram & Ramamoorthi, 1987). It inhabits mainly in the upper eulittoral zones (Hartnoll, 1976) of marine and brackish ecosystems, where it settles on rocks, mangrove prop roots and on other hard substrates (e.g., jetties) (Singh, 2019). The brown mussel *M. philippinarum* is also an Indo-Pacific species, however it is distributed as well in the Red Sea and South and East China Seas (Rajagopal *et al.,* 1999). This mussel species is a tropical mytilid that uses its byssus to attach to a rocky substrate in the intertidal or to sediment particles and seagrass roots, potentially forming extensive beds (Ozawa, 2001; Savazzi, 1989). Both bivalve species are consumed in the villages of Mecúfi and Metuge where our work took place.

Oysters and mussels are filter-feeder bivalves that can remarkably accumulate heavy metals and other persistent pollutants (Naimo, 1995). During their feeding activity, bivalves filter large amounts of water to remove particulate matter from it, contacting with many potential metals present in the water (Ciutat & Boudou, 2003). So, metal concentrations in bivalves body tissues can be 1000 times higher than in the surrounding water (Naimo, 1995). Mercury can reach coastal waters through natural (e.g., volcanic and hydrothermal activity) and anthropogenic sources (e.g., industry wastes) (Saniewska *et al.,* 2014; Stoffers *et al.,* 1999). This heavy metal bioaccumulates with trophic level increase and fish and seafood (bivalves) consumption are the most common source of mercury poisoning to humans (Saniewska *et al.,* 2014), causing damages in the gastrointestinal tract, in the kidneys and in the neurological system (Andersen *et al.,* 1993; Park & Zheng, 2012; Wheatley *et al.,* 1979).

Other broadly distributed pollutants of concern are microplastics. Microplastics are plastic debris < 5mm that can be derived from the breakdown of larger plastic pieces or they can enter our ecosystems already assuming these small proportions (Peixoto *et al.,* 2019; Strungaru *et al.,* 2019). The greatest number of plastics in marine ecosystems reached these natural environments already in the form of microplastics (Peixoto *et al.,* 2019) like spheres, pellets, irregular fragments and fibres (Wright *et al.,* 2013). Bivalves have great exposure risk to microplastics due to their filter-feeding mechanism (Li *et al.,* 2015; Wright *et al.,* 2013). Consequently, and because we consume the whole-body tissue of these organisms, they might be a pathway to human exposure of microplastics (Thiele *et al.,* 2019). It has been shown that microplastics can have potential negative impacts in human's health since they can alter metabolic mechanisms as well as cause oxidative stress, cell apoptosis and inflammatory responses (Brown *et al.,* 2001; Inkielewicz-Stepniak *et al.,* 2018; Mahadevan & Valiyaveettil, 2021; Mahler *et al.,* 2012). Besides, heavy metals have been used as plastic additives (Hahladakis *et al.,* 2018). Barboza *et al.* (2018) have shown an increased mercury bioconcentration and bioaccumulation in *Dicentrarchus labrax* organs due to higher microplastics contamination. So, microplastics can be a pathway of human exposure to harmful chemicals, namely heavy metals (Barboza *et al.,* 2018).

That said, the present work aimed to verify if *S. cucullata* oysters and *M. philippinarum* mussels collected and aquaculture-produced in Cabo Delgado coastal villages were proper for human consumption.

In the second chapter, we assessed if seasonality affected the suitability and nutritional quality of *S. cucullata* oysters and *M. philippinarum* mussels collected in Cabo Delgado. Bivalve field sampling was performed in two different seasons (dry and wet) to evaluate seasonal changes on two contaminants (mercury and microplastics) burden in oysters and mussels as well as their constitution in the three major energetic reserves (lipids, sugars and proteins) and oxidative damage (lipid peroxidation).

Moreover, it was important to ensure that bivalves produced by community-based aquaculture could be efficiently preserved for longer periods of time after being collected. So, in the third chapter, we tested different traditional preservation methods to preserve oysters and mussels: salting, smoking and sun-drying. These methods are used by coastal communities of Cabo Delgado to preserve fish and seafood (Souto, 2015) since only 19% of the population living in this region of Mozambique has access to electricity (INE, 2020c). This lack of access to electricity in the majority of Cabo Delgado population unable the preservation of alimentary products using refrigerators and freezers. So, it was relevant to evaluate which of the traditional preservation methods studied more effectively conserved oysters and mussels' nutritional quality and prevented them to be spoiled. To verify this, oysters and mussels' energetic reserves (lipid, sugar and protein content) and microbial contamination were assessed.

This work is part of Our Sea Our Life (OSOL) project coordinated by the Zoological Society of London. One of the aims of OSOL project is to establish Locally Managed Marine Areas (LMMA) (Rosendo *et al.,* 2020). LMMA are marine protect areas where fishing practices are managed by local coastal communities to ensure their livelihoods in a way that also allow the recovery of depleted resources and degraded ecosystems (Rosendo *et al.,* 2020). One of the measures compensating for the LMMA establishment is community-based aquaculture (Rosendo *et al.,* 2020) since it gives an alternative to explore marine resources while LMMA are recovering their biodiversity values. In the fourth chapter, we analysed the implementation of a non-fed community-based aquaculture of *M. philippinarum* mussels in Mecúfi and Metuge, two villages of Cabo Delgado where LMMA were created. We chose to develop a community-based aquaculture only with *M. philippinarum* mussel instead of producing *S. cucullata* oyster as well because mussel seeds are easier to collect compared to encrusted oysters. *M. philippinarum* community-based aquaculture was developed with women villagers and it aimed to verify the viability of this bivalve species production in two coastal villages, Mecúfi and Metuge.

2. Seasonal variations of pollutants contamination (mercury and microplastics) and nutrient reserves of *Saccostrea cucullata* oysters and *Modiolus philippinarum* mussels in the North of Mozambique

2.1 Introduction

Seasonality of the dry and wet seasons can affect physical, chemical and biological processes in coastal marine ecosystems (McClanahan, 1988), as well as concentrations of contaminants, such as heavy metals (Barua *et al.,* 2011; Mtanga & Machiwa, 2007). Such alterations greatly affect marine organisms that inhabit these ecosystems and might compromise the harvest of some species for human consumption (Lipp *et al.,* 2001; Rajeshkumar *et al.,* 2018). Seasonal variations of persistent and emerging pollutants have been described in coastal ecosystems (Barua *et al.,* 2011; Cheung *et al*., 2016; Mtanga & Machiwa, 2007). Mercury is among one of the major persistent pollutants that reaches coastal zones mainly through rivers due to the intensive inland anthropogenic activities (Saniewska *et al.,* 2014) or because of geological settings that might exist in coastal zones, like tectonic, volcanic and hydrothermal activity (Stoffers *et al.,* 1999). Mercury tends to mostly accumulate in coastal areas instead of reaching off-shore waters and its concentration in gulfs and bays can be multiple times higher than in off-shore waters (Horvat *et al.,* 2003; Laurier *et al.,* 2004; Saniewska *et al.,* 2014). Rainfall has been associated with higher abundance of anthropogenic debris and pollutants in coastal zones since it increases their transport by surface run-off into streams and rivers (Araújo & Costa, 2007; Barua *et al.,* 2011). In regions with different seasonal precipitation patterns, during wet season there are more rainfall and stream flow than during dry season (Ríos-Touma *et al.,* 2011; Zhang *et al.,* 2007). In tropical areas, higher river discharges into coastal environments during wet season due to heavy rainfall can affect heavy metals concentration in these ecosystems (Barua *et al.,* 2011).

Coastal waters have also been identified as a major sink of emerging contaminants, such as microplastics from inland activities (Cheung *et al*., 2016), most of them carried out by rivers (Lebreton *et al.,* 2017). Microplastics in sediments tend to be significantly higher than in the water column (Scherer *et al.,* 2020), affecting organisms differently depending on their habitat. Due to their density, size, and shape, microplastic levels in both water and sediments are likely to change from dry to wet seasons (Oni *et al.,* 2020). In fact, in tropical environments it has been detected an increase of microplastics pollution during wet season in coastal waters because of higher surface run-off and drainage of this debris into rivers due to intense rainfall (Cheung *et al*., 2016; Lima *et al.,* 2014; Moore *et al.,* 2002).

Bivalves are filter feeders present in coastal ecosystems that can accumulate large amounts of mercury (Naimo, 1995; Sajwan *et al.,* 2008) and microplastics in their body tissues (Li *et al.,* 2015; Wright *et al.,* 2013), pollutants that can suffer seasonal variations in coastal ecosystems. Biochemical composition of bivalve molluscs can also change seasonally (Costa *et al*., 2020; Mitra *et al.,* 2008;

Topić Popović *et al*., 2020) due to: physiological conditions, depending on the reproductive cycle and food availability; environmental parameters like salinity and temperature; contaminants pollution like heavy metals and microplastics (Bour *et al.,* 2018; Jana *et al.,* 2013; Paul *et al.,* 2021; Widdows, 1978). In Cabo Delgado province, the northern region of Mozambique, bivalves are collected and consumed mainly in coastal villages (Fernando *et al*., 2012). This region has a Tropical Savanna climate according to Köppen's classification and it has two distinct seasons: the dry season (May-November) and the wet season (December-April) (Pemba Climate: Temperature, Climograph and Climate Table for Pemba – Climate-Data.org, n.d.; Rrokaj & Corti, 2019).

In the present work, we evaluated the levels of mercury (persistent pollutant) and microplastics (emerging contaminant) in two valuable species of bivalves consumed in Cabo Delgado province, *Saccostrea cucullata* oyster and *Modiolus philippinarum* mussel, during 2019 dry season and 2020 wet season. The biochemical composition was also evaluated to infer about their health and nutritional values. Our working hypothesis is that seasons affect bivalves' exposure to contamination, contaminants accumulation levels and biochemical composition, and therefore their consumption suitability and quality.

2.2 Materials and methods

2.2.1 Study area

This study was carried out in the villages of Mecúfi (-13.289695 S, 40.566257 E) and Metuge (-12.971234 S, 40.414297 E) in Cabo Delgado province in the North coast of Mozambique. The study site in Mecúfi was next to a mangrove forest and a river estuary in the seacoast. In Metuge, the study site was inside Pemba Bay, also next to a mangrove forest.

2.2.2 Sampling procedure

Sampling occurred in September 2019 (dry season) and March 2020 (wet season). *M. philippinarum* mussels were collected in the seacoast nearby Mecúfi and *S. cucullata* oysters were collected from an oyster bank near Metuge. Organisms were stored at -20 °C to evaluate mercury and microplastics levels, and to address organisms' biochemical composition (i.e., oxidative damage via lipid peroxidation; energetic reserves via lipid, sugar and protein contents).

Physicochemical seawater parameters (i.e., water oxygen, temperature, pH, salinity and conductivity) were measured *in situ* during sampling, using a digital multiparameter (WTW 2FD460 Multi 3420).

2.2.3 Mercury quantification

Total mercury (µg/g wet weight) was quantified in oysters (n=10 per season) and mussels (n=10 from dry season and n=5 from wet season) collected from Mecúfi and Metuge by atomic absorption spectrophotometry using an Advanced Mercury Analyser (AMA) LECO 254. The analytical procedure based on thermal decomposition (Costley *et al*., 2000) followed the adaptations developed by Cabecinhas *et al*. (2015) and Vieira *et al*. (2015): drying time of 60 s, decomposition time of 150 s and waiting time of 45 s.

At the beginning and between samples blanks were performed until values obtained were less than 0.02 ng of Hg (equipment detection limit) to internally clean the analyser. Two certified reference materials (NRC TORT-3 Lobster hepatopancreas, and NRC DOLT-5 Fish liver) were also intercalated with samples to assess the accuracy of the data.

2.2.4 Microplastics extraction and quantification from biological tissues

S. cucullata (n=10 per season) and *M. philippinarum* (n=10 from dry season and n=5 from wet season) were prepared for microplastics extraction and quantification (microplastic particles/g wet weight). Extraction of microplastics from whole organisms followed the adapted KOH digestion protocol described by Thiele *et al*., (2019), with minor adjustments.

Briefly, defrosted soft tissues were removed from shells, placed in glass containers and digested with 10% KOH (EMSURE®; w/v, 3× tissue volume, ≈30 ml), and incubated without agitation at 50 °C for up to 96h. Afterwards, digestates were neutralised with 1M citric acid solution (ITW Reagents), gently swirled and immediately vacuum-filtered onto glass microfiber filters (0.7 µm pore size, ∅ 47 mm, Ahlstrom-Munksjö). For microplastic quantification, each filter was stained with Nile Red (10 µg NR per mL of ethanol, N-3013, Sigma Aldrich) for 3 minutes, filters were then thoroughly rinsed with filtered ultrapure water, stored in glass petri-dishes and allowed to dry at room temperature for 48h. Dried filters were photographed (Nikon D500, AF-S nikkor 18–55 mm, F5.6, ISO 100, exposure time varied according to the lightness needed) under visible light (blue light, 450nm, SPEX Forensics, U.S.A.) using an orange filter (Standard ProMaster® Orange Filter). Microplastic debris present in the filters were quantified by individually counting microplastic particles on the images using the freeware ImageJ (version 1.52K, National Institute of Health, U.S.A.). Only particles revealing a clear fluorescence on a red colour range under blue light were considered microplastics (see Figure S1).

Contamination control measures included the use of 100% cotton clothing. Glassware was acid washed and pre-cleaned with ultrapure water before use. Whenever possible, samples were covered with aluminium foil and work was performed in a fume hood. Airborne contamination was assessed by applying laboratory blanks composed of cleaned petri dishes filled with ultrapure water in each laboratory room used for the analysis and we also used blanks (to assess cross-contamination)

composed of glass tubes with 10% KOH and without any biological sample that were submitted to the whole procedure of microplastics extraction.

2.2.5 Oxidative damage and energy-related biomarkers analysis

2.2.5.1 Sample preparation

Tissue from oysters (n=10 per season) and mussels (n=10 from dry season and n=5 from wet season) were individually homogenised on ice using 4ml of ultra-pure water, using the sonicator (pulsed mode 80%, output control 1, 250 Sonifier, Branson Ultrasonics). Oysters and mussels were homogenised during 160 and 80 pulses, respectively. From each sample, two aliquots were taken for the analysis of lipid, sugar and protein contents. One aliquot containing 4% butylated hydroxytoluene (BHT) in methanol was used to determine lipid peroxidation (LPO).

All biomarkers determinations were performed spectrophotometrically, in micro-assays set up in 96 well flat-bottom plates, with the Microplate reader MultiSkan Spectrum (Thermo Fisher Scientific, USA) (Rodrigues *et al*., 2015; Silvestre *et al.,* 2021).

2.2.5.2 Lipid peroxidation

Endogenous lipid peroxidation (LPO) was determined by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm (nmol TBARS/g wet weight) (Bird & Draper, 1984). Experimental blanks were run simultaneously, i.e. experimental solution and ultra-pure water instead of bivalve sample.

2.2.5.3 Energetic reserves

Lipid, sugar, and protein content (mJ/mg wet weight) was determined using the methods described by De Coen & Janssen (1997) with slight modifications for microplate (Rodrigues *et al*., 2015).

The total lipid content of each organism was determined by adding chloroform, methanol and ultra-pure water in a 2:2:1 proportion. After centrifugation, the organic phase of each sample was transferred to clean glass tubes and H_2SO_4 was added before incubation for 15 min at 200 ºC. Tripalmitin was used as a lipid standard, and absorbance was measured at 375 nm.

Prior to total carbohydrates and protein quantification, it was required to do a samples' pre-treatment. After adding 15% TCA (Trichloroacetic acid) to the samples, they were incubated at -20 ºC for 10 min. After a 10 min centrifugation the supernatant was transferred to a clean eppendorf and used for carbohydrates quantification. The pellet was resuspended using NaOH and then samples were heated up to 60 ºC for 30 min. Using HCl, samples were neutralised and used for protein content

quantification. Carbohydrates quantification was performed by adding 5% phenol and H_2SO_4 to the pre-treatment samples in glass tubes, with glucose as a standard. Absorbance was read at 492 nm. For total protein content quantification Bradford's method (Bradford, 1976) was used. Absorbance was measured at 520 nm and we used bovine serum albumin as a standard.

Fractions of energy available were converted into energetic equivalent values using the corresponding energy of combustion: 39500 mJ/ g lipid, 17500 mJ/ g glycogen, 24000 mJ/ g protein (Gnaiger, 1983).

2.2.6 Data analysis

Differences between oysters dry and wet season and mussels dry and wet season regarding mercury, microplastics and biomarkers were analysed by Independent-Samples t-test with a significance level of 5%. Shapiro-Wilk test was used to verify if our data followed a normal distribution. When this assumption was not fulfilled, we performed a Mann-Whitney non-parametric test. All these procedures were conducted using IBM SPSS Statistics 25. Original sample size was ten in all analyses, however we considered outliers values that fell out of the interval mean ± 2*standard deviation, so sample size varied between nine and ten. Nevertheless, concerning data from mussels of the wet season sample size was only five in all the analyses performed (mercury, microplastics and biomarkers) due to transportation and methodological constraints.

We also performed a Principal Component Analysis (PCA) for oysters and mussels to verify which of the studied variables contributed the most to data segregation between dry and wet season of these two organisms. This analysis was developed in R (R Core Team, 2020), using the package ggbiplot. For graphical representations we used PC1 (x axis) and PC2 (y axis).

2.3. Results

2.3.1 Physicochemical water parameters

Physicochemical seawater parameters measured in Mecúfi and Metuge during 2019 dry season (September) and 2020 wet season (March) are presented in Table 1. We verified few differences between seawater physicochemical properties in dry and wet seasons. In both locations, oxygen was slightly lower during wet season than during dry season. Temperature between dry and wet seasons was similar in Metuge and it increased in Mecúfi. Seawater pH was similar between dry and wet seasons in both locations and salinity and conductivity were lower in wet season than in dry season in Metuge and in Mecúfi.

Table 1. Physicochemical seawater parameters in Mecúfi and Metuge in September 2019 (dry season) and March 2020 (wet season).

	Metuge		Mecúfi	
	September 2019	March 2020	September 2019	March 2020
Oxygen (mg/l)	8.00 (106.6%)	7.74 (102.5%)	8.43 (108.1%)	8.29 (109.1%)
Temperature (°C)	30.5	30.0	28.4	29.9
pH	7.80	7.96	8.05	8.00
Salinity	35.8	31.8	34.8	32.1
Conductivity (mS/cm)	53.8	48.4	52.6	48.8

2.3.2 Mercury contamination

Oysters' mercury contamination was significantly higher during wet season (Fig. 1, Table S1). Although mussel mercury levels were visibly higher in wet season than in dry season, this difference was not significant (Fig. 1, Table S1). Mussels presented 44,4% and 36,3% more mercury contamination than oysters during dry and wet seasons, respectively.

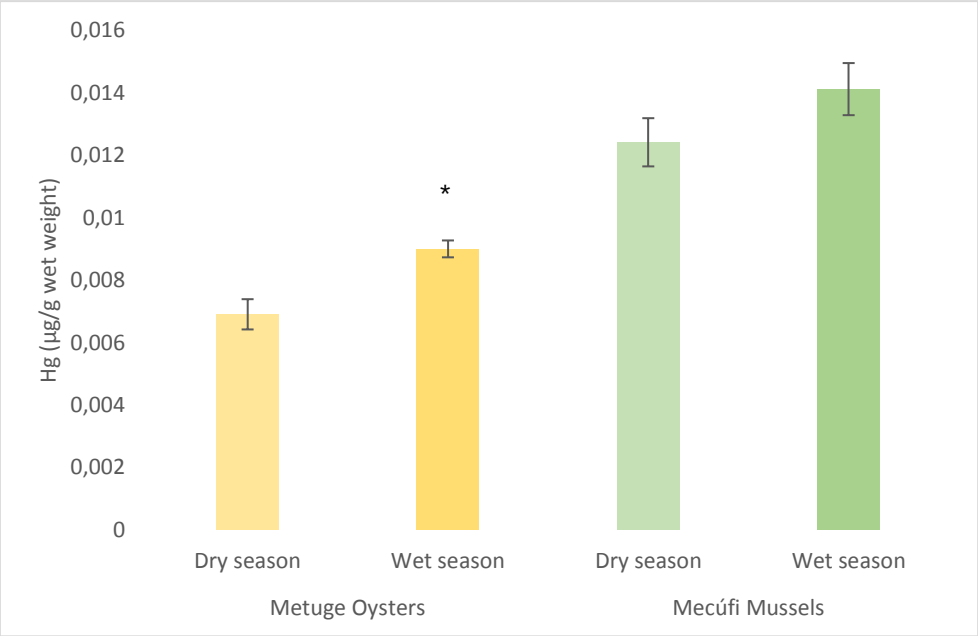

Fig. 1 – Mercury contamination (µg/g wet weight) (mean ± s.e.) of *S. cucculata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

2.3.3 Microplastics contamination

Microplastics contamination in oyster samples was significantly higher during wet season (Fig. 2, Table S1). Mussel microplastics levels were visibly higher in wet season than in dry season, however

this difference was not significant (Fig. 2, Table S1). Mussels presented higher microplastics contamination than oysters during dry and wet seasons (Fig. 2).

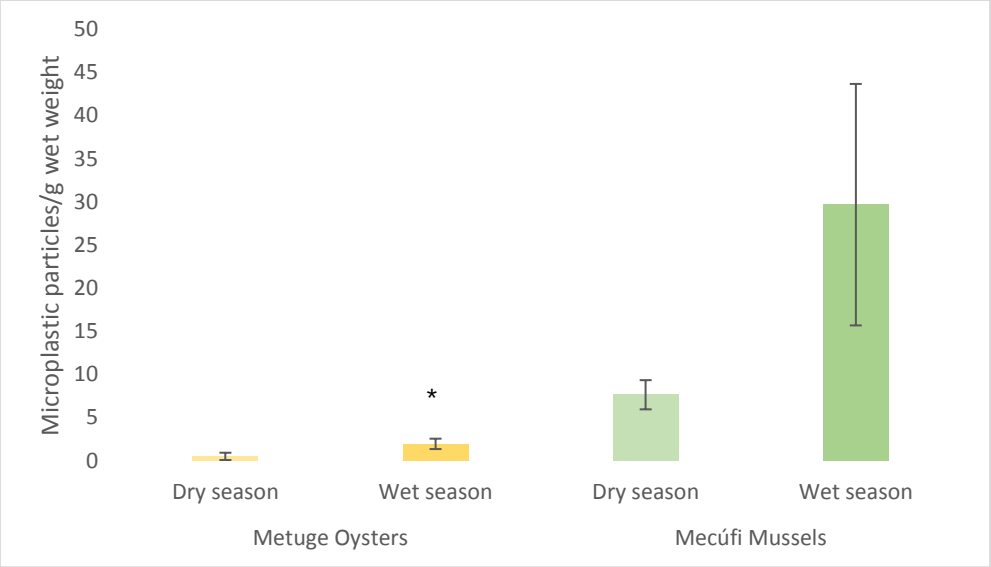

Fig. 2 – Microplastics contamination (microplastic particles/g wet weight) (mean ± s.e.) of *S. cucullata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

2.3.4 Oxidative damage and energy-related biomarkers analysis

Oysters LPO was significantly higher during dry season than during wet season and mussels LPO was significantly higher during wet season than in dry season (Fig. 3, Table S1). LPO was higher in mussels than in oysters in both dry and wet seasons (Fig. 3).

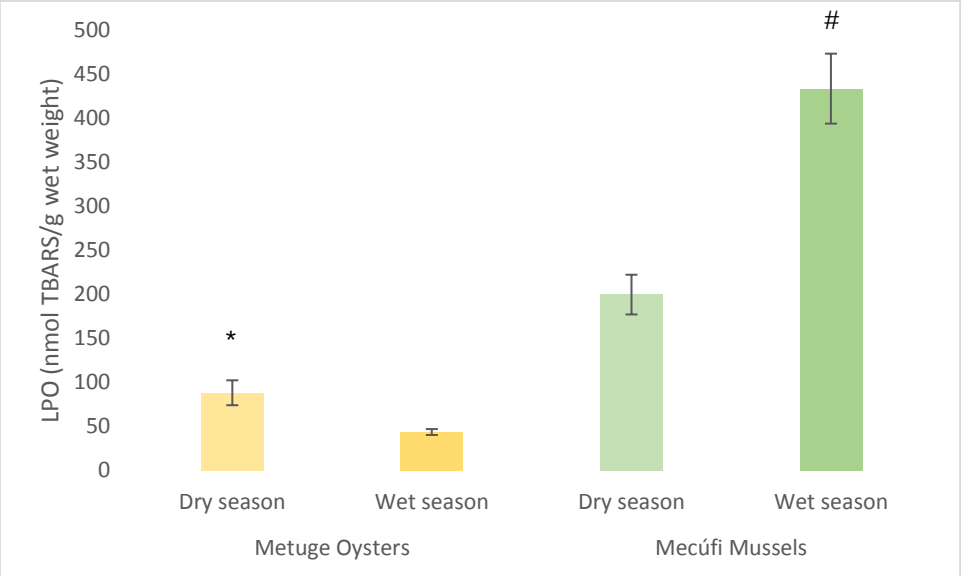

Fig. 3 – Lipid peroxidation (LPO, nmol TBARS/g wet weight) (mean ± s.e.) of *S. cucculata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

Oysters' lipid, sugar and protein content was significantly higher during dry season than during wet season (Fig. 4,5,6, Table S1). Lipid, sugar and protein content in mussel samples was also significantly higher during dry season than during wet season (Fig. 4,5,6, Table S1).

In both dry and wet seasons, oysters and mussels' lipid content was similar (Fig. 4). Sugar and protein content were higher in oysters than in mussels in dry and wet seasons (Fig. 5,6).

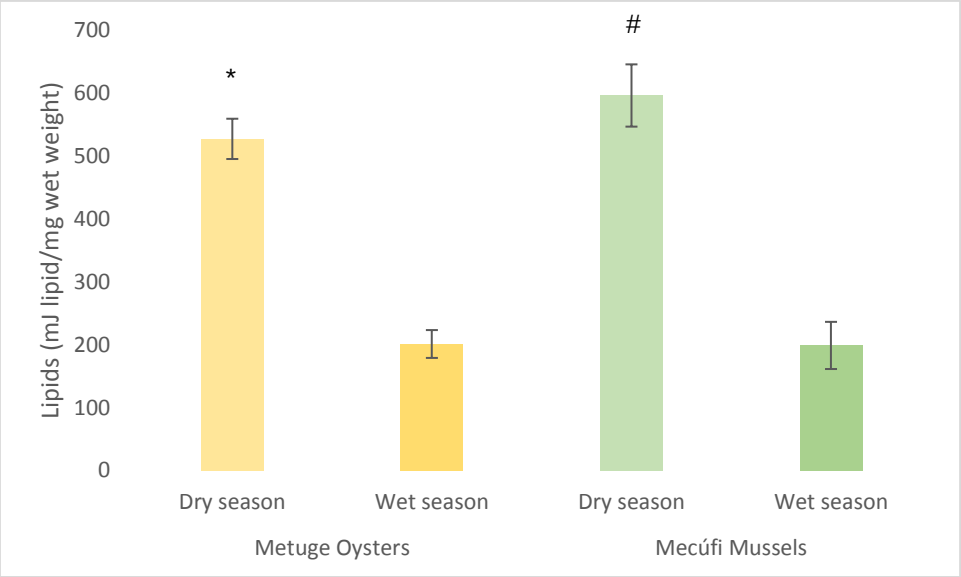

Fig. 4 – Lipids (mJ lipid/mg wet weight) (mean ± s.e.) of *S. cucculata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

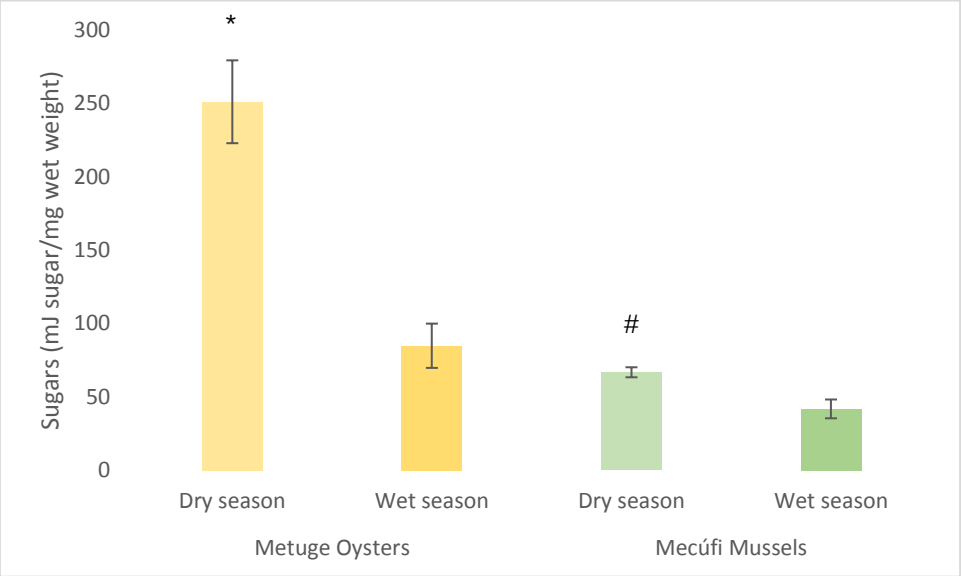

Fig. 5 – Sugars (mJ sugar/mg wet weight) (mean ± s.e.) of *S. cucculata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

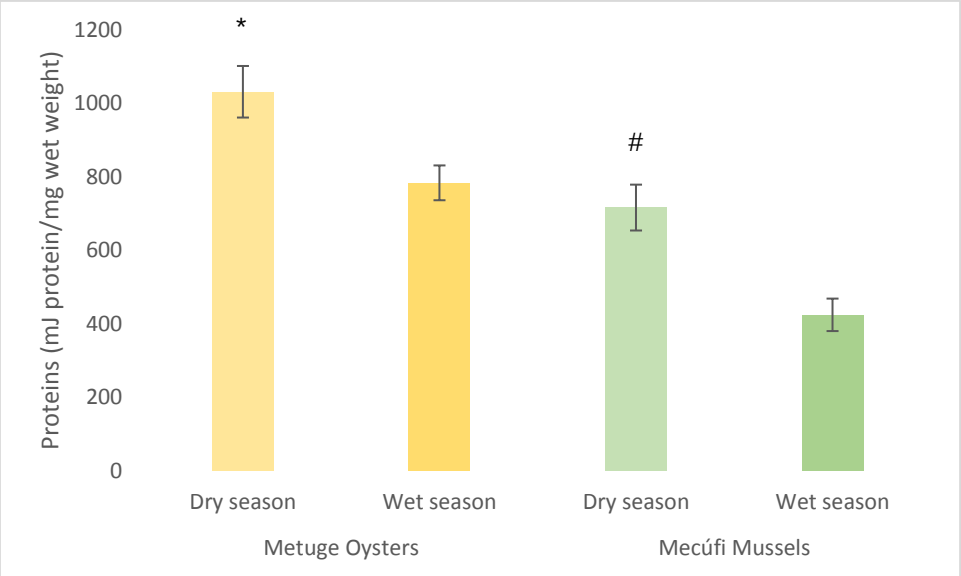

Fig. 6 – Proteins (mJ protein/mg wet weight) (mean ± s.e.) of *S. cucculata* oysters from Metuge and *M. philippinarum* mussels from Mecúfi during dry and the wet season. Significant differences detected between dry and wet seasons in oysters (*) and mussels (#) are presented.

2.3.5 PCA

Concerning oysters PCA, the first principal component (PC1) accounted for 55.2% and PC2 accounted for 20.6% of the total variability among the analysed data. We chose to analyse oysters PCA results according to values concerning PC1 because as observed in Fig. 7 PC1 provided a better visualization of separation between dry and wet season samples. Lipid and sugar content were the variables that contributed the most for oysters' data segregation between dry and wet seasons (Fig. 7, Table S2). Conversely, microplastics contamination was the variable that had a smaller influence in this segregation (Fig. 7, Table S2).

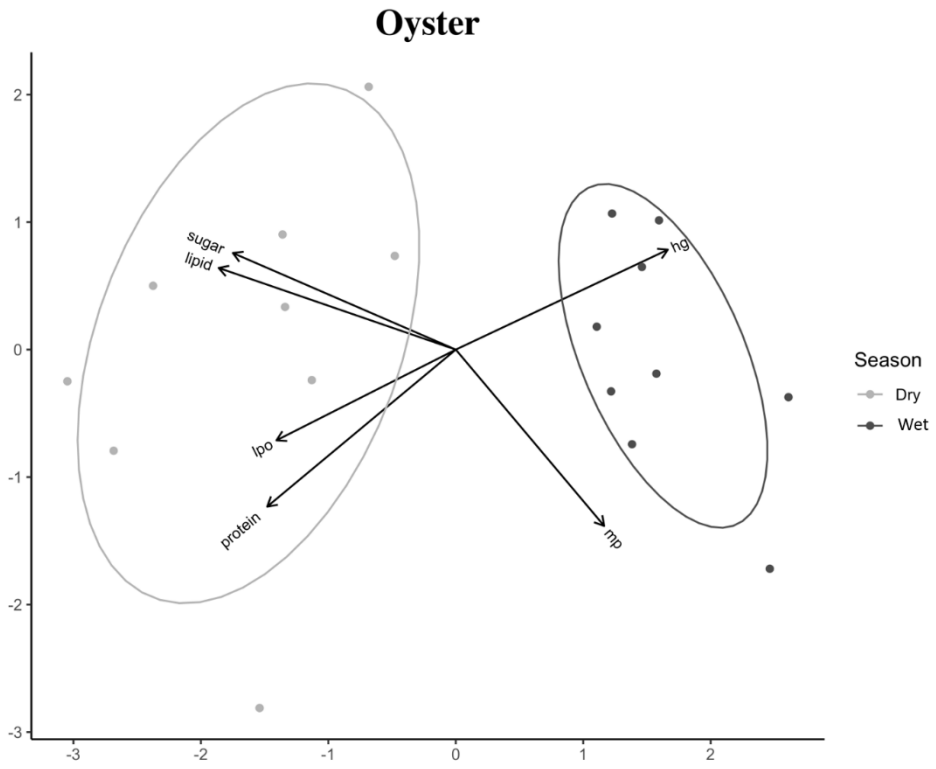

Fig. 7 – PCA of oyster samples and their pollutants contamination data (mercury, Hg and microplastics, mp) and oxidative stress and energy-related biomarkers (LPO, lipids, sugar and protein) with PC1 on x axis and PC2 on y axis.

In Mussels PCA, PC1 accounted for 50.9% and PC2 accounted for 18.6% of the total variability among the studied samples. We decided to analyse mussels PCA results according to values concerning PC1 because it enabled a clearer segregation of dry and wet season samples. LPO and lipid content were the two variables that influenced the most mussels' data segregation between dry and wet seasons (Fig. 8, Table S3). On the other hand, mercury was the variable that contributed less to this segregation (Fig. 8, Table S3).

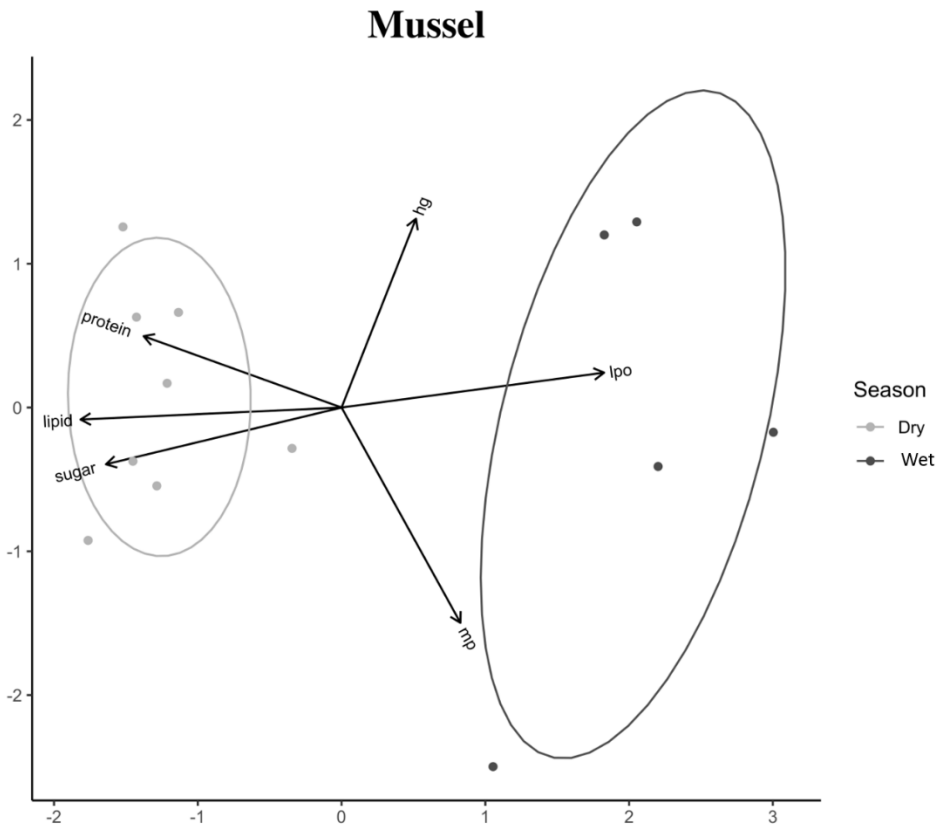

Fig. 8 – PCA of mussel samples and their pollutants contamination data (mercury, Hg and microplastics, mp) and oxidative stress and energy-related biomarkers (LPO, lipids, sugar and protein) with PC1 on x axis and PC2 on y axis.

2.4 Discussion

Differences due to seasonality in pollutants contamination in coastal ecosystems have already been shown (Barua *et al.,* 2011; Cheung *et al*., 2016; Mtanga & Machiwa, 2007). Besides, it had been suggested that rivers are a big source of mercury (Horvat *et al.,* 2003; Laurier *et al.,* 2004; Saniewska *et al.,* 2014) and microplastics pollution (Cheung *et al*., 2016) in coastal waters. Therefore, due to higher river discharges into these waters during wet season (Li & Zhang, 2010), we expected that oysters and mussels' contamination with these pollutants were higher during wet season than during dry season. Accordingly, our results showed that oysters and mussels had higher mercury and microplastics contamination during wet season. Oysters' sampling spot (Metuge) was 45km north, in a straight line parallel to the coast from mussels' sampling spot (Mecúfi). The fact that these bivalves were in different locations possibly under different pollution levels might have influenced their distinct contamination with mercury and microplastics. Besides, different species have different intrinsic metabolic rates, which affects their pollutants contamination (Boening, 1999).

Mussels collected during wet season had the highest mean mercury contamination, ≈0.01 µg/g wet weight. Mercury maximum levels in fishery products (including molluscs) for human consumption were suggested by the European Food Safety Authority (EFSA) and established by the European Commission (EC) at 0.50 µg/g wet weight (European Commission, 2006). So, mercury contamination values were below the maximum levels accepted in fishery products, which means that concerning this pollutant, oysters and mussels collected in these locations seem safe for human consumption throughout the whole year. Mean total mercury levels registered in the present study in soft tissues of *S. cucullata* oysters (≈0.030 ± 0.002 and 0.040 ± 0.001 µg/g dry weight during dry and wet season, respectively) were among the mean values detected in this species in the Persian Gulf (ranging between ≈0.035 ± 0.001 and 0.065 ± 0.001 µg/g dry weight) (Mohammadi *et al.,* 2012; Shirneshan *et al.,* 2012) and below the mean values registered in the same species in Goa coast, India (ranging from ≈0.06 to 0.10 µg/g dry weight) (Patra *et al.,* 2019). *S. cucullata* total mercury content (≈0.007 ± 0.001 and 0.009 ± 0.001 µg/g wet weight in dry and wet seasons, respectively) in Metuge, Mozambique was also lower than in its northern country neighbour, Tanzania (ranging between ≈0.055 ± 0.026 and 0.536 ± 0.036 µg/g wet weight, in two different mangrove ecosystems) (Machiwa, 2010; Mtanga & Machiwa, 2007). Concerning *M. philippinarum* mussel mercury content (≈0.057 ± 0.004 and 0.065 ± 0.004 µg/g dry weight during dry and wet season, respectively) it was lower in Mecúfi, Mozambique than in organisms of this species sold in Northern China markets (0.22 ± 0.04 µg/g dry weight) (Zhao *et al.,* 2013). We could not find studies reporting mercury content in *S. cucullata* and *M. philippinarum* or any other bivalves from the seacoast of Mozambique. Moreover, studies have shown that during warmer periods bivalves are less tolerant to heavy metals contamination (Cherkasov *et al.,* 2006; Sokolova, 2004; Sokolova & Lannig, 2008). In the context of global climate change, it has been predicted that temperatures will rise, so bivalves' survival might be affected (Sokolova & Lannig, 2008).

Microplastics in *S. cucullata* oysters have been quantified in a South China estuary (Li *et al.,* 2018) and the Kenyan coast (Awuor *et al.,* 2020) using the same digestion treatment as in this study, 10% KOH. Li *et al.* (2018) found that microplastics contamination in *S. cucullata* ranged from 1.5 to 7.2 particles/g wet weight and Awuor *et al.* (2020) reported an average of 3.36 ± 0.53 microplastic particles/g wet weight in the same species. However, in Metuge, North of Mozambique, microplastics abundance in *S. cucullata* oysters ranged from 0.5 ± 0.4 to 2.0 ± 0.6 particles/g wet weight (or 0.7 ± 0.4 and 2.1 ± 0.5 particles/individual) during dry and wet seasons, respectively. These values are lower than the mean values found in South China and Kenya, which can be related to lower microplastics contamination in oysters' living waters in the North of Mozambique. In fact, Li *et al.* (2018) suggested that oysters can reflect microplastics contamination of their surrounding waters and therefore can be used as biomonitors for the microplastic contamination in coastal waters.

Microplastics reported in *M. philippinarum* mussels from Mecúfi ranged from 7.7 ± 1.7 and 29.7 ± 14.0 particles/g wet weight (or 2.6 ± 0.6 and 7.4 ± 3.8 particles/individual) during dry and wet seasons, respectively. Although the mean value of microplastics content in mussels during wet

season was very high, it also presented high variance. Microplastics quantification of wet season mussels was performed at a different time from the other analysed samples. Even though every precaution has been taken to ensure that samples were treated in accordance with quality control measures, airborne contamination may have occurred. This time gap could have affected our results because microplastics atmospheric contamination might have been different in these two different periods, affecting the microplastics quantification. Nevertheless, we individually counted microplastics in a conservative way.

To our best knowledge, this is the first time that microplastics were quantified in *M. philippinarum* mussels and it is also the first microplastic quantification in bivalves from Mozambique. However, a previous microplastics quantification of Pemba sediments, Mozambique has already been done (Browne *et al.,* 2011). Browne *et al.* (2011) detected that in 250 mL of sediment there were 21-30 microplastic particles. There are few studies on microplastics quantification in shellfish from Southern and Eastern Africa. Besides Awuor *et al.* (2020) that reported microplastics contamination in *S. cucullata* from Kenya, microplastics were also quantified in bivalves from Cape Town, South Africa (Sparks, 2020) and Tanzanian coast (Mayoma *et al.,* 2020). In South Africa were detected 2.33 ± 0.2 particles/g wet weight (4.27 ± 0.5 particles/individual) in three mussel species (Sparks, 2020) and in Tanzanian coast ≤1 – 2.1 ± 1.8 particles/individual were reported in *Anadara antiquata* cockle (Mayoma *et al.,* 2020). Since there are no maximum levels of microplastics established for food items intended for human consumption, we compared our results to other global studies. Microplastics contamination quantified in *S. cucullata* from Metuge, Mozambique was below and/or within the average of the overall values verified in shellfish in other studies around the world (Ding *et al.,* 2020) and specifically in Southern and Eastern Africa (Awuor *et al.,* 2020; Mayoma *et al.,* 2020; Sparks, 2020). The average number of microplastics detected in *M. philippinarum* in Mecúfi, Mozambique was mostly higher than the mean values of microplastics identified in shellfish in other global studies (Ding *et al.,* 2020), including the ones developed in Southern and Eastern Africa (Awuor *et al.,* 2020; Mayoma *et al.,* 2020; Sparks, 2020), namely results from wet season mussels. We suggest that future analyses including analytical methods (e.g., FTIR) should be developed.

It has already been shown that *S. cucullata* had high nutritional value, namely due to its high percentage of protein content when comparing to its lipid and sugar content (Paul *et al.,* 2021; Umayaparvathi *et al.,* 2015). Here we also verified *S. cucullata* high protein content, which was much higher (double or more) when compared with its lipid and sugar content.

Seasonal variations in energetic reserves of bivalve molluscs have already been detected (Costa *et al*., 2020; Mitra *et al.,* 2008; Mohan & Kalyani, 1989; Topić Popović *et al*., 2020). In fact, biochemistry of these organisms suffers seasonal variations depending on environmental parameters and on physiological status (Paul *et al.,* 2021; Widdows, 1978). In this study oysters and mussels had significantly higher energetic reserves (lipids, sugars and proteins) during dry season than during wet season. Paul *et al.* (2021) also verified that *S. cucullata* protein, lipid and sugar content was higher during summer (dry season) and lower during monsoon period (wet season) in the southeast

coast of India. Concerning *M. philippinarum*, we could not find any study quantifying its nutrient reserves and their possible seasonal variations. Nevertheless, we found that nutrient reserves have been quantified in a congeneric species *Modiolus barbatus,* which just like *M. philippinarum*, it had higher protein content than lipid and sugar levels (Prato *et al.,* 2019). Among the eight bivalve species analysed by Prato *et al.* (2019), *M. barbatus* was considered one of the most suitable for consumers, because of the benefits that arise from its relatively high protein content and low lipid levels. However, *M. philippinarum* had lower levels of protein, lipid and sugar content than *M. barbatus* (Biandolino *et al.,* 2019; Prato *et al,* 2019).

S. cucullata condition index was shown to be higher during pre- and post-monsoon periods in the south-west coast of India (drier seasons) than in monsoon periods (the wettest season), when water temperature was lower (Singh, 2019). This could happen due to less food availability during low temperature days (Rao & Nayar, 1956) (that matched with monsoon period), which could lead to less food intake and lower energetic reserves in *S. cucullata* body. Although we also verified lower energetic reserves in this species during wet season, seawater temperature in Metuge was similar between dry and wet seasons. In fact, an average of 26,6°C and 28,9°C seawater temperature is reached in Pemba (North of Mozambique) during dry and wet seasons, respectively (Pemba Climate: Temperature, Climograph and Climate Table for Pemba – Climate-Data.org, n.d). So, seawater temperature does not reach low values in the North of Mozambique. However, there are other physical parameters that might be leading to seasonal variability in bivalves' energetic reserves in the North of Mozambique. Hydrodynamic processes like current velocities change seasonally, affecting food availability (Richardson & Reverdin, 1987; Sarà & Mazzola, 2004; Visbeck & Schott, 1992) and leading to seasonal changes in bivalves' energetic reserves. Besides, we noticed that salinity decreased from dry to wet season and this environmental parameter can also lead to seasonal variations in energetic reserves in bivalve molluscs (Topić Popović *et al*., 2020), since it has been associated with reproductive cycle (Mitra *et al.,* 2008). Species reproduction periods may vary according to its geographical location (Quayle, 1980). It was shown that *S. cucullata* has a well-defined spawning period in Transkei, South-eastern region of South Africa from February and March (Lasiak, 1986). However, in east Africa (Kenyan coast) spawning was shown to be related to rainfall, so *S. cucullata* spawning period coincides with wet season (October and April-May), probably due to salinity decrease (Van Someren & Whitehead, 1961). Salinity has also been pointed as having a key effect in mussels' reproductive cycle, namely in gametogenesis and spawning (Wilson, 1969). Concerning *M. philippinarum* mussel, active gametogenesis was observed between July and October in Southern Japan (Ozawa, 2001). In the present study we did not evaluate the reproductive stage of the collected organisms. However, it is known that bivalves have to storage energetic reserves prior to spawning, since they play a major role on gametogenesis (Dridi *et al.,* 2007; Mitra *et al.,* 2008; Mohan & Kalyani, 1989). Moreover, according to Van Someren & Whitehead (1961), lower salinity values registered during wet season in east Africa can be related to spawning. So, in this study we hypothesise that oysters and mussels had to storage large amounts of protein, lipid

and sugar during dry season in order to have enough energetic reserves to ensure spawning latter during wet season.

Microplastics ingested by bivalves can decrease energetic reserves of bivalve organisms (Bour *et al.,* 2018). In fact, we verified that during wet season oysters and mussels' microplastics contamination was higher and their protein, lipid and sugar content was significantly lower than in dry season. So, microplastics concentration in bivalves' tissues can be negatively affecting their energetic reserves. This can have adverse impacts in bivalves under stressful conditions like low food availability, when these organisms are forced to relocate their energy for maintenance or growth (Bour *et al.,* 2018; Smolders *et al.,* 2004). Moreover, during wet season there is an increase in suspended particulate matter and particulate organic and inorganic matter in coastal waters (Sasikumar & Krishnakumar, 2011). However, not all the particulate matter is adequate for bivalves consume (Saxby, 2002). So, during wet season bivalves actively filter a great number of particles but bivalves do not necessary use all the particles as food. This energy-consuming process might have also leaded to lower energetic reserves of oysters and mussels during wet season.

Although lipid peroxidation (LPO) was also significantly higher during dry season in oysters, in mussels the results were the opposite and LPO was significantly higher during wet season. Higher lipid peroxidation level has been related to wet season (Jana *et al.,* 2013) and with higher pollutants contamination of the seawater, namely heavy metals (Jana *et al.,* 2013; Sheehan & Power, 1999) like mercury (Carocci *et al.,* 2014). In fact, mussels' mercury and microplastics contamination was higher during wet season and so was their lipid peroxidation. So, these pollutants can be major factors contributing to why lipid peroxidation was higher in mussels during wet season. However, in oysters, even though its contamination with the analysed pollutants was higher during wet season, its lipid peroxidation was higher during dry season. Lipid peroxidation is a useful parameter to assess oxidative damage in organisms (Viarengo *et al.,* 1991). Colder seawater temperature and food availability have been pointed out as factors that can increase levels of oxidative stress (Topić Popović *et al*., 2020). Seawater temperature was similar during dry and wet seasons in Metuge and oysters' energetic reserves were also significantly higher during dry season, so we do not consider that seawater temperature or reduced food availability affected oysters' higher lipid peroxidation during dry season. Oxidative stress may vary along the year also because of seasonal changes in bivalves' metabolic status due to the reproductive cycle, namely gonad ripening (Sheehan & Power, 1999; Viarengo *et al.,* 1991). As stated before, we associated higher nutrient reserves along dry season to the need of storing larger amounts of energy for gametogenesis. So, higher lipid peroxidation in oysters during dry season might be due to gonad ripening and gametogenesis. Even though we considered mussels to be also ripening their reproductive organs during dry season, maybe pollutants in these organisms had bigger impact on their lipid peroxidation. Therefore, this oxidative stress indicator matched its higher levels with higher pollutants contamination during wet season in *M philippinarum* mussel.

PCA analysis revealed higher importance of energetic reserves in segregating values from dry and wet season and less importance of pollutants contents in this segregation in both oysters and mussels. Maybe this is due to variations of bivalves' energetic reserves that occur throughout the different stages of the reproductive cycles, which depends on environmental parameters (like salinity) that change from dry to wet season. Besides, mercury and microplastics contamination in *M. philippinarum* between dry and wet seasons did not reveal significant differences. On the other side, although these two pollutants revealed seasonal significant differences in *S. cucullata*, values concerning lipid and sugar content had greater gaps between dry and wet seasons.

2.5 Conclusions

Our study highlights that consumption suitability and quality of *S. cucullata* oysters and *M. philippinarum* mussels were better during dry season, as pollutants contamination was lower and nutrient reserves were higher in this season. However, since that period might overlap bivalves pre-spawning season, the number of bivalves harvested should have an established maximum quota and a size-controlled collection during dry season should be implemented. Further studies on seasonal variations of the reproductive cycle of these species in the North of Mozambique could verify our assumption.

Rising temperatures in the context of climate change will reduce bivalves' tolerance to heavy metals and other contaminants, which will increase bivalves' oxidative stress and reduce their energetic reserves. This can have adverse impacts in bivalve nutritional quality and, at worst their survival might be affected.

3. Preservation methods of oysters and mussels in the North of Mozambique: potential implications for human health

3.1 Introduction

Food preservation methods are essential to increase the storage life of alimentary products by preventing foods spoilage by microorganisms and avoiding their poisoning by pathogens that can harm consumers (Smid & Gorris, 2007). However, the preservation methods can affect food nutritional values (Masette & Kwetegyeka, 2013), so it is important to select a preservation technique that conserves food nutritional attributes as much as possible.

In Cabo Delgado in 2017, only 19% of the population had access to electricity or a power generator/solar panel (INE, 2020c). This lack of electricity access in this region hinders food safety since refrigerators and freezers for food preservation for long periods are inaccessible for most of the population. Therefore, to preserve fish and seafood for longer periods, coastal communities in Cabo Delgado use traditional preservation methods: salting, smoking and sun-drying (Souto, 2015).

Salting is the oldest preservation method used by humans to preserve food (Pittia & Antonello, 2016; Turan *et al.,* 2007). Salting can be "wet" where bivalves are immersed in a brine solution or "dry" where they are stacked in salt (Turan *et al.,* 2007). Salt (NaCl) is a strong depressor of water activity (Pittia & Antonello, 2016). By reducing water activity in food products, salt inhibits microbial growth, enabling longer preservation of bivalves, since regular fisheries spoilage bacteria cannot support high salt concentrations (Horner, 1997; Turan *et al.,* 2007).

In developing countries, smoking is a preservation method used to reduce post-harvest loss of fishing resources because it is a cheap and easy technique (Patterson, 2004). For bivalves, such process combines the preservative effect of salting, drying, heating and smoking, since prior to smoking bivalves' meat is brine salted and left to dry (Turan *et al.,* 2008). Increased salt content and reduced moisture retards the growth of bacteria and increases food storage life (Horner, 1997; Patterson, 2004; Turan *et al.,* 2008). In fact, the drying process is the main responsible for increasing storage life of smoked bivalves instead of the deposition of chemical compounds on bivalves' meat by smoke, like antioxidant (phenolic) and antimicrobial constituents (Horner, 1997; Patterson, 2004; Turan *et al.,* 2008).

Sun-drying is a technique still used nowadays in many parts of the world, where food items are simply left to dry in the open air, taking advantage of solar energy to evaporate the water and air currents to spread away the vapour (Horner, 1997). Water removal from food products is also the mechanism that allows the drying method to preserve alimentary products inhibiting spoilage bacteria to growth (Horner, 1997; Patterson, 2004).

Bivalves can heavily accumulate pollutants (e.g., mercury and microplastics) in their tissues due to their filter-feeding behaviour (Li *et al.,* 2015; Naimo, 1995; Wright *et al.,* 2013). Mercury can reach

coastal waters through natural (geological settings like volcanic activity) and anthropogenic sources (e.g., inland industry and rivers discharges) (Saniewska *et al.,* 2014; Stoffers *et al.,* 1999). Either way, bivalves inhabiting coastal waters contaminated with this heavy metal will increase mercury content in their body tissues and according to EFSA if mercury levels exceed 0.50 µg/g wet weight, then bivalves are no longer proper for human consumption (European Commission, 2006; Naimo, 1995). In fact, mercury can induce damages in human kidneys, gastrointestinal tract and neurological system (Andersen *et al*., 1993; Park & Zheng, 2012; Wheatley *et al*., 1979). Although microplastics maximum levels are not yet established for food intended for human consumption, microplastics can also negatively affect human health (Brown et al., 2001; Mahler et al., 2012). These debris that outcome from anthropogenic activities can cause oxidative stress, cell apoptosis and inflammatory responses (Brown *et al*., 2001; Inkielewicz-Stepniak *et al*., 2018; Mahadevan & Valiyaveettil, 2021; Peixoto *et al.,* 2019). These potential negative impacts of mercury and microplastics on human health ensures the importance of assessing these two pollutants content in bivalves consumed by humans.

In this study, we preserved rock oysters *S. cucullata* and *M. philippinarum* mussels collected in Cabo Delgado using food traditional preservation methods: salting, smoking and sun-drying. The main goal of this study was to assess which was the preservation method that ensured a higher food safety and nutritional attributes of *S. cucullata* and *M. philippinarum* for human consumption. For that, we analysed bivalves' mercury, microplastics and microbial contamination, and their oxidative damage (estimated as lipid peroxidation) and biochemical composition (lipids, sugars and proteins) after their preservation using different artisanal methods.

3.2. Materials and methods

3.2.1 Study area

In March 2020 *S. cucullata* oysters were collected inside Pemba Bay, near Metuge and *M. philippinarum* mussels were collected from the seacoast nearby Mecúfi.

3.2.2 Experimental procedure

Bivalve shells were cleaned before cooking. Together with women villagers from Mecúfi, we developed five different artisanal preservation methods of mussels and oysters: brine salting, dry salting, smoking, smoking with oil dipping and sun-drying.

Bivalve preparation before developing the five preservation methods was the same for all of them and followed the one usually made by the villagers. Mussels were cooked on a pan in the fire and were left to boil in their own juices for 6 min. After that, shells were wide open and mussels' meat was easily removed from the shells and placed in a container with freshwater from the village well to swell. Oysters were cooked with a small amount of freshwater on a pan in the fire (Fig. 9). After 18

min boiling, they were removed from the fire and the shells were opened by hand or with a knife. The edible part was placed in a container with freshwater just like the mussels. All the preservation methods procedures were done separately for mussels and oysters.

Fig. 9 – *S. cucullata* oysters being cooked prior to their preservation.

Brine and dry salting followed the procedure developed by Turan *et al.* (2007), with a few adjustments. For the brine salting, mussels and oysters were immersed in brine containing 250 g of thick granular salt/ 1 l of freshwater from villagers well and they were stored in falcon tubes. For the dry salting, we weighed oysters and then we mixed them with thick granular salt. The amount of salt used in this process was 25% of total oyster weight. We repeated the dry salting process using mussels. Dry salted mussels and oysters were also stored in falcon tubes.

We followed Turan *et al.* (2008) procedures to do mussels and oysters smoking, with minor adjustments. Bivalve molluscs were taken from the freshwater and immersed in brine containing 250 g of thick granular salt/ 1 l of freshwater for 5 min. Mussels and oysters were then left to dry in a separate reed sieve in the sun for 5 min. Next, we split in half mussel and oyster samples and the first group was dipped in soy vegetable oil prior to being smoked in a grill next to a bonfire for 45 min. The second group followed the same procedure except that these bivalves were not dipped in oil before being smoked. After samples cooled down they were stored in falcon tubes. The use of brine and oil before smoking helped improving consistency and taste of the bivalve molluscs (Turan *et al.,* 2008).

Sun-drying procedure was developed just like the villagers used to do. Mussels and oysters were taken out from the freshwater they were in and each of the bivalve molluscs was well spread in a separate reed sieve (Fig. 10). The sieves were placed in the sun and left to dry for seven to eight hours. After that samples were stored in falcon tubes.

Fig. 10 – *M. philippinarum* mussels spread in a reed sieve under the sun.

Fresh organisms, that were not submitted to any preservation method (t0) were stored at -20 °C as well as preserved bivalve molluscs after three (t3) and fifteen (t15) days of their preservation. We evaluated bivalves' mercury and microplastics levels, their microbial contamination and their biochemical composition (i.e., oxidative damage via lipid peroxidation; energetic reserves via lipid, sugar and protein contents). Although some preserved bivalves were drier (e.g., sun-dried samples) than others (e.g., brine salted organisms), we did not transform all bivalves weight into wet or dry weight since we wanted to assess the actual pollutants levels and nutrient reserves in preserved bivalve own weight.

3.2.3 Mercury quantification

Mercury was quantified (µg/g organism) in oysters and mussels (n=5 per method) according to the protocol described in chapter 2. We analysed fresh (t0) and t3 bivalve molluscs. We did not use t15 oysters and mussels because we did not expected differences in this quantification between bivalve molluscs t3 and t15.

3.2.4 Microplastics extraction and quantification from biological tissues

Oysters and mussels (n=5 per method) were collected from the surroundings of Metuge and Mecúfi, respectively and microplastics quantification (microplastic particles/g organism) followed the protocol described in chapter 2. We analysed fresh (t0) and t3 bivalve molluscs. We did not use t15 oysters

and mussels because we did not expected differences in this quantification between bivalve molluscs t3 and t15.

3.2.5 Oxidative damage and energy-related biomarkers analysis

S. cucullata and *M. philippinarum* (n=5 per method and sampling time: t0, t3 and t15) were homogenised and oxidative damage (LPO, nmol TBARS/g organism) and energy-related biomarkers (lipid, sugar and protein content, mJ/mg organism) analysis followed the methodology described in chapter 2.

3.2.6 Microbiology analysis

3.2.6.1 Culture medium preparation

We used three different media: TSA (Tryptic soy agar) with 1% NaCl, TCBS (Thiosulfate Citrate Bile Salts Sucrose Agar) and CCA (Chromocult coliform agar). TSA (VWR Chemicals ref 84602.0500) with 1% NaCl was used to culture every microorganism that grows in salt water. TCBS (VWR Chemicals ref 84641.0500) was used to verify if there were any *Vibrio* spp. in our samples. Finally, we used CCA (Merck ref 1.10426.0500) medium to access the amount of coliform microorganisms present in preserved bivalve molluscs. Media were prepared in distilled water according to the producer indications and they were stored at 4 ºC prior to their inoculation.

3.2.6.2 Shellfish samples preparation

Samples preparation followed the Portuguese Standard NP – 3006 (1985). Microbiology analysis was performed on oysters and mussels preserved three days before the analysis (t3) by the five different preservation methods. Containers with preserved mussels and oysters were aseptically (next to the flame) opened and cut in smaller pieces in a sterile Petri dish using a sterile scissors.

3.2.6.3 Preparation of the initial suspension, dilutions and plates inoculation

Dilutions' preparation followed the Portuguese Standard NP – 3005 (1985). Weighted samples were introduced in a sterile homogenizer bag, diluted 1:2 by adding 0.1% sterile peptone water in the same volume (ml) of each of the samples' weight (g) and homogenized using a Stomacher homogenizer for 3 min (1,5 + 1,5 min). Each bivalve homogenate was further diluted 1:5 in 0.1% sterile peptone water to obtain an initial suspension of 10^{-1}. Decimal dilutions (from 10^{-2} to 10^{-7}) of each homogenate were prepared in 0.1% sterile peptone water. We spread plated 100 µL from each of the six dilutions in three replicates of each three used media. Cultures in CCA media were incubated at 37ºC for 24h

and cultures inoculated in TSA and TCBS media were incubated at 28ºC for 48h. When incubation period ended, we counted the number of colonies in each plate and colony counts were converted to log CFU (colony-forming unit)/g.

3.2.7 Data analyses

Differences among mercury, microplastics, LPO and energetic reserves of oysters and mussels preserved by distinct methods were analysed by one-way analysis of variance (ANOVA) with a significance level of 5%, followed by SNK multiple comparison tests when significant differences were found. The Shapiro-Wilk test was used to verify if our data followed a normal distribution and the Levene test was used to verify the assumption of homogeneity of variance. When these two assumptions were not fulfilled, we performed a Kruskal-Wallis non-parametric test followed by a Dunn-Bonferroni *post hoc* test.

Concerning oxidative stress and energy-related biomarkers, we analysed differences between fresh and preserved bivalves with three (t3) and fifteen (t15) preservation days using an ANOVA with a significance level of 5%, followed by Dunnett multiple comparison tests (considering the fresh treatment as control) when significant differences were found. The same tests mentioned before were used to verify the required ANOVA assumptions. When these normality and homogeneity of variance assumptions were not fulfilled, we performed a Kruskal-Wallis non-parametric test followed by a Dunn-Bonferroni *post hoc* test.

Comparisons between oxidative stress and energy-related biomarkers of oysters and mussels preserved by different methods after three (t3) and fifteen (t15) preservation days were made by Independent-Samples t-test with a significance level of 5%. The Shapiro-Wilk test was used to verify if our data followed a normal distribution. When this assumption was not fulfilled, we performed a Mann-Whitney non-parametric test. All these procedures were conducted using IBM SPSS Statistics 25. Whenever it was needed, data were transformed to fulfil these parametric tests assumptions. Original sample size was five in all analyses. Even though we looked for outliers using the formula mean ± 2*standard deviation we did not detect values that fell out of this interval.

Differences between microbial growth of preserved oysters and mussels were assessed using non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) since normality and homogeneity of variance (when applicable) assumptions were not verified. This procedure was also conducted using IBM SPSS Statistics 25.

3.3. Results

3.3.1 Mercury contamination

Fresh oysters had significantly lower mercury levels than preserved organisms (Fig. 11, Table S4). Moreover, the highest mercury content was observed for sun-dried bivalves, followed by smoked and smoked with oil organisms, and brine and dry salted individuals (Fig. 11, Table S4).

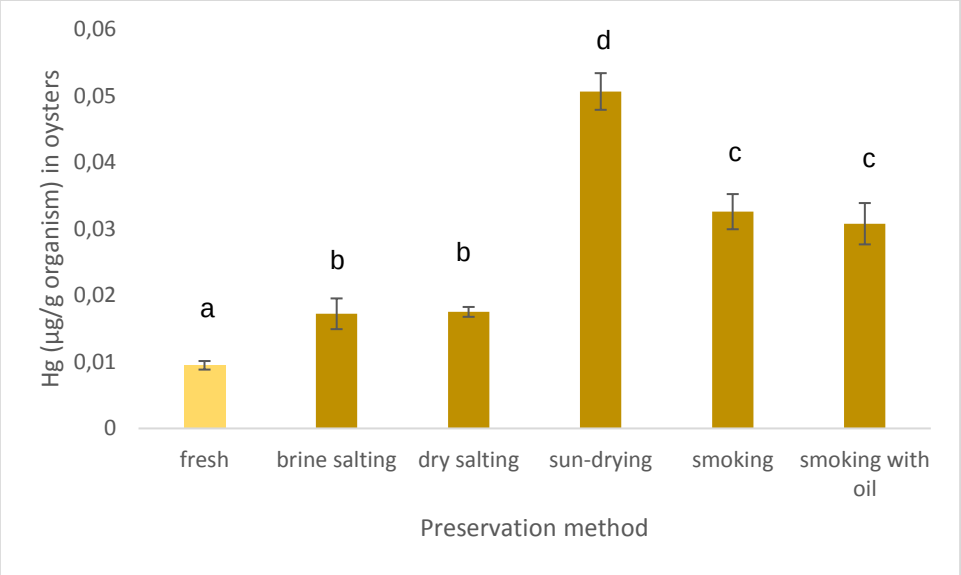

Fig. 11 - Mercury contamination (µg/g organism) (mean ± s.e.) of fresh and preserved *S. cucullata* oysters. Different letters represent significant differences among treatments (Dunn-Bonferroni *post hoc* test).

Mussels' mercury contamination was significantly lower in fresh and brine salted individuals than in the remaining treatments (Fig. 12, Table S4). On the contrary, sun-dried organisms presented the highest mercury contamination, followed by smoked with oil mussels. Dry salted and smoked mussels presented intermediate similar mercury values (Fig. 12, Table S4).

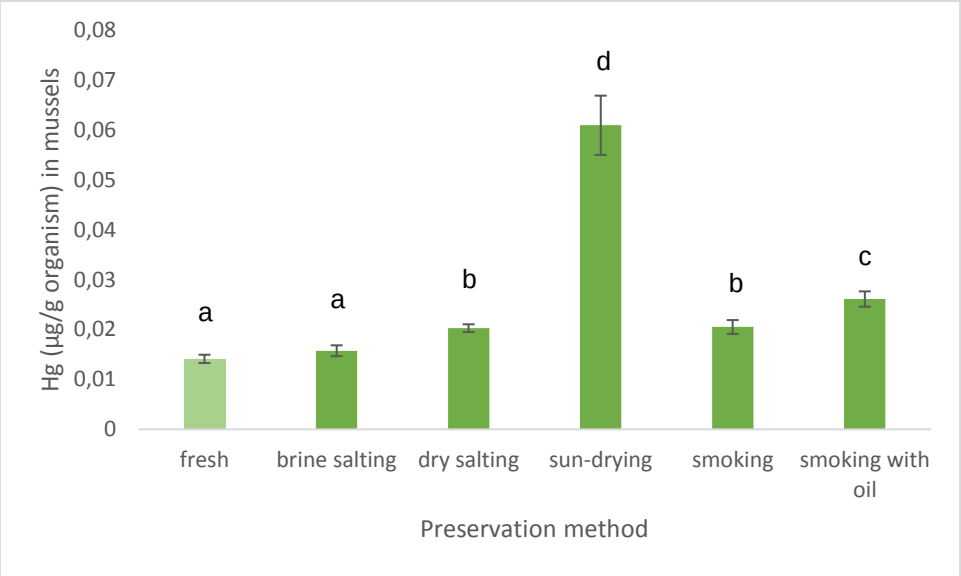

Fig. 12 - Mercury contamination (µg/g organism) (mean ± s.e.) of fresh and preserved *M. philippinarum* mussels. Different letters represent significant differences among treatments (SNK *post hoc* test).

3.3.2 Microplastics contamination

Fresh oysters had significantly lower microplastics contamination than preserved oysters (Fig. 13, Table S4). Significant differences were not found among oysters preserved by different conservation methods concerning microplastics contamination (Table S4).

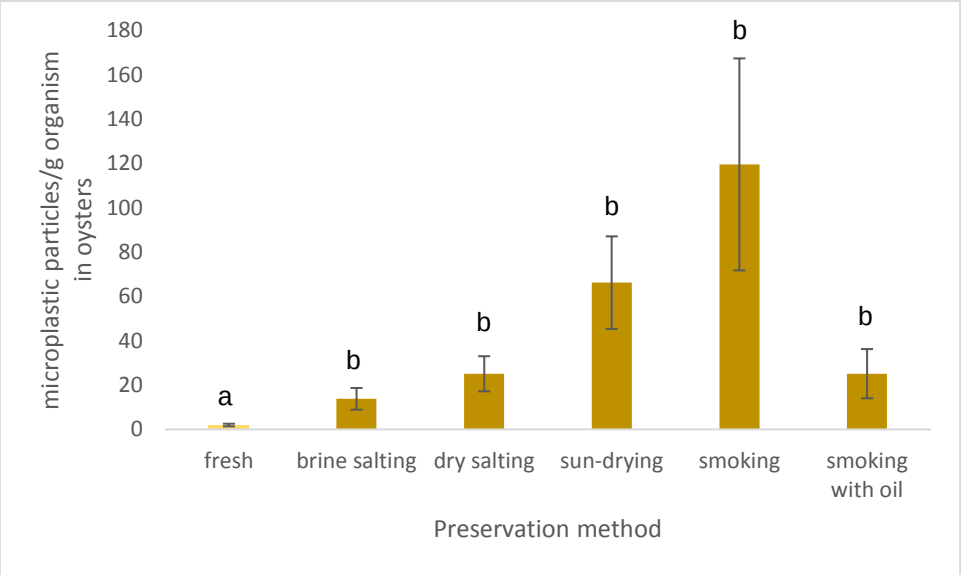

Fig. 13 - Microplastics contamination (microplastic particles/g organism) (mean ± s.e.) of fresh and preserved *S. cucullata* oysters. Different letters represent significant differences among treatments (Dunn-Bonferroni *post hoc* test).

Microplastics contamination in mussels did not change significantly among treatments (Fig. 14, Table S4).

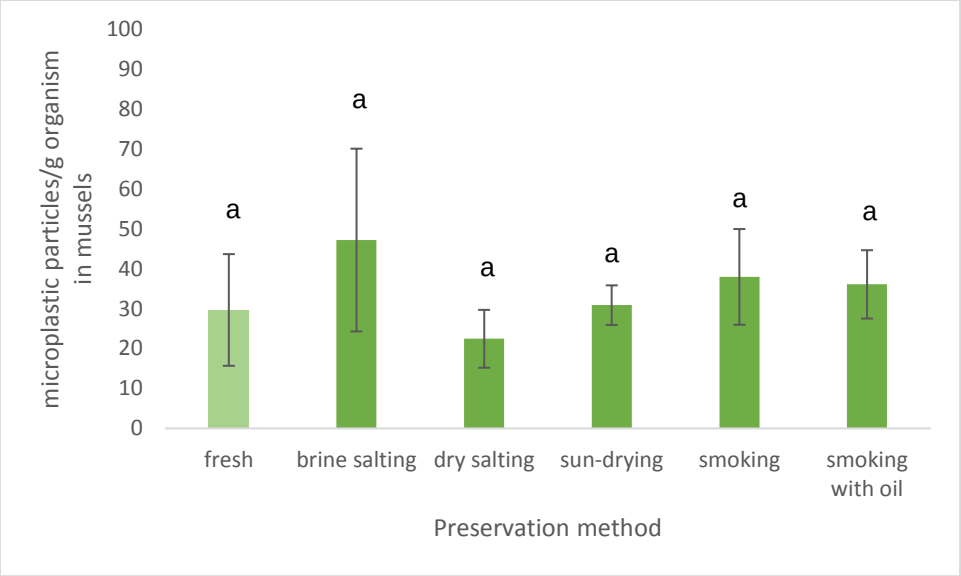

Fig. 14 - Microplastics contamination (microplastic particles/g organism) (mean ± s.e.) of fresh and preserved *M. philippinarum* mussels.

3.3.3 Oxidative damage and energy-related biomarkers analysis

Oysters LPO was significantly lower in fresh organisms (47.34 ± 5.76 nmol TBARS/g organism) than in dry salted, sun-dried, smoked and smoked with oil oysters after 3 and 15 days of preservation (Table S4, S5). LPO levels were significantly higher in t3 sun-dried and smoked with oil oysters than in the remaining t3 treatments (Fig. 15, Table S4). Concerning the different preservation methods after 15 days of storage, oysters LPO was significantly lower in brine and dry salted individuals and it was significantly higher in sun-dried oysters (Fig. 15, Table S4). Smoked oysters had significantly higher LPO levels in t15 than in t3 samples (Table S6). In all the remaining preserved oysters there were no significant differences between 3 and 15 days of storage (Table S6).

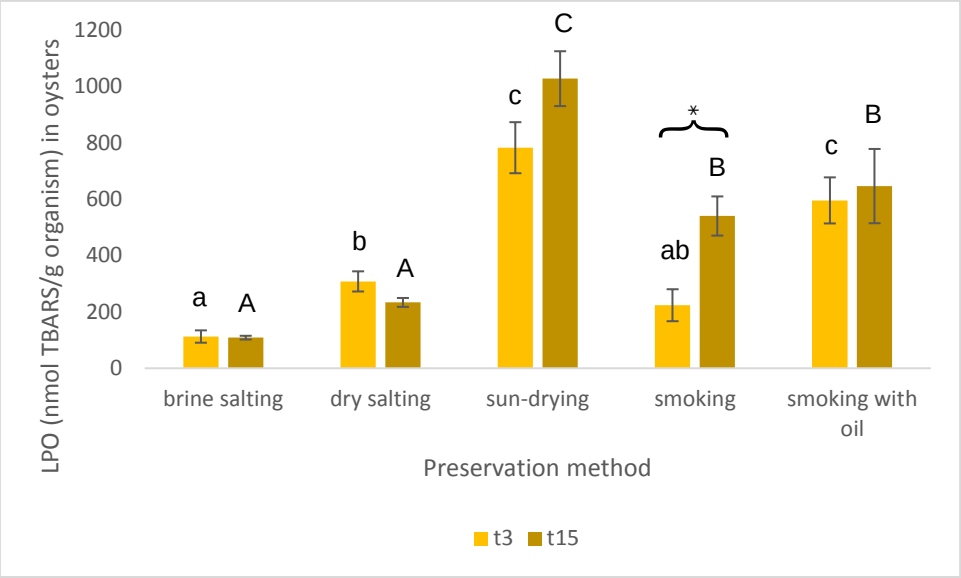

Fig. 15 – LPO (nmol TBARS/g organism) (mean ± s.e.) of preserved *S. cucullata* oysters after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved oysters (SNK *post hoc* test). Significant differences between t3 and t15 samples for each preservation method are represented by *.

Fresh mussels had significantly higher LPO levels (433.68 ± 39.70 nmol TBARS/g organism) than preserved mussels after 3 and 15 days of preservation (Table S4, S5). Concerning t3 treatments, mussels LPO was significantly higher in sun-dried organisms (Fig. 16, Table S4). Among t15 treatments, mussels LPO was significantly lower in brine salted mussels and significantly higher in sun-dried and smoked with oil individuals (Fig. 16, Table S4). Smoked with oil mussels were the only preserved mussels that revealed significant differences between samples with 3 and 15 days of storage (Table S7). Smoked with oil mussels with fifteen days of preservation had significantly higher LPO levels than t3 smoked with oil mussels (Table S7).

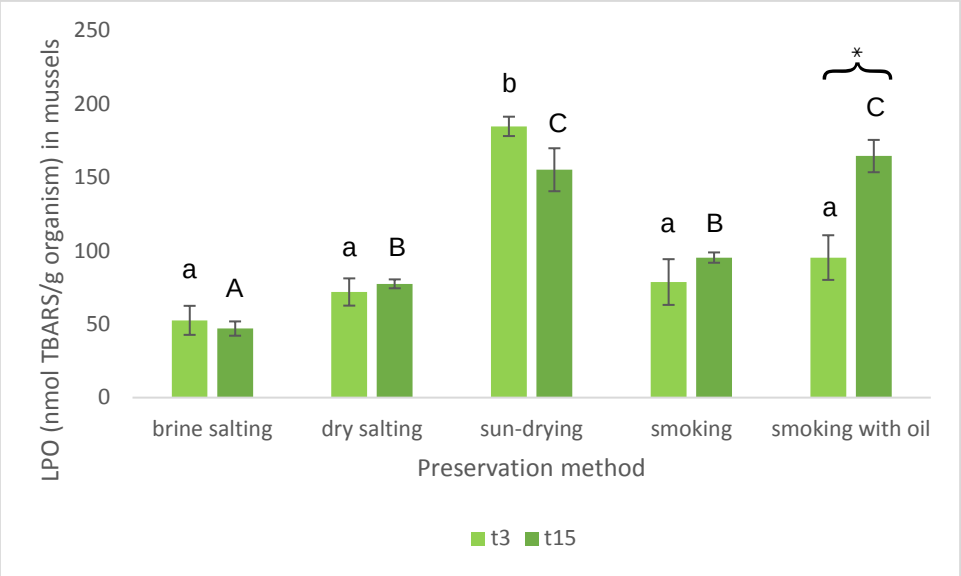

Fig. 16 – LPO (nmol TBARS/g organism) (mean ± s.e.) of preserved *M. philippinarum* mussels after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved mussels (SNK *post hoc* test). Significant differences between t3 and t15 samples for each preservation method are represented by *.

Concerning t3 and t15 treatments, fresh oysters had significantly lower lipid content (198.53 ± 21.05 mJ lipid/mg organism) than smoked and smoked with oil organisms (Table S4, S5). Smoked with oil oysters with three preservation days (t3) had significantly higher lipid content than the other t3 preserved oysters (Fig. 17, Table S4). We verified the same pattern for t15 preserved oysters, since smoked with oil samples also had significantly higher lipid content than the remaining preserved oysters (Fig. 17, Table S4). There were no significant differences between t13 and t15 oysters preserved by the five different preservation methods (Table S6).

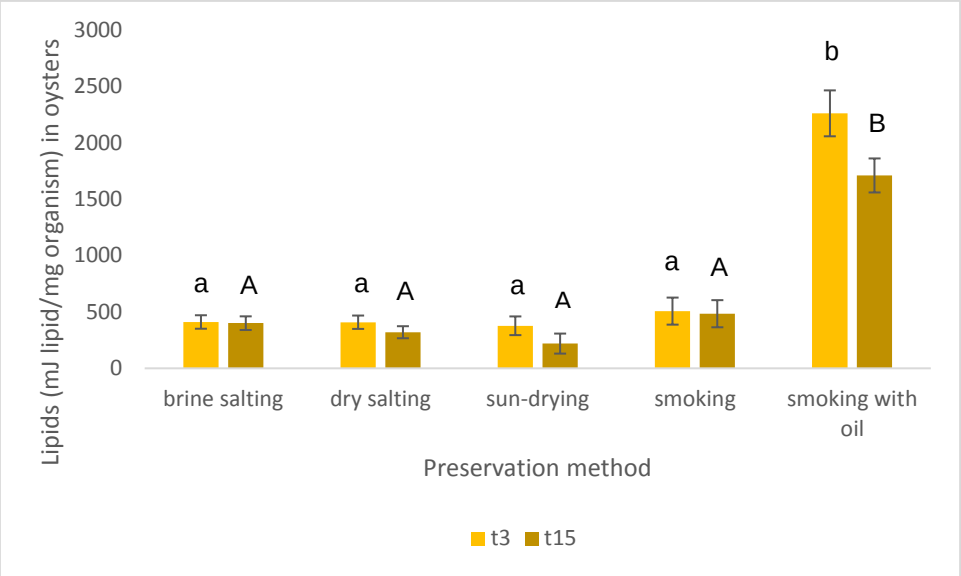

Fig. 17 - Lipids (mJ lipid/mg organism) (mean ± s.e.) of preserved *S. cucullata* oysters after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved oysters (SNK *post hoc* test).

Just like fresh oysters, fresh mussels also had significantly lower lipid content (198.98 ± 37.48 mJ lipid/mg organism) than smoked and smoked with oil organisms in both t3 and t15 treatments (Table S4, S5). Smoked with oil mussels with three preservation days (t3) had significantly higher lipid content than the rest of t3 preserved organisms (Fig. 18, Table S4). Concerning t15 treatments, smoked with oil mussels had significantly the highest lipid content and smoked individuals had significantly higher lipid levels than brine salted, dry salted and sun-dried mussels (Fig. 18, Table S4). There were no significant differences between t3 and t15 mussels except for smoked organisms, which revealed higher lipid content in t15 than in t3 mussels (Table S7).

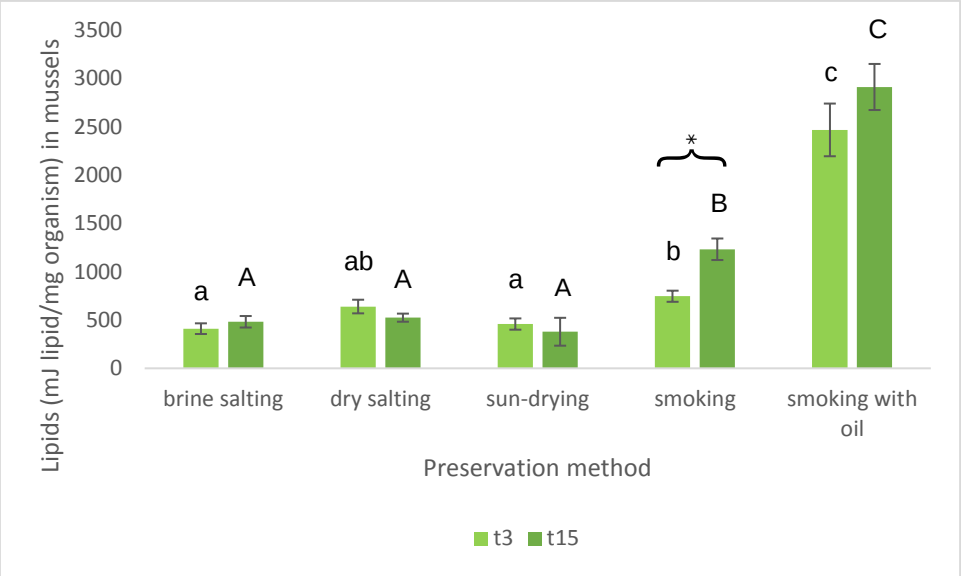

Fig. 18 - Lipids (mJ lipid/mg organism) (mean ± s.e.) of preserved *M. philippinarum* mussels after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved mussels (SNK *post hoc* test). Significant differences between t3 and t15 samples for each preservation method are represented by *.

Oysters sugar content was significantly lower in fresh organisms (78.68 ± 28.65 mJ sugar/mg organism) than in t3 dry salted and sun-dried oysters (Table S4, S5). However, concerning t15 treatments fresh oysters revealed significantly lower sugar content than t15 sun-dried and smoked oysters (Table S4, S5). Although significant differences were detected among t3 and t15 oyster treatments, a clear pattern was not revealed by the multiple comparison test (Table S4). Nevertheless, oysters sugar content was higher in t3 sun-dried organisms than in the remaining t3 treatments and it was lower in t15 brine and dry salted oysters than the others t15 organisms (Fig. 19, Table S4). Brine and dry salted oysters revealed significantly lower sugar content in organisms with 15 days of preservation than in individuals with 3 preservation days (Table S6).

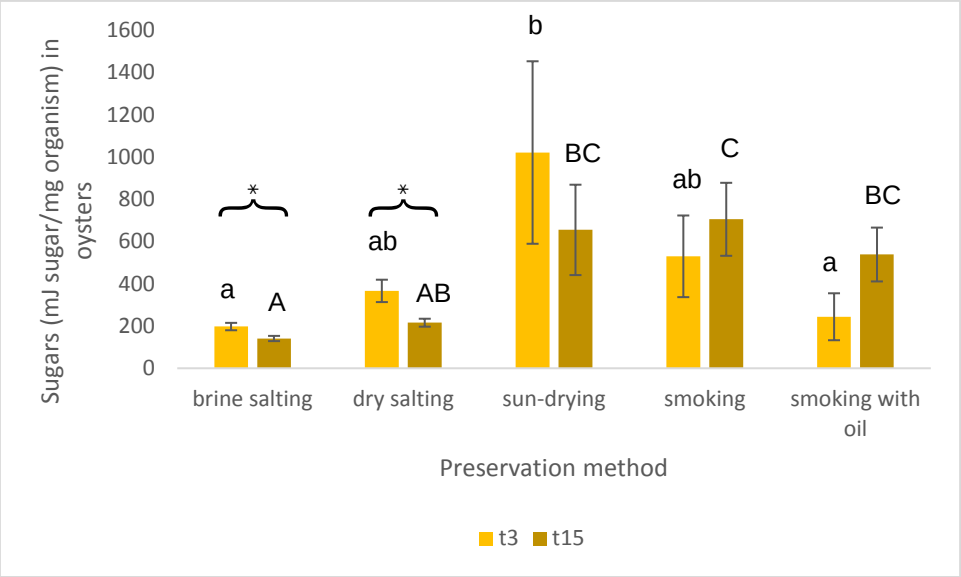

Fig. 19 - Sugars (mJ sugar/mg organism) (mean ± s.e.) of preserved *S. cucullata* oysters after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved oysters (Dunn-Bonferroni *post hoc* test for t3 treatments and SNK *post hoc* test for t15 treatments). Significant differences between t3 and t15 samples for each preservation method are represented by *.

Mussels sugar content was significantly lower in fresh organisms (41.80 ± 6.40 mJ sugar/mg organism) than in sun-dried and smoked mussels in both t3 and t15 organisms (Table S4, S5). Fresh mussels also revealed lower sugar content than t15 smoked with oil organisms (Table S4, S5). Sugar content was significantly higher in t3 sun-dried oysters than in the remaining t3 treatments (Fig. 20, Table S4). Concerning t15 treatments, sugar content was significantly lower in brine salted mussels and significantly higher in sun-dried, smoked and smoked with oil mussels (Fig. 20, Table S4). Significant differences between t3 and t15 treatments were not detected in any preservation method (Table S7).

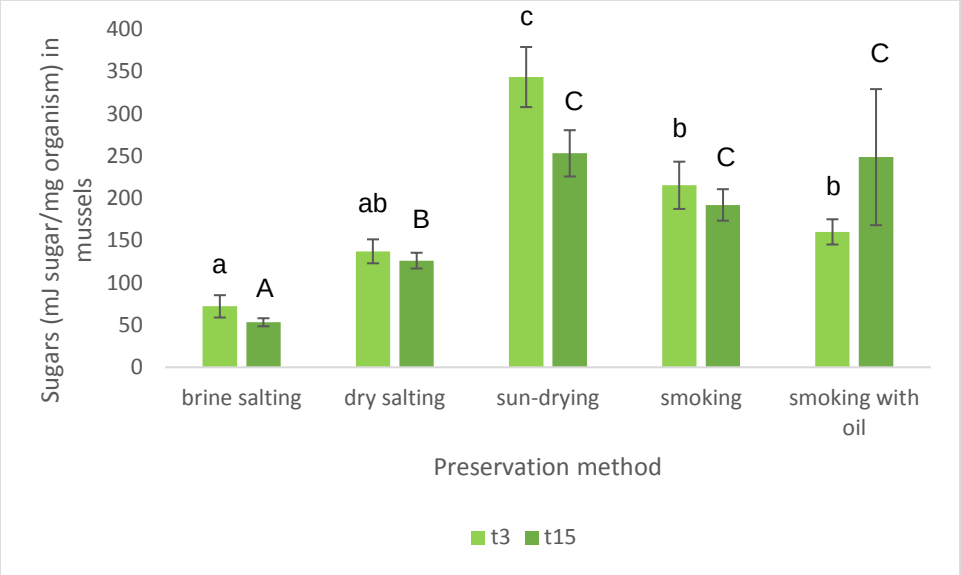

Fig. 20 - Sugars (mJ sugar/mg organism) (mean ± s.e.) of preserved *M. philippinarum* mussels after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved mussels (SNK *post hoc* test for t3 treatments and Dunn-Bonferroni *post hoc* test for t15 treatments).

Fresh samples (812.04 ± 79.99 mJ protein/mg organism) did not reveal significant differences from all t3 and t15 treatments (Table S4, S5). Brine and dry salted oysters with three preservation days (t3) had significantly higher protein content than the remaining t3 treatments (Fig. 21, Table S4). Concerning t15 treatments, sun-dried had the lowest protein content and brine salted, dry salted and smoked with oil oysters had one of the highest protein levels (Fig. 21, Table S4). There were no significant differences between t3 and t15 preserved oysters (Table S6).

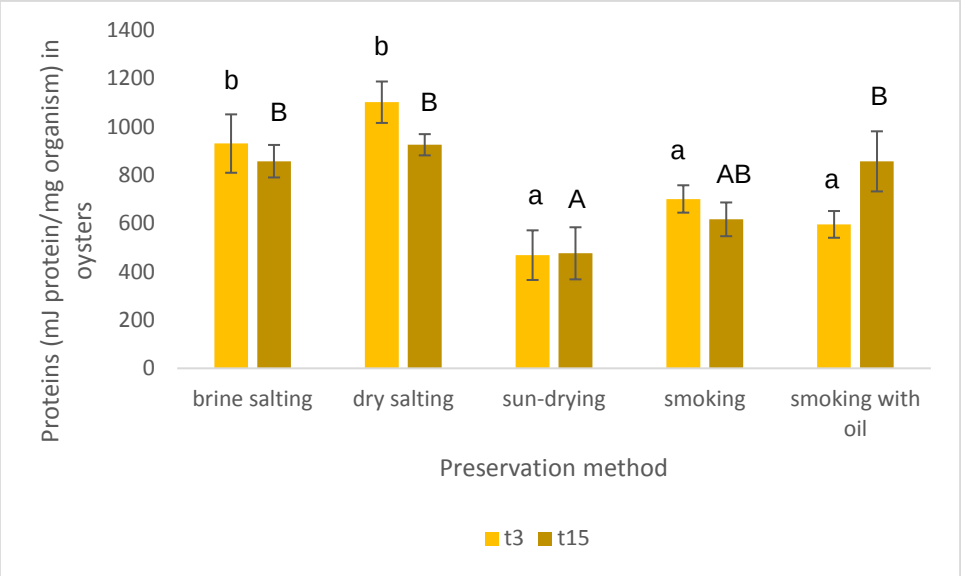

Fig. 21 - Proteins (mJ protein/mg organism) (mean ± s.e.) of preserved *S. cucullata* oysters after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved oysters (Dunn-Bonferroni *post hoc* test).

Mussel protein content was significantly lower in fresh individuals (424.57 ± 44.22 mJ protein/mg organism) than in t3 smoked mussels and in t15 dry salted organisms (Table S4, S5). There were not significant differences among t3 preserved mussels' protein content (Fig. 22, Table S4). Brine salted, dry salted and sun-dried mussels with fifteen preservation days (t15) had significantly higher protein content than t15 smoked and smoked with oil mussels (Fig. 22, Table S4). Smoked and smoked with oil mussels had significantly lower protein content in t15 than in t3 organisms (Table S7). Brine salted, dry salted and sun-dried mussels did not reveal significant differences between t3 and t15 individuals (Table S7).

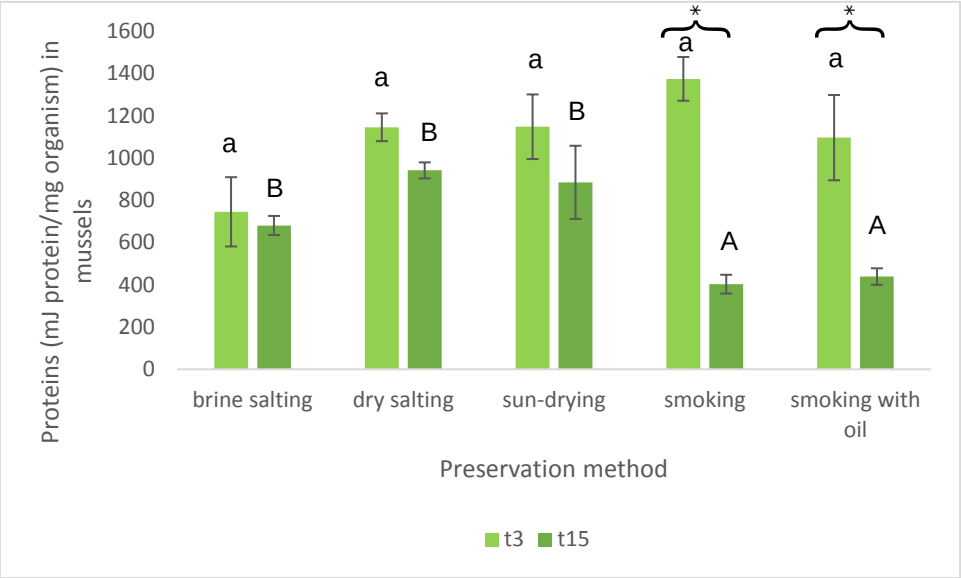

Fig. 22 - Proteins (mJ protein/mg organism) (mean ± s.e.) of preserved *M. philippinarum* mussels after three (t3) and fifteen (t15) days of its preservation. Different letters represent significant differences detected among t3 (lower case letters) and t15 (upper case letters) preserved mussels (SNK *post hoc* test). Significant differences between t3 and t15 samples for each preservation method are represented by *.

3.3.4 Microbiology

It was only possible to estimate microbial growth of sun-dried oysters (log cfu/g) in CCA and TCBS media (Table 2). All microbial growth (log cfu/g) concerning the remaining preserved oysters was low or even none, reflecting their minimal microbial contamination with specimens able to grow in the analysed culture media. For example, in TCBS medium brine salted oysters were the only treatment that revealed microbial growth in TCBS medium besides sun-dried oysters. Concerning TSA medium, microbial growth was higher in sun-dried oysters. However, significant differences were not detected among the analysed preserved oysters (Table S8).

Microbial growth of preserved mussels was significantly lower in dry salted and smoked with oil samples in TSA medium (Table 3, S8). Although there were no significant differences among preserved mussels in CCA and TCBS media, sun-dried samples revealed higher microbial growth (Table 3). All microbial growth (log cfu/g) that were not possible to estimate was due to samples low or none microorganisms growth, which revealed their reduced microbial contamination with microorganisms that proliferate in these specific media.

Table 2. Microbial growth (mean ± s.e.) in three different media (CCA, TSA and TCBS) in oysters preserved by five preservation methods. Values in the same column with different letters (a, b) are significantly different ($p < 0.05$).

Preservation method	Microbiology growth (log cfu/g)		
	CCA	TSA	TCBS
Brine salting	ND[1]	ND	ND
Dry salting	ND	5.83±2.7 a	ND
Sun-drying	7.82±1.43	10.30±0.10 a	6.98±3.2
Smoking	ND	5.61±0.38 a	ND
Smoking with oil	ND	5.63±0.07 a	ND

[1]ND, non-detected

Table 3. Microbial growth (mean ± s.e.) in three different media (CCA, TSA and TCBS) in mussels preserved by five preservation methods. Values in the same column with different letters (a, b, c) are significantly different ($p < 0.05$).

Preservation method	Microbiology growth (log cfu/g)		
	CCA	TSA	TCBS
Brine salting	6.82±2.1 a	7.26±0.20 b	ND
Dry salting	5.54±0.85 a	5.95±1.67 a	ND
Sun-drying	7.98±0.78 a	9.95±0.73 c	8.55±0.65 a
Smoking	6.64±0.05 a	8.66±0.8 bc	5.69±0.9 a
Smoking with oil	ND[1]	5.53±0.35 a	ND

[1]ND, non-detected

3.4 Discussion

Food preservation methods should be carefully selected to ensure that food spoilage will be prevented and that food nutritional values will be preserved as much as possible (Masette & Kwetegyeka, 2013; Smid & Gorris, 2007). Here we evaluated five distinct bivalves preservation methods to verify which was the technique that allowed higher nutritional values preservation and lower microbiological and other pollutants contamination to ensure bivalves quality and suitability for human consumption.

Mercury maximum levels in fishery products (including molluscs) for human consumption were suggested by EFSA and established by EC at 0.50 µg/g wet weight (European Commission, 2006). Considering mercury levels of preserved bivalves per g wet weight, we verified that oysters mercury contamination ranged from ≈0.018 ± 0.001 to ≈0.007 ± 0.001 µg/g wet weight in dry salted and smoked with oil samples, respectively; and mussels mercury levels ranged from ≈0.020 ± 0.001 to ≈0.004 ± 0.001 µg/g wet weight in dry salted and smoked organisms, respectively. Therefore, oysters and mussels preserved by the presented artisanal methods seem safe for human consumption concerning mercury contamination, since this pollutant values were at least 25 times lower than the maximum levels accepted in fishery products.

Cooked (boiled and grilled) and smoked samples experience water loss during culinary treatment which leads to an increase in total mercury level in sea products (Afonso *et al.,* 2015; Knowles *et al.,* 2003). It also has been shown that dried and salted fish have higher mercury values than fresh ones since dehydration concentrates the metal level in fish muscle tissues (Elrais *et al.,* 2018; Jeevanaraj *et al.,* 2020). In fact, preserved oysters had significantly higher mercury levels than fresh samples and the driest samples (sun-dried oysters) had the highest mercury contamination, ≈0.051 ± 0.003 µg/g organism. Preserved mussels (except for brine salted organisms) also had significantly higher mercury levels than fresh mussels and sun-dried mussels had the highest mean mercury contamination, ≈0.061 ± 0.006 µg/g organism. Furthermore, brine salting was the developed preservation method that removed less water content from samples, since bivalves were immersed in a watery solution with NaCl. So, this lower water loss might explain the similar mercury levels of fresh and brine salted mussels.

Handling and de-shelling of bivalve samples prior and during its preservation might have affected their microplastic contamination (e.g., airborne contamination with synthetic fibres from clothes) (Li *et al.,* 2018; Lusher *et al.,* 2017) since they were more manipulated and exposed than fresh bivalves, that were not extracted from their shells. In fact, higher microplastics contamination has been detected in processed mussels (frozen and pre-cooked) rather than in live/fresh mussels provided by supermarkets in the U.K., possibly due to handling along the processing chain (Li *et al.,* 2018). Moreover, water loss due to preservation techniques can lead to higher concentration of pollutants in dry, salted and smoked organisms (Jeevanaraj *et al.,* 2020; Knowles *et al.,* 2003). Microplastics might have increased their concentration in preserved samples because of their dehydration. Actually, oyster samples revealed this manipulation and water loss impact as oysters preserved with the five distinct conservation methods had significantly higher microplastics contamination than fresh *S. cucullata* organisms. However, mussel samples did not show this pattern and there were not significant differences among fresh and preserved *M. philippinarum* individuals. The boiling treatment applied to all oyster and mussel samples prior to their preservation could have also affected the number of microplastic particles detected in the analysed organisms. Some types of microplastic particles can melt and be completely lost when exposed to temperatures over 70°C (Munno *et al.,* 2018). So, some microplastic particle might have been destroyed due to the initial boiling treatment, namely in mussels. Mussels used in this study were small (shell length were less than 3 cm) and their shells opened really fast during the boiling treatment. This could have contributed to higher particle losses in mussels hiding the microplastics increment due to water loss and manipulation in preserved organisms and their differentiation from fresh mussels. However, as all oyster and mussels' samples were submitted to this boiling treatment it did not influence differences among distinct preservation methods.

In this study we used 10% KOH to digest biological tissues for microplastics quantification. Alkaline solutions disintegrate soft tissues by dissolving proteins and fats (Masse *et al.,* 2001; Undeland *et al.,* 2002) and the suitability of this alkaline solution for bivalve digestion without inducing several

plastic polymers degradation have already been shown (Kühn *et al.,* 2017). Nevertheless, even though we left our samples digesting with 10% KOH for 96h, some of our filters still had a bit of biological material, possibly affecting our microplastics counting. In fact, we noticed the presence of fat compounds in some filters, which prevented the most accurate counting of microplastic particles in filters "polluted" area. Due to their reduced water content, KOH might have taken longer to get into soft tissues from preserved drier samples (sun-dried and smoked organisms) so digestion was slower. Strong acid solutions could have digested our samples biological tissues more efficiently since they accelerate soft tissues break down (Karami *et al.,* 2017). However, they are the least advisable reagents for microplastics quantification since acid solutions can degrade plastic polymers, fusing and discolouring them potentially underestimating microplastics in organisms (Avio *et al.,* 2015; Claessens *et al.,* 2013; Dehaut *et al.,* 2016). So, an increased period of time of samples under the effect of KOH might have enhanced soft tissues break down and decreased filters contamination with biological compounds.

We noticed high variability in data concerning microplastics contamination in both oyster and mussel samples. Due to microplastics diverse density, size and shape they tend to have a heterogeneous dispersion throughout the water column and sediments (Claessens *et al.,* 2013; Oni *et al.,* 2020) which can lead to a great variability in bivalve contamination with this contaminant. Due to this great variability, we could not detect significant differences in microplastics contamination among the five preservation methods applied to oyster and mussel samples.

Microplastic particles in commercial sea, lake and rock salts from different countries have already been detected (Gündoğdu, 2018; Karami *et al.,* 2017; Seth & Shriwastav, 2018). In fact, Seth & Shriwastav (2018) verified that microplastics contamination of commercialised sea salts from India could reach high values (103 ± 39 particles/kg of salt). So, salted samples might have revealed an increased microplastics contamination because the salt used to preserve them could have microplastics that contributed to their contamination (Lee *et al.,* 2019).

Oil patches in smoked with oil bivalve filters might have affected microplastics counting, since oil reveals red-orange fluorescence patches with Nile Red under blue light, possibly hindering microplastics identification. In addition, oil is also used to extract microplastics, so its application in the preservation method could, somehow, had influenced their transition and elimination from bivalve tissues (Mani *et al.,* 2019).

There is no legislation concerning microplastics contamination in food items for human consumption (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). The average number of microplastics detected in preserved *S. cucullata* oysters and *M. philippinarum* mussels from Mozambique was mostly higher than the mean values of microplastics identified in shellfish in other global studies (Ding *et al*., 2020). We suggest that analytical methods (e.g., FTIR) should be developed in future studies to verify the amount of microplastics content and to ensure the quality of preserved oysters and mussels by artisanal methods.

Fresh organisms presented the highest LPO levels among mussel samples and one of the lowest among oyster samples. LPO might have been high in fresh mussels due to constraints during their transportation after being gathered in Mecúfi village. This period between their collection from the seacoast and their freezing could have enhanced mussels LPO levels, since their shells opened easily, and they were exposed to undesirable conditions. In contrast, oysters kept their shells tightly closed after being collected from the oyster bank, so they were not as much exposed as mussels to unsuitable environmental conditions. Preserved bivalves were boiled prior to their preservation and boiling can increase LPO levels proportionally to the boiling time (Qiu *et al.,* 2019). In fact, preserved oysters had higher LPO levels than fresh oysters and due to their longer boiling time they also had higher LPO levels than preserved mussels. Concerning preserved bivalves, LPO was significantly higher in sun-dried and in smoked with oil preserved oysters and mussels. Sun-drying method can accelerate lipid oxidation due to photosensitization, since sunlight exposure stimulates the transformation of triplet to singlet oxygen by photosensitizers (e.g., myoglobin) which is a very strong oxidant and enhances lipid oxidation (Chaijan *et al.,* 2017). Also, smoked with oil oysters and mussels had an increased lipid content due to samples oil dipping, so there was a higher amount of lipids likely to be oxidated. Besides, smoked oysters and smoked with oil mussels significantly increased LPO values from day 3 to 15. During smoking process phenolic antioxidant compounds in the smoke are absorbed by food items and retard lipid oxidation (Pittia & Antonello, 2016). Increment of LPO values from day 3 to 15 in smoked oysters and smoked with oil mussels might reflect the short time of our smoking process (45 min) and the fewer deposition of antioxidant compounds present in the smoke on bivalves. Furthermore, it has been shown that salt can have a pro-oxidant effect on fish, leading to higher lipid oxidation (Shimizu *et al.,* 2009) since it can disrupt cell membrane integrity enabling greater access of oxidative components to lipid substrates (Rhee, 1999). However, Kong *et al.* (2008) did not detect effects of salt on fish LPO levels. In this study brine and dry salted bivalves had low LPO levels, so salt did not have major impacts on oysters and mussels' samples during the first fifteen days of their storage. Moreover, dry salted oysters after 3 days of preservation and dry salted mussels after 15 days of storage revealed significantly higher LPO levels than brine salted bivalves with the same preservation days. In fact, other studies had already shown that dry salting leaded to higher LPO and lower oxidative stability in fish than wet/brine salting (Chaijan, 2011; Vidal *et al.,* 2015) because of limited oxygen levels in the brine solution (Horner, 1997).

Salting, sun-drying and smoking preservation techniques reduce water content in bivalves and fish (Kyriazi-Papadopoulou *et al.,* 2003; Masette & Kwetegyeka, 2013; Turan *et al.,* 2007). It has been shown that smoking reduces mussels' moisture content, leading to an increase in their protein and lipid content (Kyriazi-Papadopoulou *et al.,* 2003; Turan *et al.,* 2008). Water loss in mussels also leads to an increase of their carbohydrates content (Goulas, 2008). In fact, we verified that fresh bivalves revealed significantly lower energetic reserves (lipids, sugar and protein) than some preserved samples, which was due to water loss and nutrients concentration in preserved oysters and mussels.

Concerning bivalves' lipid content, it was significantly higher in smoked with oil oysters and mussels since bivalves were dipped in oil prior to the smoking process, which contributed to an increase of samples lipidic content masking bivalve intrinsic lipid content.

Sugar content was significantly higher in sun-dried mussels (t3) and it was also higher in sun-dried oysters (t3). Sun-drying was the developed preservation technique that resulted in drier samples in the short storage period analysed (3 and 15 days after preservation). So, due to less water content sun-dried oysters and mussels presented higher sugar levels. Moreover, brine salted bivalves (specially mussels) had low sugar content instead of showing higher content as organisms preserved with the remaining preservation methods. As mentioned before, brine salting consisted of immersing bivalves in a watery solution with NaCl, so these samples experienced less water loss during the storage period analysed than individuals preserved with the remaining preservation techniques. Longer storage periods might reduce brine and dry salted bivalves sugar content since it decreased from 3 to 15 preservation days in salted oysters.

Proteins can undergo partial denaturation and lose some amino acids when are submitted to high temperatures (e.g., high heat smoking process) reducing their bioavailability (Horner, 1997; Pittia & Antonello, 2016). In fact, sun-dried, smoked and smoked with oil oysters after 3 days of preservations had significantly lower protein content than salted individuals after 3 days of storage. Although all samples were boiled prior to their preservation, these three preservation methods implied oyster submission to higher temperatures for longer periods of time due to solar exposure and smoking process. So, this longer exposure to high temperatures might have reduced sun-dried, smoked and smoked with oil oyster protein content. Moreover, some smoke components can react with amino acids from food items, also reducing their protein content (Horner, 1997). In fact, smoked and smoked with oil mussels after 15 days of preservation also had significantly lower protein content than the remaining t15 mussel treatments. Additionally, smoked and smoked with oil mussels reduced their protein content from 3 to 15 preservation days, which can be related to the negative effects of smoke components on mussel proteins.

Microbial growth was higher in sun-dried oysters and mussels in all the three media (CCA, TSA and TCBS). Sun-dried samples were placed in sieves and left to dry under solar exposure without any covering. Samples were not protected from flies and sieves could had been contaminated with microorganisms, which might have contributed to sun-dried coliform (CCA), salt-water microorganisms (TSA) and *Vibrio* spp. (TCBS) contamination. Besides, in sun-dried bivalves the only mechanism preventing microbial growth is water removal from oysters and mussels' tissues, contrary to salted and smoked samples, where salt and smoke components contribute to hinder microbial proliferation. Moreover, smoked mussels also revealed high microbial growth in the three media. The retarding effect of smoking on the growth of bacteria that can potential spoil food items depends on different factors like the duration of smoking, the concentration of active compounds in the smoke and the temperature of heating (Kyriazi-Papadopoulou *et al.,* 2003). Our smoking process only lasted 45 min, so we believe that there was not enough time for bivalves to lose the ideal water content to

efficiently prevent microbial growth. Besides, longer smoking processes enable higher absorption of compounds present in the smoke on bivalves.

All bivalve samples were immersed in freshwater from the village well prior their preservation. Access to potable water is scarce in Cabo Delgado, so water for domestic use is taken from artisanal sources, like village wells with uncontrolled water quality (Araújo & Silva, 2015; INE, 2021). This water could have contributed to microbial contamination of our samples (coliforms and *Vibrio* spp.), namely the brine salted bivalves whose brine was made using well's water. We also verified that microbial growth in TSA medium was significantly lower in dry salted and smoked with oil mussels. In fact, oil creates an anaerobic environment in the food item and inhibits bacterial grow (Ofulla *et al.,* 2011) and the decreasing water content and increasing salt concentration in bivalves and fish flesh hinder spoilage microbial growth (Horner, 1997; Ofulla *et al.,* 2011; Turan *et al.,* 2007).

Sun-dried oysters and mussels and smoked mussels had higher microbial growth in TCBS medium where *Vibrio* spp. preferentially growth. The *Vibrio* genus includes different pathogenic species, namely bacteria that cause gastroenteritis (*V. parahaemolyticus*) and the well-known cholera disease (*V. cholerae*) (Donovan & Van Netten, 1995). So, these preserved samples should be avoided for human consumption.

3.5 Conclusions

We verified that dry salting was the method that allowed a higher preservation of oysters and mussel protein content while having low LPO, mercury, microplastic and microbial contamination.

Future studies should include fatty acids profile to verify if important polyunsaturated fatty acid (PUFA) with valuable properties for human health (e.g., prevention of cardiovascular diseases), like eicosapentaenoic acid (EPA) C20:5 *n*-3 and docosahexaenoic acid (DHA) C22:6 *n*-3 (Biandolino *et al.,* 2021; Stark, 2008) are affected by the different bivalves' preservation methods. Moreover, future studies could include an evaluation of sensory attributes (smell, taste and texture) of bivalves preserved through distinct preservation techniques to assess consumers preference.

4. Community-based aquaculture of *Modiolus philippinarum* mussels in the North of Mozambique: production optimization

4.1 Introduction

Community-based aquaculture founded on the principles of common interests of a community is a practice able to fulfil people needs through an implementation of a low-technological and extensive production leaded by a community group (Ananth *et al*., 2014). This aquaculture system is a sustainable alternative to traditional and artisanal fishing practices providing protein source and income to local coastal communities and diversifying their livelihoods (Ananth *et al*., 2014; Ateweberhan *et al*., 2014). Besides, community-based aquaculture contributes to alleviate the pressure on marine resources harvesting and to improve coastal marine ecosystems conservation (Ateweberhan *et al*., 2014).

Marine resources in Cabo Delgado province, North of Mozambique have been declining due to overfishing and unsustainable fishing practices (Rosendo *et al*., 2020; Wanyonyi *et al*., 2011). Coastal communities in this region heavily rely on marine resources for food and income (Bilika *et al*., 2019; Souto, 2015). So, to enable coastal marine resources to recover and to ensure these communities livelihoods based on marine resources, it is urgent to implement a better fishing practices management (Rosendo *et al*., 2020).

Implementation of a community-based aquaculture in Cabo Delgado villages fulfil different Mozambique policies and strategies aiming to reduce poverty, to increase sustainable and community management of marine resources and to improve biodiversity conservation. The Fisheries Law (Law n. 22/2013) and the Conservation Law (Law n. 5/2017) of Mozambique highlights the importance of community management of marine resources to ensure their rights to fishing resources and the role of local communities in marine ecosystems conservation through a more sustainable exploitation of their resources (Rosendo *et al*., 2020). Moreover, Mozambique's National Development Strategy (2015-2030), the Fisheries Master Plan (2010-2019) objective 2 and the new Sea Policy and Strategy (2017) cornerstone C prioritise, respectively: sustainable management of natural resources; increase welfare of small-scale aquaculture productions and artisanal fishing communities; preservation of natural resources for the communities' wellbeing (Rosendo *et al*., 2020).

Bivalves are organisms that can be easily produced by community-based aquaculture. These organisms are filter-feeders removing large quantities of organic matter from the water column (Cranford *et al.,* 2003; Kellogg *et al*., 2013), so communities do not have to provide them food since it is provided by the environment itself. Due to their filtering capacity, bivalves help avoiding excessive phytoplankton blooms in estuaries and coastal waters that might occur because of increased nitrogen levels coming from anthropogenic activities (Gallardi, 2014). Bivalves also have the ability to enhance

water clarity by removing suspended inorganic sediments, allowing higher light penetration in the water column (Gallardi, 2014; Newell, 2004; Newell & Koch, 2004). This can improve seagrasses growth which are important nursery and carbon sink habitats (Duarte *et al.,* 2010; Newell & Koch, 2004). Bivalves are also low trophic organisms so their extensive aquaculture production will have a reduced environmental impact and it will contribute to carbon sequestration, a regulating ecosystem service (Gentry *et al.,* 2020; Olivier *et al*., 2020).

Even though bivalves filter-feeding behaviour presents great advantages to the surrounding environment and to producers that implement a community-based aquaculture with these organisms, it can also have some drawbacks. Bivalves can heavily accumulate contaminants in their body tissues (e.g., heavy metals and microplastics) due to the large amount of water filtered by them during their feeding activity (Li *et al.,* 2015; Naimo, 1995; Wright *et al.,* 2013). Contaminants like mercury and microplastics can increase their levels in coastal waters through river discharges and surface runoff that carry out waste products and debris resulting from anthropogenic activities (Cheung *et al*., 2016; Lebreton *et al.,* 2017; Saniewska *et al.,* 2014). So, bivalves' community-based aquaculture implemented in coastal waters polluted with mercury and microplastics will potentially reduce their suitability for human consumption due to negative impacts of these contaminants in human health (Mahler *et al.,* 2012; Park & Zheng, 2012; Saniewska *et al.,* 2014; Thiele *et al.,* 2019).

Most of bivalves captures in Cabo Delgado are undertaken by women in mangrove and seagrass meadows intertidal ecosystems (Fernando *et al*., 2012). Developing a community-based aquaculture of bivalves leaded by women is a way to avoid the intensive resource exploitation of those important coastal ecosystems (Rosendo *et al*., 2020). Moreover, women are among the lowest socioeconomic status in Cabo Delgado, and they hardly can manage how their family income is spent (De Brauw, 2015). Besides, men are commonly fed first than women, even if the food is scarce due to socio-cultural factors (Lusambili *et al*., 2020). So, a bivalve community-based aquaculture leaded by women can be a strategy to increase women entrepreneurship, income, and livelihoods in Cabo Delgado.

We implemented a community-based aquaculture of the native *Modiolus philippinarum* mussel in two villages of Cabo Delgado, Mecúfi and Metuge with the help of villagers. We performed biometric measurements and we analysed mercury and microplastics concentration and oxidative damage and energy-related biomarkers of aquaculture-produced mussels in the two villages. Our main goal was to verify which was the best location for *M. philippinarum* community-based aquaculture.

4.2 Materials and methods

4.2.1 Study area

This study was carried out in the villages of Mecúfi (-13,288677 S, 40,558873 E) and Metuge (-12.971234 S, 40.414297 E), in the North coast of Mozambique. In Mecúfi the study site was placed in a river estuary, surrounded by mangrove and in Metuge the study site was also next to a mangrove forest, inside Pemba Bay.

4.2.2 Experimental procedure

In September 2019 we collected mussels from the seacoast nearby Mecúfi with women villagers (Fig. 23). Wood-tables 2,5x1 m were made with the villagers of Mecúfi and Metuge using wood of *Moringa* sp. and *Casuarina* sp., which was picked up by them in the surrounding of the villages. Wood sticks were attached to each other with plastic clamps (Fig. 24).

Fig. 23 – *M. philippinarum* mussels gathered by women villagers to introduce in aquaculture bags.

In Mecúfi we built two wood-tables, one closer to the mangrove and one further away. Four plastic oyster bags 100x50 cm, mesh size 1 cm, were tied up with sisal rope to each of the wood-tables at 1 m from the ground. In each bag we introduced 200 mussels (n=4). The table further the mangrove was submerged in sand and our samples were lost. So, from now on we will consider only the table placed next to the mangrove forest when we refer to Mecúfi samples.

In Metuge we built one wood-table next to an oyster bank following the same procedure as in Mecúfi. We tied up two bags to the table and each one of them was divided in two, so we also had four replicates (n=4). In each half we introduced 145 mussels (collected in Mecúfi seacoast).

Physicochemical water parameters (water oxygen, temperature, pH, salinity and conductivity) were measured in Mecúfi in September 2019 (t_i) and in this same village and in Metuge six months after, in March 2020 (t_f) using a digital multiparameter (WTW 2FD460 Multi 3420).

Fig. 24 – Aquaculture wood-table built in Mecúfi village.

Using mussel samples from the beginning of the aquaculture experiment and six months after we did biometric measurements, mercury analysis, microplastics quantification and oxidative damage (lipid peroxidation) and biochemical biomarkers analysis (lipids, sugars and proteins).

4.2.3 Biometric measurements

In September, we measured 96 mussels collected from their original locations in Mecúfi: half (48) were included in Mecúfi aquaculture tables (t_0 Mecúfi), and the other half were grown in aquaculture tables in Metuge (t_0 Metuge). In Mecúfi and Metuge, for each of the four replicates we measured 12 mussels in March (t_f). With a calliper with an accuracy of 0.5 mm we measured the maximum length, width and height of mussels (cm) and using a mini electronic balance with an accuracy of 0.01 g we accessed mussels' total, shell and edible weight (g). We calculated the increment in mussel's shell measurements and weights throughout the experiment in the two different locations by performing the difference between the mean value of each parameter in September (t_0) and March (t_f). We also registered mussels' survival six months after the beginning of the experiment (t_f) in the tables of the two villages.

4.2.4 Mercury quantification

Mercury was quantified (µg/g wet weight) in mussels (n=10 in t_i and t_f) collected from the seacoast of Mecúfi (t_i) and from aquaculture wood-tables in Mecúfi and Metuge (t_f) by atomic absorption

spectrophotometry using an Advanced Mercury Analyser (AMA) LECO 254 according to the methodology described in chapter 2.

4.2.5 Microplastics extraction and quantification from biological tissues

M. philippinarum (n=10 in t_i and t_f) was collected from its original location in Mecúfi seacoast (t_i) and from aquaculture tables in Mecúfi and Metuge (t_f) and microplastics quantification (microplastic particles/g wet weight) followed the protocol described in chapter 2.

4.2.6 Oxidative damage and energy-related biomarkers analysis

Mussels (n=10 in t_i and t_f) were collected from their original location in Mecúfi (t_i) and from aquaculture sites in Mecúfi and Metuge (t_f) and oxidative damage (nmol TBARS/g wet weight) and energy-related (mJ/mg wet weight) biomarkers analysis followed the protocol described in chapter 2.

4.2.7 Data analysis

Differences among *M. philippinarum* collected from Mecúfi seacoast in September (t_i) (that were subsequently introduced in aquaculture tables) and *M. philippinarum* collected in March (t_f) from aquaculture wood-tables in Mecúfi and Metuge were analysed. Concerning mussels' biometric measurements, we analysed differences between mussels' measurements used in aquaculture tables in both locations in t_0 and t_f. Weight measurements were not compared in t_0 between both locations because although we measured mussels' length, width and height of individuals that were integrated in Metuge aquaculture tables, we did not measure their initial weight. We also compared mussel biometric measurements in the beginning of the aquaculture experiment (t_0) and six months after (t_f) in Mecúfi and in Metuge. These comparisons were made by Independent-Samples t-test with a significance level of 5%. The Shapiro-Wilk test was used to verify if our data followed a normal distribution. When this assumption was not fulfilled, we performed a Mann-Whitney non-parametric test. Original sample size was 48 in all biometric measurements, but we considered outliers values that fell out of the interval mean ± 2*standard deviation, so sample size varied between 46 and 48.

Regarding mercury, microplastics and biomarkers these differences were analysed by one-way analysis of variance (ANOVA) with a significance level of 5%, followed by SNK multiple comparison tests when significant differences were found. The Shapiro-Wilk test was used to verify if our data followed a normal distribution and the Levene test was used to verify the assumption of homogeneity of variance. When needed, data were transformed in order to fulfil these two assumptions. Original sample size was ten in mercury, microplastics and biomarkers, however we considered outliers values that fell out of the interval mean ± 2*standard deviation, so sample size varied between nine and ten. All these procedures were conducted using IBM SPSS Statistics 25.

4.3 Results

4.3.1 Physicochemical water parameters

Physicochemical seawater parameters measured in Mecúfi in September 2019 (t_i) and in Mecúfi and Metuge in March 2020 (t_f) are presented in Table 1 (chapter 2). In March (t_f), seawater oxygen was slightly higher in Mecúfi (8.29 mg/l) than in Metuge (7.74 mg/l). Seawater temperature, pH, salinity and conductivity were similar in both locations in March. In Mecúfi, seawater temperature increased a little from t_i (28.4°C) to t_f (29.9°C) and salinity and conductivity decreased from t_i (34.8 and 52.6 mS/cm) to t_f (32.1 and 48.8 mS/cm).

4.3.2 Biometric measurements and survival

Mussels mean percentage of survival was slightly higher in Metuge (36.2 ± 18.3%) than in Mecúfi bags (31.5 ± 6.5%).

As expected, all mussel biometric measurements were significantly higher in t_f than in t_0 in Mecúfi and Metuge tables (Table S9). So, mussels grown in aquaculture wood-tables in both locations significantly increased their shell measurements and weight.

Concerning shells measurements, (Fig. 25) in t_0 these values were significantly lower in mussels introduced in Metuge aquaculture table than in Mecúfi table (Table S9). However, in t_f there were no significant differences between mussels grown in these two locations (Table S9). These observations revealed that mussels grown in Metuge aquaculture tables had higher length, width and height increment than mussels grown in Mecúfi tables (Fig. 25).

Concerning mussel weight (Fig. 26), in t_0 there were no significant differences between mussels introduced in Mecúfi and in Metuge aquaculture tables (Table S9). Nevertheless, in t_f mussel weights were significantly higher in Metuge than in Mecúfi (Table S9). So, higher increments were also detected in mussels grown in Metuge tables than in Mecúfi tables.

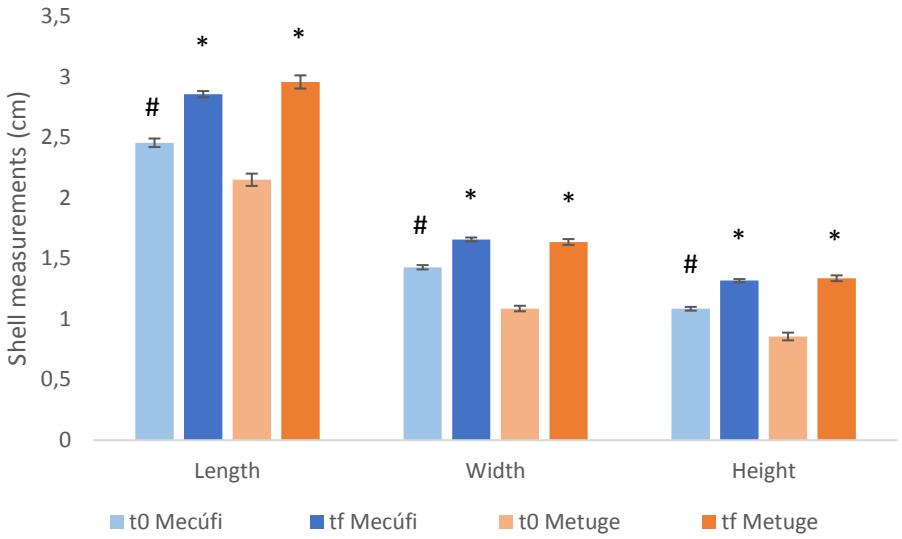

Fig. 25 – Length, width and height (mean ± s.e.) of *M. philippinarum* mussels introduced in aquaculture tables in September (t_0) in Mecúfi and Metuge and mussels from these same tables in March (t_f). Significant differences detected between t_0 and t_f in Mecúfi and Metuge are represented by * and significant differences detected during the same sampling period (t_0 and/or t_f) in Mecúfi and Metuge are represented by #.

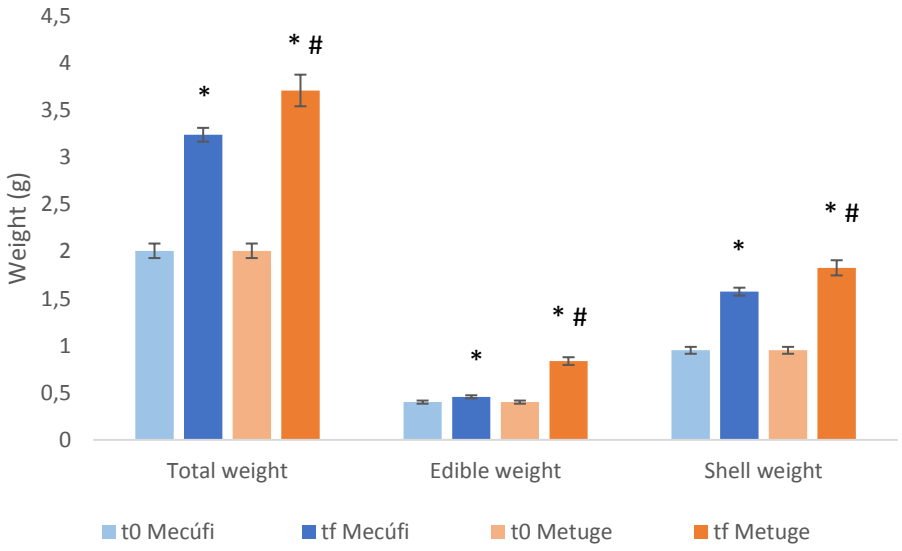

Fig. 26 – Total, edible and shell weight (mean ± s.e.) of *M. philippinarum* mussels introduced in aquaculture tables in Mecúfi and Metuge in September (t_0) and mussels from these same tables in March (t_f). Significant differences detected between t_0 and t_f in Mecúfi and Metuge are represented by * and significant differences detected during the same sampling period (t_0 and/or t_f) in Mecúfi and Metuge are represented by #.

4.3.3 Mercury contamination

Mercury contamination of *M. philippinarum* mussels was significantly higher in t_i than in t_f in Mecúfi and Metuge (Fig. 27, Table S10). In t_f, there were no significant differences in mercury contamination Between *M. philippinarum* individuals grown in Mecúfi and Metuge.

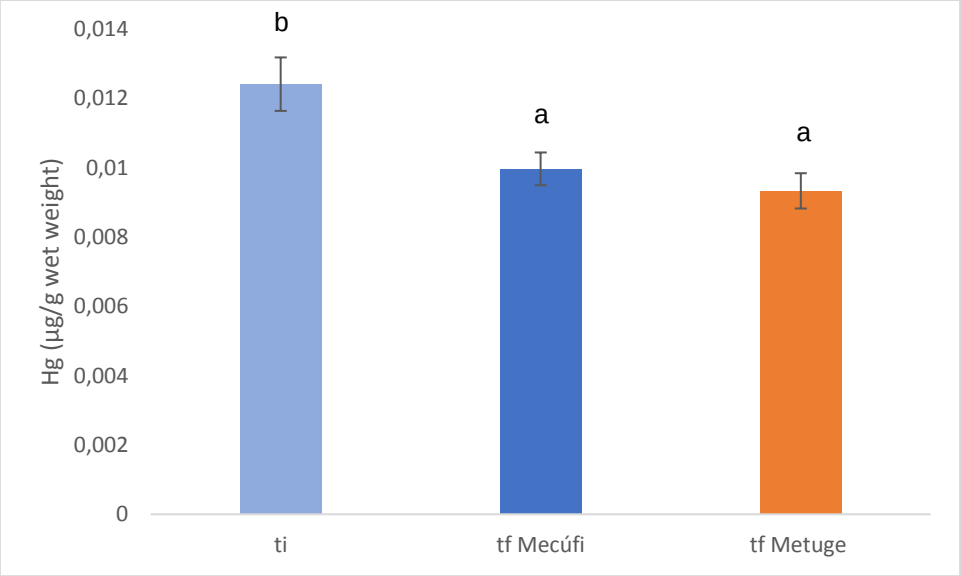

Fig. 27 - Mercury (µg/g wet weight) contamination (mean ± s.e.) of *M. philippinarum* mussels from the beginning of the aquaculture experiment from Mecúfi seacoast (t_i) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b) (SNK *post hoc* test).

4.3.4 Microplastics contamination

Mussel microplastics contamination was significantly higher in t_f Mecúfi than in t_i and t_f Metuge (Fig. 28, Table S10).

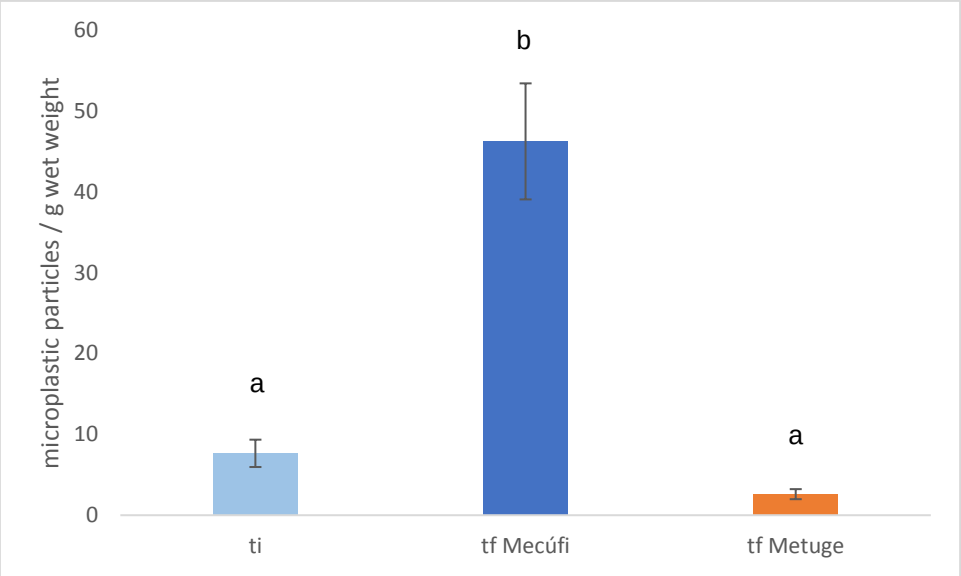

Fig. 28 - Microplastics contamination (microplastic particles/g wet weight) (mean ± s.e.) of *M. philippinarum* mussels from Mecúfi seacoast in September (t_{fi}) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b) (SNK *post hoc* test).

4.3.5 Oxidative damage and energy-related biomarkers analysis

We did not find significant differences between mussel LPO in t_i and t_f in Mecúfi (Fig. 29, Table S10). However, LPO was significantly higher in t_f mussels grown in aquaculture tables in Metuge than in the two previously described scenarios (Fig. 29, Table S10).

There were no significant differences in mussels' lipid content concerning t_i individuals and t_f mussels collected from aquaculture wood-tables in Metuge (Fig. 30, Table S10). On the other hand, lipid content of t_f mussels from Mecúfi aquaculture tables was significantly lower than in the two previously described scenarios (Fig. 30, Table S10).

The highest sugar content was detected in t_i mussels. Concerning t_f mussels, the ones that grew in Metuge had significantly higher sugar content than the ones that grew in Mecúfi (Fig. 31, Table S10).

There were no significant differences in the protein content of t_i and t_f mussels grown in the aquaculture table in Mecúfi. However, t_f mussels collected from aquaculture bags in Metuge had significantly higher protein than in t_i and t_f in Mecúfi (Fig. 32, Table S10).

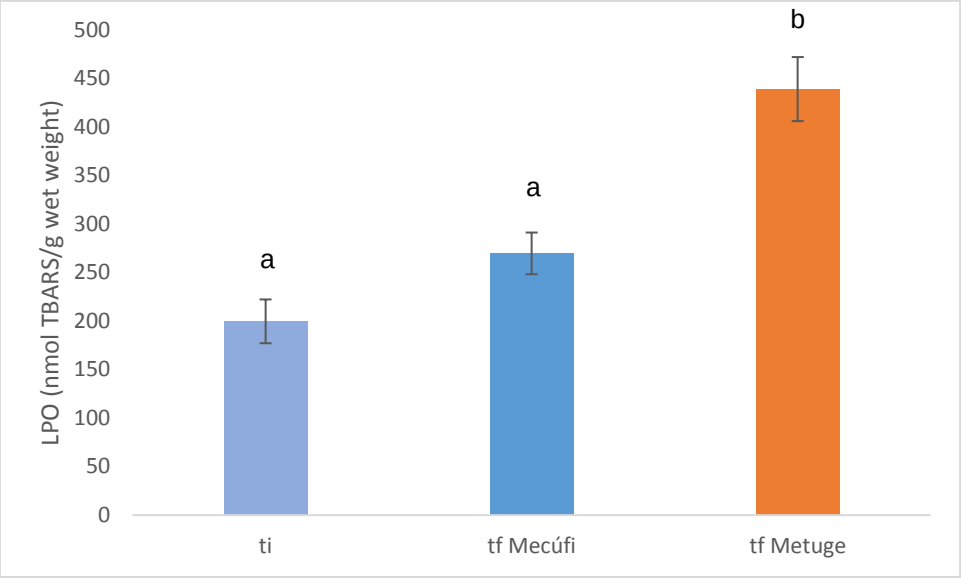

Fig. 29 - LPO (nmol TBARS/g wet weight) (mean ± s.e.) of *M. philippinarum* mussels from the beginning of the aquaculture experiment from Mecúfi seacoast (t_i) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b) (SNK *post hoc* test).

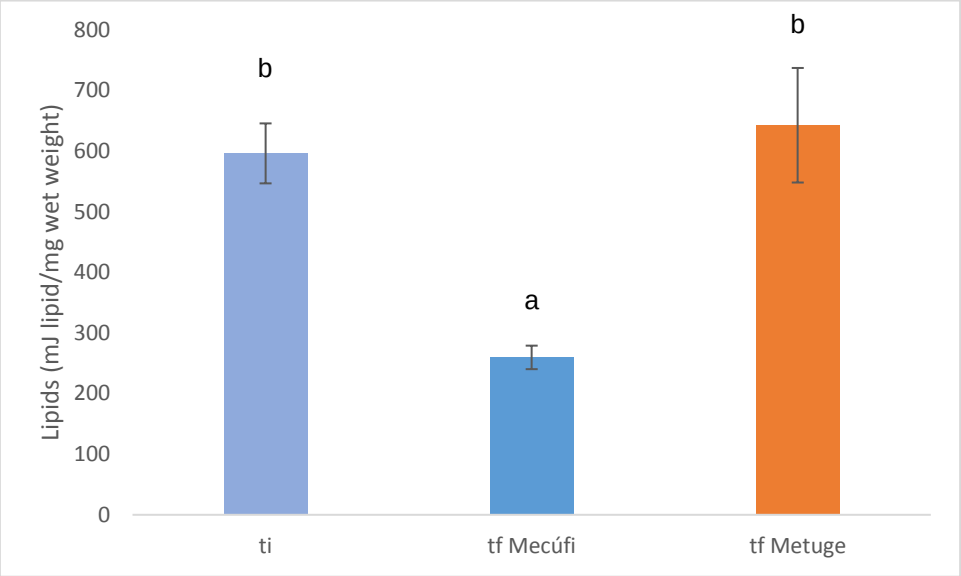

Fig. 30 - Lipids (mJ lipid/mg wet weight) (mean ± s.e.) of *M. philippinarum* mussels from Mecúfi seacoast (t_i) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b) (SNK *post hoc* test).

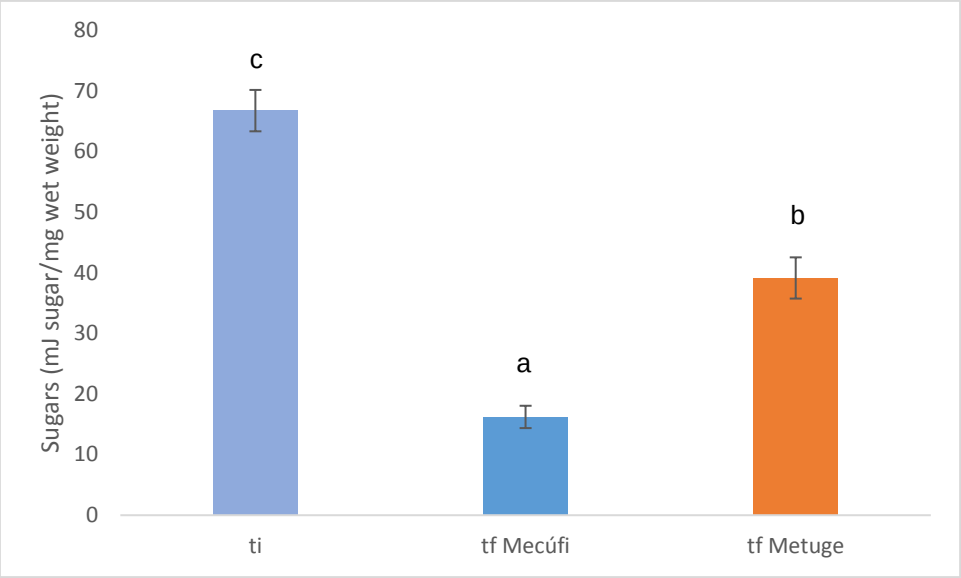

Fig. 31 - Sugars (mJ sugar/mg wet weight) (mean ± s.e.) of *M. philippinarum* mussels from the beginning of the aquaculture experiment from Mecúfi seacoast (t_i) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b,c) (SNK *post hoc* test).

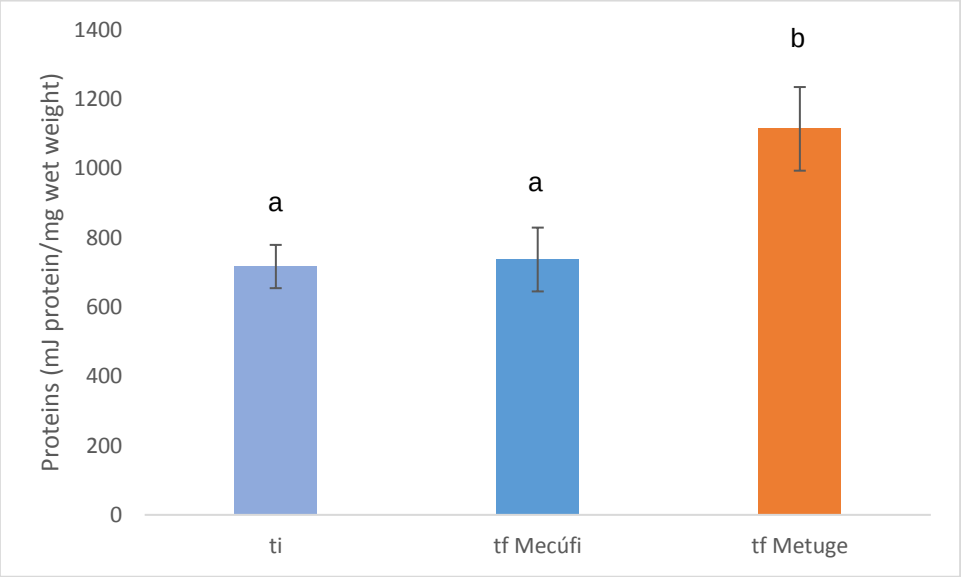

Fig. 32 - Proteins (mJ protein/mg wet weight) (mean ± s.e.) of *M. philippinarum* mussels from Mecúfi seacoast (t_i) and from aquaculture wood-tables in Mecúfi and Metuge six months after (t_f). Significant differences detected are represented by different letters (a,b) (SNK *post hoc* test).

4.4 Discussion

Unsustainable fishing practices have been depleting Cabo Delgado marine resources, namely bivalve molluscs stocks (Rosendo *et al*., 2020; Wanyonyi *et al*., 2011). A community-based aquaculture of *M. philippinarum* mussels leaded by women will favour this species recovery in its natural habitat and set the mote for future similar projects contributing to Cabo Delgado's marine ecosystems conservation and to rise women entrepreneurship and income. So, to implement these aquaculture projects it is essential to verify the best location to optimize species growth and production to help ensuring local coastal communities' livelihoods and income (Ananth *et al*., 2014; Ateweberhan *et al*., 2014). In the present study, *M. philippinarum* grown in aquaculture tables in Mecúfi and Metuge revealed different survival rates and biometric increments. Mussels grown in Metuge had higher survival rate and higher shell and weight increment during our six-month aquaculture experiment. Although their survival rate was higher in Metuge (36.2 ± 18.3%) than in Mecúfi bags (31.5 ± 6.5%), it was still a bit low in a production perspective. We hypothesise that this reduced value is owed to predators, since mangrove areas provide a large range of niches supporting high biodiversity levels (Hendy *et al.,* 2014). In accordance, while collecting our samples from Metuge aquaculture-bags we noticed the presence of crabs. Moreover, in Mecúfi we detected a higher number and diversity of *M. philippinarum* predators, which included crabs and sea snails. Despite that, biometric measurements highlighted that both areas are favourable to the growth of mussels since they grew and fattened in both locations, from t_0 to t_f. However, shell and weight increment were significantly higher in *M. philippinarum* grown in aquaculture bags in Metuge than in Mecúfi. In Mecúfi the study site was placed in a river estuary next to a mangrove forest, where we expected to be abundant organic matter in the water which would favour bivalve growth. However, this mangrove forest has been continuously destroyed to build salt pans. Moreover, in Metuge the mangrove forest in the surrounding area of the aquaculture table was better preserved. Mangroves provide significant amounts of dissolved and microparticulate organic matter to estuarine areas (Dittmar *et al.,* 2001; Jaffé *et al.,* 2004). Better preserved mangrove forests can contribute in a higher scale to increase organic matter in the surrounding waters than mangrove habitats that have been degraded and devastated. Therefore, the mangrove forest nearby mussels' aquaculture table in Metuge provides higher organic matter inputs to the surrounding waters increasing food availability to bivalves. So, mussels grown in Metuge aquaculture bags will be more likely to have higher shell and weight increment than mussels grown in Mecúfi aquaculture table.

Mercury levels decreased with mussel growth from t_i to t_f possibly due to growth dilution effect (Otchere *et al.,* 2003). Moreover, since individuals present in the bags were gathered in Mecúfi seacoast in September, aquaculture tables might have been placed in locations with lower mercury contamination. Besides the location, seasons might also have influenced mussels' mercury contamination. In the North of Mozambique there are two seasons: the dry season (May-November) and the wet season (December-April) (Pemba Climate: Temperature, Climograph and Climate Table for Pemba – Climate-Data.org, n.d.; Rrokaj & Corti, 2019). During dry season, in September (t_i),

mussels were gathered from the seacoast of Mecúfi and introduced in aquaculture tables placed in the final part of a river estuary in Mecúfi and inside Pemba Bay, next to a mangrove forest in Metuge. Due to heavy rains, during wet season, pollutants get flushed out from estuaries (Chakraborty *et al.,* 2019). In fact, in March (t_f, during wet season), mussels mercury contamination from Mecúfi aquaculture bags was lower than specimens from this village seacoast in September (t_i, dry season). Since Mecúfi aquaculture table was inside the river estuary instead of being in the exposed seacoast (outside the estuary), mercury might have been flushed out by rain from the estuarine region and mussels did not accumulate much of this heavy metal in this location during this period. Mercury contamination in mussels from Metuge (≈0.010 ± 0.001 µg/g wet weight) was similar to the one detected in oysters from this same location during wet season (≈0.009 ± 0.001 µg/g wet weight) (see Chapter 2). In Chapter 2 we have already noticed that mercury pollution levels in bivalves from Metuge were lower than in bivalves from Mecúfi seacoast. This distinct *M. philippinarum* mercury contamination might reflect the different pollution levels of this heavy metal in its occurring habitats, since due to their filter feeding activity, bivalves reflect their surrounding seawater pollution (Newman & McIntosh, 1982). We also verified this different mercury contamination of *M. philippinarum* mussels when looking at other study involving this species. *M. philippinarum* mercury content was lower in Mecúfi (≈0.057 ± 0.004 and ≈0.046 ± 0.002 µg/g dry weight in September and March, respectively) and in Metuge (≈0.043 ± 0.002 µg/g dry weight in March) than in specimens of this species sold in Northern China markets (0.22 ± 0.04 µg/g dry weight) (Zhao *et al*., 2013). According to EFSA, mercury maximum levels in fishery products, including molluscs are 0.50 µg/g wet weight (European Commission, 2006). Mercury contamination values in the analysed samples were quite below the maximum levels accepted, so concerning this pollutant, mussels from Mecúfi and Metuge seem safe for human consumption.

Mussels microplastics contamination was significantly higher in organisms grown in aquaculture bags in Mecúfi (t_f) (≈46.2 ± 7.2 particles/g wet weight) than in t_i mussels (≈7.7 ± 1.7 particles/g wet weight) and t_f mussels collected from Metuge aquaculture table (≈2.6 ± 0.6 particles/g wet weight). Microplastics contamination of mussels collected from aquaculture bags in Metuge in March was similar to oysters' contamination with this pollutant collected from this same location at the same time of the year (≈2.0 ± 0.6 particles/g wet weight) (see Chapter 2). Metuge might have less anthropogenic pressure and consequently less microplastics pollution in its waters than Mecúfi estuarine region. Moreover, mangrove trees have been pointed out as good phytoremediators, as they extract heavy metals and other pollutants, like total petroleum hydrocarbons (TPH's) (Chowdhury *et al.,* 2015; Moreira *et al.,* 2011; Rezaei *et al.,* 2021). So, preserved mangrove forests accumulate larger amounts of contaminants, extracting them from the seawater and making them less accessible to marine organisms. This might be happening with microplastics. As mentioned before, Metuge mangrove forests are better preserved than the ones in Mecúfi, which can help explaining why mussels grown in Metuge aquaculture bags had significantly less microplastics than organisms grown in the Mecúfi table.

To our best knowledge, the present study is the first one quantifying microplastics in *M. philippinarum* mussels. As there are no maximum levels of microplastics established for food products for human consumption, we compared our results to other global studies. Microplastics detected in *M. philippinarum* in Metuge aquaculture table were within the average of the overall values verified in shellfish in other studies around the world (Awuor *et al*., 2020; Ding *et al*., 2020; Mayoma *et al*., 2020; Sparks, 2020). However, the number of microplastics quantified in *M. philippinarum* grown in Mecúfi aquaculture bags was above the average values of microplastics identified in shellfish in other global studies (Awuor *et al*., 2020; Ding *et al*., 2020; Mayoma *et al*., 2020; Sparks, 2020). We suggest that future studies including analytical methods (e.g., FTIR) should be developed to increase knowledge about microplastics content in bivalves inhabiting the North of Mozambique.

Mussels LPO was significantly higher in Metuge than in Mecúfi (t_i and t_f). Biotic factors like predation risk leads to higher LPO levels (Janssens & Stoks, 2013). Moreover, LPO in bivalve molluscs can be increased because of reduced water quality due to heavy metals (e.g., mercury) and microplastics pollution (Bonnail *et al.,* 2019; Oliveira *et al.,* 2018). However, we did not consider that these biotic and abiotic factors leaded to higher LPO levels in mussels grown in Metuge table, since they had less mussel predators, lower microplastic contamination and similar mercury levels to mussels from Mecúfi bags. Additionally, LPO helps assessing oxidative stress in organisms which may vary throughout the year due to bivalves' reproductive cycle, namely gonad ripening (Sheehan & Power, 1999; Viarengo et al., 1991). While preparing mussel samples for mercury, microplastics and biomarkers analysis we noticed that some individuals from Metuge had internal orange masses with roe eggs. This maturation stage was only verified in organisms from Metuge, we did not detect any t_i and t_f individual from Mecúfi with roe eggs. So, considering that mercury and microplastics contamination were lower in this location, mussels higher LPO in Metuge might be mainly explained by gonad ripening and gametogenesis.

Concerning energetic reserves measured in t_f mussels from aquaculture tables, lipid, sugar and protein contents were significantly higher in organisms grown in Metuge than in Mecúfi. This might be due to higher food availability in Metuge because of a better-preserved mangrove forest in that region. Besides, higher energetic reserves in mussels grown in Metuge table might also be due to gonad ripening. Energetic reserves are crucial for bivalve growth and reproduction (Lodeiros *et al.,* 2001). These organisms have to storage energetic reserves before spawning, since they play a key role on gametogenesis (Dridi *et al*., 2007; Mitra *et al*., 2008; Mohan & Kalyani, 1989). In fact, as mentioned before we noticed that some t_f mussels collected from Metuge aquaculture table had roe eggs. So, their higher energetic reserves might be related to their evident gonad ripening, which was not detected in any analysed mussel from Mecúfi aquaculture bags. Additionally, microplastics ingested by bivalves can decrease their energetic reserves (Bour *et al*., 2018). In fact, mussels grown in aquaculture bags in Mecúfi had significantly the highest microplastics contamination and significantly the lowest lipid and sugar contents.

4.5 Conclusions

Six months after we started the aquaculture experiment, mussels grown on aquaculture table in Metuge had higher shell and weight increment, less microplastics contamination and significantly higher lipid, sugar and protein content than mussels grown on aquaculture bags in Mecúfi. So, a community-based aquaculture of *M. philippinarum* would be better succeeded if installed in Metuge rather than in Mecúfi, in those specific locations and concerning the analysed variables. Mecúfi t_f mussels had significantly higher microplastic contamination and significantly lower sugar and lipid content than t_i mussels, that had been gathered from Mecúfi seacoast six months earlier and introduced in the aquaculture table. So, we suggest that to implement a community-based aquaculture of this mussel species in Mecúfi a most suitable location nearby the village to promote mussels' growth and development should be selected.

Although we did not detect *M. philippinarum* seeds in natural banks in Metuge, it is known that after bivalves' reproduction their larvae might settle on spat collector near the production site, which means that producers will reduce the collection of new individuals from natural banks to introduce in their production (Poirier *et al.,* 2019; Sievers *et al.,* 2014). We suggest that future studies include spat collectors nearby the aquaculture tables to gather new mussel seeds to ensure shellfish production continuity and avoiding gathering from natural banks and depletion of marine natural resources.

5. General conclusion

In this study we analysed two native bivalve species, *S. cucullata* oysters and *M. philippinarum* mussels and their potential for community-based aquaculture in two villages in the North of Mozambique where LMMA have been established, Mecúfi and Metuge.

We verified that both oysters and mussels were proper for human consumption throughout the whole year (during dry and wet seasons), concerning mercury contamination and oxidative damage and energy-related biomarkers. Moreover, the best period to collect oysters and mussels was during dry season since it was when they had higher nutrient contents and less pollutants (mercury and microplastics) contamination. However, according to literature, their higher energetic reserves could mean that these bivalve molluscs were in the pre-spawning phase, so a balance is needed in their collection to enable their spawning and future recruitment.

Dry salting was the artisanal preservation method that most efficiently prevented microbial growth and that best kept protein nutritional qualities of oysters and mussels. So, it would be the best preservation technique to be applied in future aquaculture produced bivalves since it would better avoid their spoilage and enable their preservation for longer periods of time.

M. philippinarum community-based aquaculture installed in Mecúfi and Metuge had different production performances, possibly due to the distinct preservation levels of the mangrove forests in the surrounding area. Mecúfi mangrove forest has been deeply destroyed contrary to Metuge mangrove forest and better-preserved mangrove forests can contribute with higher amounts of organic matter to the surrounding waters increasing food availability to bivalves. In fact, after six months of the community-based aquaculture implementation, mussels grown in Metuge had significantly higher shell and weight increment, less microplastics contamination and significantly higher nutritional content than mussels grown in Mecúfi. Therefore, we considered that a community-based aquaculture of *M. philippinarum* mussels would be better succeeded if installed in Metuge, concerning the analysed variables. To start a community-based aquaculture of *M. philippinarum* mussels in Mecúfi we suggest that a different location nearby the village should be chosen.

We did not implement a community-based aquaculture of *S. cucullata* since their seeds were hard to collect from the wild. However, we verified that *S. cucullata* oyster seeds widely settled on aquaculture bags from mussel aquaculture table in Metuge. We noted that *S. cucullata* seeds also settled on mussels' empty shells from individuals that died/were predated in Metuge aquaculture table. The fact that Metuge aquaculture table where we grew *M. philippinarum* mussels was close to an oyster bank might have enhanced this settlement. We have already verified that *S. cucullata* oysters are proper for human consumption throughout the whole year (dry and wet seasons), concerning the analysed variables and that dry salting is the most suitable method to preserve them for longer periods after being collected. Moreover, we observed that *S. cucullata* seeds greatly settle on spat collectors, including natural ones (empty shells). So, the use of natural spat collectors to

gather seeds from this oyster species could be a good strategy to start a community-based aquaculture of *S. cucullata* oysters in Mecúfi and Metuge.

We verified that in March, only six months after we have built aquaculture tables the one installed in Mecúfi was already occupied and used as refugee by different fish species, like longfin batfish (*Platax teira*), lionfish (*Pterois* spp.), butterfly fish (*Chaetodon* spp.) and puffer fish (*Canthigaster* spp.). This structure might function as an artificial reef and it can improve conservation of a wide range of marine species. Moreover, the colonization of aquaculture tables by fish species increases organic matter levels in the water enhancing bivalve growth.

Community-based aquaculture of bivalves is also a great solution to ensure a reliable income for women as well as livelihoods for the whole coastal communities. These are small, sustainable and non-fed productions that can promote restoration and conservation of marine resources, reducing the impact in marine protected areas and in wild stocks. In fact, shellfish community-based aquaculture in the surrounding of marine protected areas can enhance water clarity and avoid eutrophication by filtering suspended sediments from the water (Gallardi, 2014; Newell & Koch, 2004; Rice, 2001). This increases light penetration in the water column improving seagrasses growth, which are important nursery and carbon sink habitats (Duarte *et al.,* 2010; Newell & Koch, 2004) that exist nearby the aquaculture tables that we installed. So, bivalves' community-based aquaculture not only reduces wild marine resources exploitation as it can improve important marine ecosystems.

6. References

Afonso, C., Costa, S., Cardoso, C., Oliveira, R., Lourenço, H. M., Viula, A., Batista, I., Coelho, I., & Nunes, M. L. (2015). Benefits and risks associated with consumption of raw, cooked, and canned tuna (Thunnus spp.) based on the bioaccessibility of selenium and methylmercury. *Environmental research*, *143*, 130-137.

Ananth, P. N., Sahoo, P. R., Dash, A. K., Pati, B. K., Jayasankar, P., & Singh, S. R. (2014). A study on community-based aquaculture promoted by KVK-Khordha, Odisha, India. *Current World Environment*, *9*(3), 947-951.

Andersen, A., Ellingsen, D. G., Mørland, T., & Kjuus, H. (1993). A neurological and neurophysiological study of chloralkali workers previously exposed to mercury vapour. *Acta neurologica scandinavica*, *88*(6), 427-433.

Araújo, M. C., & Costa, M. (2007). An analysis of the riverine contribution to the solid wastes contamination of an isolated beach at the Brazilian Northeast. *Management of Environmental Quality: An International Journal*, *18*(1), 6-12.

Araújo, M. G. M., & Silva, T. C. (2015). Comunidades Costeiras de Moçambique. In: Silva, T. C., Araújo, M. G. M., Souto, A. N. (Orgs.), Comunidades Costeiras: Perspectivas e Realidades. CESAB, Maputo, pp. 25-60.

Ateweberhan, M., Hudson, J., Rougier, A., Harris, A., Jiddawi, N., & Msuya, F. E. (2014). Community based aquaculture in the Western Indian Ocean: Challenges faced and lessons learned. *Blue Ventures Conservation. London, UK*.

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. *Marine environmental research*, *111*, 18-26.

Awuor, W., Muthumbi, A. W. N., & Robertson-Andersson, D. V. (2020). Presence of microplastics in benthic macroinvertebrates along the Kenyan coast. *African Journal of Marine Science*, *42*(4), 405-411.

Barboza, L. G. A., Vieira, L. R., Branco, V., Carvalho, C., & Guilhermino, L. (2018). Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in Dicentrarchus labrax juveniles. *Scientific reports*, *8*(1), 1-9.

Barua, P., Mitra, A., Banerjee, K., & Chowdhury, M. S. N. (2011). Seasonal variation of heavy metals accumulation in water and oyster (Saccostrea cucullata) inhabiting central and western sector of Indian sundarbans. *Environmental Research Journal, 5*(3), 121-130.

Biandolino, F., Di Leo, A., Parlapiano, I., Papa, L., Giandomenico, S., Spada, L., & Prato, E. (2019). Nutritional quality of edible marine bivalves from the southern coast of Italy, Mediterranean Sea. *Polish Journal of Food and Nutrition Sciences*, *69*(1), 71-81.

Biandolino, F., Parlapiano, I., Denti, G., Nardo, V. D., & Prato, E. (2021). Effect of Different Cooking Methods on Lipid Content and Fatty Acid Profiles of Mytilus galloprovincialis. *Foods*, *10*(2), 416.

Bilika, F., Farooq, H., Simão, C., Soares, A., & Morgado, F. (2019). Composição específica da comunidade de peixes da Baía de Pemba (Norte Moçambique). *Revista Captar: Ciência e Ambiente para Todos*, *8*(1), 144-155.

Bird, R.P., & Draper, H.H. (1984). Comparative studies on different methods of malonaldehyde determination. *Methods in enzymology, 105*, 299–305.

Boening, D. W. (1999). An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. *Environmental monitoring and assessment*, *55*(3), 459-470.

Bonnail, E., Macías, F., & Osta, V. (2019). Ecological improvement assessment of a passive remediation technology for acid mine drainage: Water quality biomonitoring using bivalves. *Chemosphere*, *219*, 695-703.

Bour, A., Haarr, A., Keiter, S., & Hylland, K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. *Environmental pollution*, *236*, 652-660.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Brown, D. M., Wilson, M. R., MacNee, W., Stone, V., & Donaldson, K. (2001). Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and applied pharmacology*, *175*(3), 191-199.

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: sources and sinks. *Environmental science & technology*, *45*(21), 9175-9179.

Cabecinhas, A. S., Novais, S. C., Santos, S. C., Rodrigues, A. C., Pestana, J. L., Soares, A. M., & Lemos, M. F. (2015). Sensitivity of the sea snail Gibbula umbilicalis to mercury exposure–Linking endpoints from different biological organization levels. *Chemosphere*, *119*, 490-497.

Carocci A., Rovito N., Sinicropi M.S., Genchi G. (2014) Mercury Toxicity and Neurodegenerative Effects. In: Whitacre D. (eds) Reviews of Environmental Contamination and Toxicology. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), vol 229, 1-18.

Chaijan, M. (2011). Physicochemical changes of tilapia (Oreochromis niloticus) muscle during salting. *Food Chemistry*, *129*(3), 1201-1210.

Chaijan, M., Panpipat, W., & Nisoa, M. (2017). Chemical deterioration and discoloration of semi-dried tilapia processed by sun drying and microwave drying. *Drying Technology*, *35*(5), 642-649.

Chakraborty, P., Jayachandran, S., Lekshmy, J., Padalkar, P., Sitlhou, L., Chennuri, K., Shetye, S., Sardar, A., & Khandeparker, R. (2019). Seawater intrusion and resuspension of surface sediment

control mercury (Hg) distribution and its bioavailability in water column of a monsoonal estuarine system. *Science of The Total Environment*, *660*, 1441-1448.

Cherkasov, A. S., Ringwood, A. H., & Sokolova, I. M. (2006). Combined effects of temperature acclimation and cadmium exposure on mitochondrial function in eastern oysters Crassostrea virginica Gmelin (Bivalvia: Ostreidae). *Environmental Toxicology and Chemistry: An International Journal*, *25*(9), 2461-2469.

Cheung, P. K., Cheung, L. T. O., & Fok, L. (2016). Seasonal variation in the abundance of marine plastic debris in the estuary of a subtropical macro-scale drainage basin in South China. *Science of the Total Environment*, *562*, 658-665.

Chowdhury, R., Favas, P. J., Pratas, J., Jonathan, M. P., Ganesh, P. S., & Sarkar, S. K. (2015). Accumulation of trace metals by mangrove plants in Indian Sundarban Wetland: prospects for phytoremediation. *International journal of phytoremediation*, *17*(9), 885-894.

Ciutat, A., & Boudou, A. (2003). Bioturbation effects on cadmium and zinc transfers from a contaminated sediment and on metal bioavailability to benthic bivalves. *Environmental Toxicology and Chemistry: An International Journal*, *22*(7), 1574-1581.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B., & Janssen, C. R. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine pollution bulletin*, *70*(1-2), 227-233.

Costa, S., Lopes, J., Coppola, F., Correia, S., Henriques, B., Leite, C., Soares, A. M., Zengjie, J., Pereira, E., Chiesa, S., & Freitas, R. (2020). Bioaccumulation and biochemical patterns of Ruditapes philippinarum clams: Responses to seasonality and low contamination levels. *Estuarine, Coastal and Shelf Science*, *243*, 106883.

Costley, C. T., Mossop, K. F., Dean, J. R., Garden, L. M., Marshall, J., & Carroll, J. (2000). Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. *Analytica Chimica Acta*, *405*(1-2), 179-183.

Cranford, P. J., Dowd, M., & Grant, J. (2003). Ecosystem level effects of marine bivalve aquaculture. *Canadian Technical Reports of Fisheries and Aquatic Sciences, 2450*(1), 51-96.

Cruz-Romero, M. C., Kerry, J. P., & Kelly, A. L. (2008). Fatty acids, volatile compounds and colour changes in high-pressure-treated oysters (Crassostrea gigas). *Innovative Food Science & Emerging Technologies*, *9*(1), 54-61.

De Brauw, A. (2015). Gender, control, and crop choice in northern Mozambique. *Agricultural Economics (United Kingdom)*, *46*(3), 435-448.

De Coen, W., & Janssen, C.R., (1997). The use of biomarkers in Daphnia magna toxicity testing. IV.Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. *Journal of Aquatic Ecosystem Stress and Recovery, 6*, 43–55.

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, 223-233.

Ding, J., Li, J., Sun, C., Jiang, F., He, C., Zhang, M., Ju, P., & Ding, N. X. (2020). An examination of the occurrence and potential risks of microplastics across various shellfish. *Science of The Total Environment*, *739*, 139887.

Dittmar, T., Lara, R. J., & Kattner, G. (2001). River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Marine Chemistry*, *73*(3-4), 253-271.

Donovan, T. J., & Van Netten, P. (1995). Culture media for the isolation and enumeration of pathogenic Vibrio species in foods and environmental samples. *Progress in industrial microbiology*, *34*, 203-217.

Dridi, S., Romdhane, M. S., & Elcafsi, M. H. (2007). Seasonal variation in weight and biochemical composition of the Pacific oyster, Crassostrea gigas in relation to the gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia. *Aquaculture*, *263*(1-4), 238-248.

Duarte, C. M., Marbà, N., Gacia, E., Fourqurean, J. W., Beggins, J., Barrón, C., & Apostolaki, E. T. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles*, *24*(4), GB4032.

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *Efsa Journal*, *14*(6), e04501.

Elrais, A., Hassanien, F., Hassan, M. A., Latif, H. M., & El-Zahaby, D. (2018). Detection of some Heavy metals in fresh and salted fish. *Benha Veterinary Medical Journal*, *35*(2), 169-177.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off J Eur Union*, *364*, 365–324.

Fernando, S. M.C., Murama, J. P., & Pires, P. L. (2012). Estudo da biodiversidade de recursos pesqueiros acessíveis à pesca artesanal nas zonas entre marés na província de Cabo Delgado. Instituto Nacional de Investigação Costeira- Boletim de Divulgação n52. Maputo. 27pp.

Gaertner, H., Hamad, A. A., & Walz, J. R. (2020). The Community Influence of Sponge and Coral Aquaculture in Zanzibar. *DU Undergraduate Research Journal Archive*, *1*(2), 1.

Gallardi, D. (2014). Effects of bivalve aquaculture on the environment and their possible mitigation: a review. *Fisheries and Aquaculture Journal*, *5*(3).

Gentry, R. R., Alleway, H. K., Bishop, M. J., Gillies, C. L., Waters, T., & Jones, R. (2020). Exploring the potential for marine aquaculture to contribute to ecosystem services. *Reviews in Aquaculture, 12*(2), 499-512.

Gnaiger, E. (1983). Calculation of Energetic and Biochemical Equivalents of Respiratory Oxygen

Consumption, in: Gnaiger, E., Forstner, H. (Eds.), Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 337–345.

Goulas, A. E. (2008). Combined effect of chill storage and modified atmosphere packaging on mussels (Mytilus galloprovincialis) preservation. *Packaging Technology and Science: An International Journal*, *21*(5), 247-255.

Gündoğdu, S. (2018). Contamination of table salts from Turkey with microplastics. *Food Additives & Contaminants: Part A*, *35*(5), 1006-1014.

Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., & Purnell, P. (2018). An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *Journal of hazardous materials*, *344*, 179-199.

Hartnoll, R. G. (1976). The ecology of some rocky shores in tropical East Africa. *Estuarine and Coastal Marine Science*, *4*(1), 1-21.

Hendy, I. W., Michie, L., & Taylor, B. W. (2014). Habitat creation and biodiversity maintenance in mangrove forests: teredinid bivalves as ecosystem engineers. *PeerJ*, *2*, e591.

Horner, W. F. A. (1997). Preservation of fish by curing (drying, salting and smoking). In *Fish processing technology* (pp. 32-73). Springer, Boston, MA.

Horvat, M., Kotnik, J., Logar, M., Fajon, V., Zvonarić, T., & Pirrone, N. (2003). Speciation of mercury in surface and deep-sea waters in the Mediterranean Sea. *Atmospheric environment*, *37*, 93-108.

INE, (2020a). *Projecções anuais da população total, urbana e rural, dos distritos da província de Cabo Delgado – 2017-2050*. Maputo: INE.

INE, (2020b). *IV Recenseamento Geral da População e Habitação, 2017: Resultados definitivos.* Maputo: INE.

INE, (2020c). *IV Recenseamento Geral da População e Habitação, 2017: Resultados definitivos*. *Província de Cabo Delgado*. Maputo: INE.

INE, (2021). *Quadro 51. Habitações particulares, agregados familiares e seus membros por tipo de habitação, segundo área de residência e condições básicas existentes. Província de Cabo Delgado, 2017.* Maputo: INE.

Inkielewicz-Stepniak, I., Tajber, L., Behan, G., Zhang, H., Radomski, M. W., Medina, C., & Santos-Martinez, M. J. (2018). The role of mucin in the toxicological impact of polystyrene nanoparticles. *Materials*, *11*(5), 724.

Jaffé, R., Boyer, J. N., Lu, X., Maie, N., Yang, C., Scully, N. M., & Mock, S. (2004). Source characterization of dissolved organic matter in a subtropical mangrove-dominated estuary by fluorescence analysis. *Marine Chemistry*, *84*(3-4), 195-210.

Jana, H., Mondal, K. C., Maity, C., Ghosh, K., Mitra, A., Banerjee, K., Dey, S., & Pati, B. R. (2013). Variation of antioxidant biomarkers in the edible oyster Saccostrea cucullata collected from three different water bodies of Sundarbans. *Chemistry and Ecology*, *29*(8), 745-753.

Janssens, L., & Stoks, R. (2013). Predation risk causes oxidative damage in prey. *Biology Letters*, *9*(4), 20130350.

Jeevanaraj, P., Foat, A. A., Tholib, H., & Ahmad, N. I. (2020). Heavy metal contamination in processed seafood and the associated health risk for Malaysian women. *British Food Journal*, *122*(10), 3099-3114.

Kalyanasundaram, M., & Ramamoorthi, K. (1987). Larval development of the oyster Saccostrea cucullata (Born). *Mahasagar*, *20*(1), 53-58.

Karami, A., Golieskardi, A., Choo, C. K., Larat, V., Galloway, T. S., & Salamatinia, B. (2017). The presence of microplastics in commercial salts from different countries. *Scientific Reports*, *7*(1), 1-11.

Karami, A., Golieskardi, A., Choo, C. K., Romano, N., Ho, Y. B., & Salamatinia, B. (2017). A high-performance protocol for extraction of microplastics in fish. *Science of the Total Environment*, *578*, 485-494.

Kellogg, M. L., Cornwell, J. C., Owens, M. S., & Paynter, K. T. (2013). Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series*, *480*, 1-19.

Knowles, T. G., Farrington, D., & Kestin, S. C. (2003). Mercury in UK imported fish and shellfish and UK-farmed fish and their products. *Food Additives & Contaminants*, *20*(9), 813-818.

Kong, F., Oliveira, A., Tang, J., Rasco, B., & Crapo, C. (2008). Salt effect on heat-induced physical and chemical changes of salmon fillet (O. gorbuscha). *Food Chemistry*, *106*(3), 957-966.

Kühn, S., Van Werven, B., Van Oyen, A., Meijboom, A., Rebolledo, E. L. B., & Van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, *115*(1-2), 86-90.

Kyriazi-Papadopoulou, A., Vareltzis, K., Bloukas, J. G., & Georgakis, S. (2003). Effect of Smoking on Quality Characteristics and Shelf Life of Mediterranean Mussel (Mytilus galloprovincialis) Meat under Vacuum in Chilled Storage. *Italian journal of food science*, *15*(3), 371-382.

Lasiak, T. (1986). The reproductive cycles of the intertidal bivalves Crassostrea cucullata (Born, 1778) and Perna perna (Linnaeus, 1758) from the Transkei Coast, southern Africa. *The Veliger*, *29*(2), 226-230.

Laurier, F. J. G., Mason, R. P., Gill, G. A., & Whalin, L. (2004). Mercury distributions in the North Pacific Ocean—20 years of observations. *Marine Chemistry*, *90*(1-4), 3-19.

Lebreton, L. C., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nature communications*, *8*(1), 1-10.

Lee, H., Kunz, A., Shim, W. J., & Walther, B. A. (2019). Microplastic contamination of table salts from Taiwan, including a global review. *Scientific reports*, *9*(1), 1-9.

Li, J., Yang, D., Li, L., Jabeen, K., & Shi, H. (2015). Microplastics in commercial bivalves from China. *Environmental pollution*, *207*, 190-195.

Li, H. X., Ma, L. S., Lin, L., Ni, Z. X., Xu, X. R., Shi, H. H., Yan, Y., Zheng, G.M., & Rittschof, D. (2018). Microplastics in oysters Saccostrea cucullata along the Pearl River estuary, China. *Environmental pollution*, *236*, 619-625.

Li, S., & Zhang, Q. (2010). Risk assessment and seasonal variations of dissolved trace elements and heavy metals in the Upper Han River, China. *Journal of Hazardous Materials*, *181*(1-3), 1051-1058.

Lima, A. R. A., Costa, M. F., & Barletta, M. (2014). Distribution patterns of microplastics within the plankton of a tropical estuary. *Environmental Research*, *132*, 146-155.

Lipp, E. K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. R., & Rose, J. B. (2001). The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries, 24*(2), 266-276.

Lodeiros, C. J., Rengel, J. J., Guderley, H. E., Nusetti, O., & Himmelman, J. H. (2001). Biochemical composition and energy allocation in the tropical scallop Lyropecten (Nodipecten) nodosus during the months leading up to and following the development of gonads. *Aquaculture*, *199*(1-2), 63-72.

Lusambili, A., Naanyu, V., Manda, G., Mossman, L., Wisofschi, S., Pell, R., Jadavji, S., Obure, J., & Temmerman, M. (2020). Nutritional influences on the health of women and children in Cabo Delgado, Mozambique: a qualitative study. *International Journal of Environmental Research and Public Health*, *17*(17), 1-27.

Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2017). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods*, *9*(9), 1346-1360.

Machiwa, J. F. (2010). Coastal Marine Pollution in Dar es Salaam (Tanzania) relative to Recommended Environmental Quality Targets for the Western Indian Ocean. *Western Indian Ocean Journal of Marine Science*, *9*(1), 17-30.

Mahadevan, G., & Valiyaveettil, S. (2021). Understanding the interactions of poly (methyl methacrylate) and poly (vinyl chloride) nanoparticles with BHK-21 cell line. *Scientific Reports*, *11*(1), 1-15.

Mahler, G. J., Esch, M. B., Tako, E., Southard, T. L., Archer, S. D., Glahn, R. P., & Shuler, M. L. (2012). Oral exposure to polystyrene nanoparticles affects iron absorption. *Nature nanotechnology*, *7*(4), 264.

Mani, T., Frehland, S., Kalberer, A., & Burkhardt-Holm, P. (2019). Using castor oil to separate microplastics from four different environmental matrices. *Analytical methods*, *11*(13), 1788-1794.

Masette, M., & Kwetegyeka, J. (2013). The effect of artisanal preservation methods on nutritional security of “Mukene” Rastrineobola argentea caught from Lakes Victoria and Kyoga in Uganda. *Uganda Journal of Agricultural Sciences*, *14*(2), 95-107.

Masse, L., Kennedy, K. J., & Chou, S. (2001). Testing of alkaline and enzymatic hydrolysis pretreatments for fat particles in slaughterhouse wastewater. *Bioresource Technology*, *77*(2), 145-155.

Mayoma, B. S., Sørensen, C., Shashoua, Y., & Khan, F. R. (2020). Microplastics in beach sediments and cockles (Anadara antiquata) along the Tanzanian coastline. *Bulletin of Environmental Contamination and Toxicology*, *105*(4), 513-521.

McClanahan, T. R. (1988). Seasonality in East Africa's coastal waters. *Marine Ecology Progress Series, 44,* 191-199*.*

Mitra, A., Basu, S., & Banerjee, K. (2008). Seasonal variation in biochemical composition of edible oyster (Saccostrea cucullata) from Indian Sundarbans. *Fishery Technology*, *45*(2), 209-216.

Mohammadi, M., Bakhtiari, A. R., Sary, A. E., Bani, A. (2012). Biomonitoring Total Mercury in the Persian Gulf Using the Rock Oyster (Saccostrea cucullata). *Caspian Journal of Environmental Sciences, 10*(2), 145-155.

Mohan, D., & Kalyani, M. (1989). Seasonal variations in biochemical composition of green mussel Perna viridis (Linnaeus). *Mahasagar*, *22*(3), 113-120.

Moore, C. J., Moore, S. L., Weisberg, S. B., Lattin, G. L., & Zellers, A. F. (2002). A comparison of neustonic plastic and zooplankton abundance in southern California’s coastal waters. *Marine pollution bulletin*, *44*(10), 1035-1038.

Moreira, I. T., Oliveira, O. M., Triguis, J. A., dos Santos, A. M., Queiroz, A. F., Martins, C. M., Silva, C. S., & Jesus, R. S. (2011). Phytoremediation using Rizophora mangle L. in mangrove sediments contaminated by persistent total petroleum hydrocarbons (TPH's). *Microchemical Journal*, *99*(2), 376-382.

Mtanga, A., & Machiwa, J. F. (2007). Heavy metal pollution levels in water and oysters, *Saccostrea cucullata*, from Mzinga Creek and Ras Dege mangrove ecosystems, Tanzania. *African Journal of Aquatic Science*, *32*(3), 235-244.

Munno, K., Helm, P. A., Jackson, D. A., Rochman, C., & Sims, A. (2018). Impacts of temperature and selected chemical digestion methods on microplastic particles. *Environmental toxicology and chemistry*, *37*(1), 91-98.

Naimo, T. J. (1995). A review of the effects of heavy metals on freshwater mussels. *Ecotoxicology, 4*(6), 341-362.

Newell, R. I. (2004). Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish research*, *23*(1), 51-62.

Newell, R. I., & Koch, E. W. (2004). Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. *Estuaries*, *27*(5), 793-806.

Newman, M. C., & McIntosh, A. W. (1982). The influence of lead in components of a freshwater ecosystem on molluscan tissue lead concentrations. *Aquatic Toxicology*, *2*(1), 1-19.

Norma Portuguesa (NP) 3005. (1985). Microbiologia Alimentar. Preparação de diluições para análise microbiológica. Instituto Português da Qualidade.

Norma Portuguesa (NP) 3006. (1985). Microbiologia Alimentar. Pescado. Preparação da amostra para análise microbiológica. Instituto Português da Qualidade.

Ofulla, A. V., Onyuka, J. H., Wagai, S., Anyona, D., Dida, G. O., & Gichuki, J. (2011). Comparison of Different Techniques for Processing and Preserving fish Rastrineobola argentea from Lake Victoria, Kenya. *World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *5*(12), 906-910.

Oliveira, P., Barboza, L. G. A., Branco, V., Figueiredo, N., Carvalho, C., & Guilhermino, L. (2018). Effects of microplastics and mercury in the freshwater bivalve Corbicula fluminea (Müller, 1774): Filtration rate, biochemical biomarkers and mercury bioconcentration. *Ecotoxicology and environmental safety*, *164*, 155-163.

Olivier, A. S., Jones, L., Vay, L. L., Christie, M., Wilson, J., & Malham, S. K. (2020). A global review of the ecosystem services provided by bivalve aquaculture. *Reviews in Aquaculture*, *12*(1), 3-25.

Oni, B. A., Ayeni, A. O., Agboola, O., Oguntade, T., & Obanla, O. (2020). Comparing microplastics contaminants in (dry and raining) seasons for Ox-Bow Lake in Yenagoa, Nigeria. *Ecotoxicology and environmental safety*, *198*, 110656.

Otchere, F. A., Joiris, C. R., & Holsbeek, L. (2003). Mercury in the bivalves Anadara (Senilia) senilis, Perna perna and Crassostrea tulipa from Ghana. *Science of the total environment*, *304*(1-3), 369-375.

Ozawa, H. (2001). Reproductive cycle and spawning of Modiolus philippinarum (Bivalvia: Mytilidae) in a seagrass bed in Kin Bay, Okinawa Island, Southern Japan. *Venus (Journal of the Malacological Society of Japan)*, *60*(3), 173-181.

Park, J. D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of preventive medicine and public health*, *45*(6), 344-352.

Patra, P., Mohandass, C., & Chakraborty, P. (2019). Snapshot of environmental condition in different tropical estuarine systems by using S. cucullata (an edible oyster) as bio-indicator. *Environmental Science and Pollution Research*, *26*(11), 11342-11354.

Patterson, J. (2004). Smoking–an ideal method to preserve mollusc meat. *SPC Trochus Information Bulletin*, *11*, 8-10.

Paul, P., Siva, J., Priya, A., & Stella, C. (2021). Biochemical Studies on edible oysters Crassostrea madrasensis and Saccostrea cucullata. *Sustainability, Agri, Food and Environmental Research*, *9*(3), 347-368.

Peixoto, D., Pinheiro, C., Amorim, J., Oliva-Teles, L., Guilhermino, L., & Vieira, M. N. (2019). Microplastic pollution in commercial salt for human consumption: A review. *Estuarine, Coastal and Shelf Science*, *219*, 161-168.

Pemba Climate: Temperature, Climograph and Climate Table for Pemba – Climate-Data.org. https://en.climate-data.org/africa/mozambique/cabo-delgado/pemba-3995/. Accessed: 10-05-2021

Pittia, P., & Antonello, P. (2016). Safety by control of water activity: Drying, smoking, and salt or sugar addition. In *Regulating safety of traditional and ethnic foods* (pp. 7-28). Academic Press.

Poirier, L. A., Clements, J. C., Davidson, J. D., Miron, G., Davidson, J., & Comeau, L. A. (2019). Sink before you settle: Settlement behaviour of Eastern oyster (Crassostrea virginica) larvae on artificial spat collectors and natural substrate. *Aquaculture Reports*, *13*, 100181.

Prato, E., Biandolino, F., Parlapiano, I., Giandomenico, S., Denti, G., Calò, M., Spada, L., & Di Leo, A. (2019). Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health. *Science of the total environment*, *648*, 153-163.

Qiu, X., Chen, S., & Lin, H. (2019). Oxidative stability of dried seafood products during processing and storage: A review. *Journal of Aquatic Food Product Technology*, *28*(3), 329-340.

Quayle, D. B. (1980). *Tropical oysters: culture and methods*. IDRC, Ottawa, ON, CA.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.r-project.org

Rajagopal, S., Van der Velde, G., Nair, K. V. K., & Jenner, H. A. (1999). Response of the tropical mussel Modiolus philippinarum (Hanley) to heat treatment: An experimental study for antifouling application. *Marine & Freshwater Behaviour & Phy*, *32*(4), 239-253.

Rajeshkumar, S., Liu, Y., Zhang, X., Ravikumar, B., Bai, G., & Li, X. (2018). Studies on seasonal pollution of heavy metals in water, sediment, fish and oyster from the Meiliang Bay of Taihu Lake in China. *Chemosphere, 191*, 626-638.

Rao, K. V., & Nayar, K. N. (1956). Rate of growth in spat and yearlings of the Indian backwater oyster Ostrea madrasensis Preston. *Indian Journal of Fisheries*, *3*(2), 231-260.

Rezaei, M., Kafaei, R., Mahmoodi, M., Sanati, A. M., Vakilabadi, D. R., Arfaeinia, H., Dobaradaran, S., Sorial, G. A., Ramavandi, B., & Boffito, D. C. (2021). Heavy metals concentration in mangrove tissues and associated sediments and seawater from the north coast of Persian Gulf, Iran: Ecological and health risk assessment. *Environmental Nanotechnology, Monitoring & Management*, *15*, 100456.

Rhee, K. S. (1999). Storage stability of meat products as affected by organic and inorganic additives and functional ingredients. In *Quality attributes of muscle foods* (pp. 95-113). Springer, Boston, MA.

Rice, M.A. (2001) Environmental impacts of shellfish aquaculture: Filter feeding to control eutrophication. In Tlusty, M., Bengtson, D., Halvorson, H.O., Oktay, S., Pearce, J., & Rheualt, R. (Eds.). *Marine aquaculture and the marine environment: A meeting for the stakeholders in the northeast.* pp. 77-86. Held Jan. 11-13, 2001 at the University of Massachusetts, Boston. Cape Cod Press, Falmouth MA.

Richardson, P. L., & Reverdin, G. (1987). Seasonal cycle of velocity in the Atlantic North Equatorial Countercurrent as measured by surface drifters, current meters, and ship drifts. *Journal of Geophysical Research: Oceans*, *92*(C4), 3691-3708.

Ríos-Touma, B., Encalada, A. C., & Prat Fornells, N. (2011). Macroinvertebrate assemblages of an Andean high-altitude tropical stream: The importance of season and flow. *International review of hydrobiology*, *96*(6), 667-685.

Rodrigues, A.C.M., Gravato, C., Quintaneiro, C., Golovko, O., Žlábek, V., Barata, C., Soares, A.M.V.M., Pestana, J.L.T. (2015). Life history and biochemical effects of chlorantraniliprole on Chironomus riparius. *Science of the Total Environment, 508*, 506–513.

Rosendo, S., Pinto, A., Andriamalala, G., Huet, J. (eds) (2020). Toolkit for LMMA establishment: A case study of Our Sea Our Life's approach to community-based marine conservation in northern Mozambique. London: Zoological Society of London and Pemba: Associação do Meio Ambiente.

Rrokaj, S., & Corti, B. (2019). *Flood risk assessment and mitigation for Rio Maguide in Cabo Delgado, Mozambique*. https://www.politesi.polimi.it/handle/10589/151590

Sajwan, K. S., Kumar, K. S., Paramasivam, S., Compton, S. S., & Richardson, J. P. (2008). Elemental status in sediment and American oyster collected from Savannah marsh/estuarine ecosystem: A preliminary assessment. *Archives of environmental contamination and toxicology, 54*(2), 245-258.

Saniewska, D., Bełdowska, M., Bełdowski, J., Saniewski, M., Szubska, M., Romanowski, A., & Falkowska, L. (2014). The impact of land use and season on the riverine transport of mercury into the marine coastal zone. *Environmental monitoring and assessment*, *186*(11), 7593-7604.

Sasikumar, G., & Krishnakumar, P. K. (2011). Aquaculture planning for suspended bivalve farming systems: The integration of physiological response of green mussel with environmental variability in site selection. *Ecological indicators*, *11*(2), 734-740.

Savazzi, E. (1989). Shell torsion and life habit in the Recent mytilid bivalve Modiolus philippinarum. *Palaeogeography, palaeoclimatology, palaeoecology*, *72*, 277-282.

Saxby, S. A. (2002). *A review of food availability, sea water characteristics and bivalve growth performance at coastal culture sites in temperate and warm temperate regions of the world*. Fisheries Research Report, No. 132*,* 42 pp.

Seth, C. K., & Shriwastav, A. (2018). Contamination of Indian sea salts with microplastics and a potential prevention strategy. *Environmental Science and Pollution Research*, *25*(30), 30122-30131.

Shimizu, Y., Kiriake, S., Ohtubo, S., & Sakai, T. (2009). Effect of NaCl on protein and lipid oxidation in frozen yellowtail meat. *Bioscience, biotechnology, and biochemistry*, *73*(4), 923-925.

Sievers, M., Dempster, T., Fitridge, I., & Keough, M. J. (2014). Monitoring biofouling communities could reduce impacts to mussel aquaculture by allowing synchronisation of husbandry techniques with peaks in settlement. *Biofouling, 30*(2), 203-212.

Singh, Y. T. (2019). Biometrics, condition index and meat yield of edible rock oyster, Saccostrea cucullata (Born, 1778). *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom*, *99*(2), 385-392.

Smid, E. J., & Gorris, L. G. (2007). Natural Antimicrobials for Food Preservation In: Rahman, M. S. (Ed.), Handbook of food preservation. CRC press, Boca Raton, Florida, pp. 237-258.

Souto, A. N. (2015). O lado humano do Oceano Indico: As comunidades de pescadores artesanais em Cabo Delgado. In: Silva, T. C., Araújo, M. G. M., Souto, A. N. (Orgs.), Comunidades Costeiras: Perspectivas e Realidades. CESAB, Maputo, pp. 133-190.

Stark, K. D. (2008). The percentage of n-3 highly unsaturated fatty acids in total HUFA as a biomarker for omega-3 fatty acid status in tissues. *Lipids*, *43*(1), 45-53.

Strungaru, S. A., Jijie, R., Nicoara, M., Plavan, G., & Faggio, C. (2019). Micro-(nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology. *TrAC Trends in Analytical Chemistry*, *110*, 116-128.

Thiele, C. J., Hudson, M. D., & Russell, A. E. (2019). Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine pollution bulletin*, *142*, 384-393.

Topić Popović, N., Beer Ljubić, B., Strunjak-Perović, I., Babić, S., Lorencin, V., Jadan, M., Čižmek, L., Matulić, D., Bojanić, K., & Čož-Rakovac, R. (2020). Seasonal antioxidant and biochemical properties of the Northern Adriatic Pecten jacobaeus. *Plos one*, *15*(3), e0230539.

Turan, H., SÖNMEZ, G., ÇELİK, M. Y., YalCIn, M., & Kaya, Y. (2007). Effects of different salting process on the storage quality of mediterranean mussel (Mytilus Galloprovincialis L. 1819). *Journal of Muscle Foods*, *18*(4), 380-390.

Turan, H., SÖNMEZ, G., ÇELİK, M. Y., YalCIn, M., & Kaya, Y. (2008). The effects of hot smoking on the chemical composition and shelf life of mediterranean mussel (Mytilus galloprovincialis L. 1819) under chilled storage. *Journal of food processing and preservation*, *32*(6), 912-922.

Umayaparvathi, S., Arumugam, M., Meenakshi, S., & Balasubramanian, T. (2015). Antioxidant properties of protein hydrolysate obtained from oyster Saccostrea cucullata (Born, 1778). *Journal of Aquatic Food Product Technology*, *24*(5), 502-515.

Undeland, I., Kelleher, S. D., & Hultin, H. O. (2002). Recovery of functional proteins from herring (Clupea harengus) light muscle by an acid or alkaline solubilization process. *Journal of agricultural and food chemistry*, *50*(25), 7371-7379.

Van Someren, V. D., & Whitehead, P. J. (1961). An investigation of the biology and culture of an East African oyster Crassostrea cucullata. *Colonial Office Fishery Publications, 14,* 1-41.

Viarengo, A., Canesi, L., Pertica, M., & Livingstone, D. R. (1991). Seasonal variations in the antioxidant defence systems and lipid peroxidation of the digestive gland of mussels. *Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology*, *100*(1-2), 187-190.

Vidal, N. P., Goicoechea, E., Manzanos, M. J., & Guillén, M. D. (2015). 1H NMR study of the changes in brine-and dry-salted sea bass lipids under thermo-oxidative conditions: Both salting methods reduce oxidative stability. *European Journal of Lipid Science and Technology*, *117*(4), 440-449.

Vieira, H. C., Morgado, F., Soares, A. M. V. M., & Abreu, S. N. (2015). Real and potential mercury accumulation in human scalp of adolescents: a case study. *Biological trace element research*, *163*(1-2), 19-27.

Visbeck, M., & Schott, F. (1992). Analysis of seasonal current variations in the western equatorial Indian Ocean: Direct measurements and GFDL model comparison. *Journal of physical oceanography*, *22*(10), 1112-1128.

Wanyonyi, I., Crona, B., & Rosendo, S. (2011) *Migrant fishers and fishing in the Western Indian Ocean: Socio-economic dynamics and implications for management.* Final Report of Commissioned Research Project MASMA/CR/2008/02. 159 pp

Wheatley, B., Barbeau, A., Clarkson, T. W., & Lapham, L. W. (1979). Methylmercury poisoning in Canadian Indians—the elusive diagnosis. *Canadian Journal of Neurological Sciences*, *6*(4), 417-422.

Widdows, J. (1978). Combined effects of body size, food concentration and Season on physiology of Mytilus edulis. *Journal of the Marine Biological Association of the United Kingdom*, *58*(1), 109-124.

Wilson, B. R. (1969). Survival and reproduction of the mussel Xenostrobus securis (Lamarck)(Mollusca; Bivalvia; Mytilidae) in a Western Australian estuary. Pt. II: reproduction, growth and longevity. *Journal of natural History*, *3*(1), 93-120.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, *178*, 483-492.

Zhang, Q., Shi, X., Huang, B., Yu, D., Öborn, I., Blombäck, K., Wang, H., Pagella, T. F., & Sinclair, F. L. (2007). Surface water quality of factory-based and vegetable-based peri-urban areas in the Yangtze River Delta region, China. *Catena*, *69*(1), 57-64.

Zhao, L., Yang, F., & Yan, X. (2013). Concentrations of selected trace elements in marine bivalves and the dietary risk to residents of Dalian City, Northern China. *Human and Ecological Risk Assessment: An International Journal*, *19*(1), 145-150.

7. Supplemental material

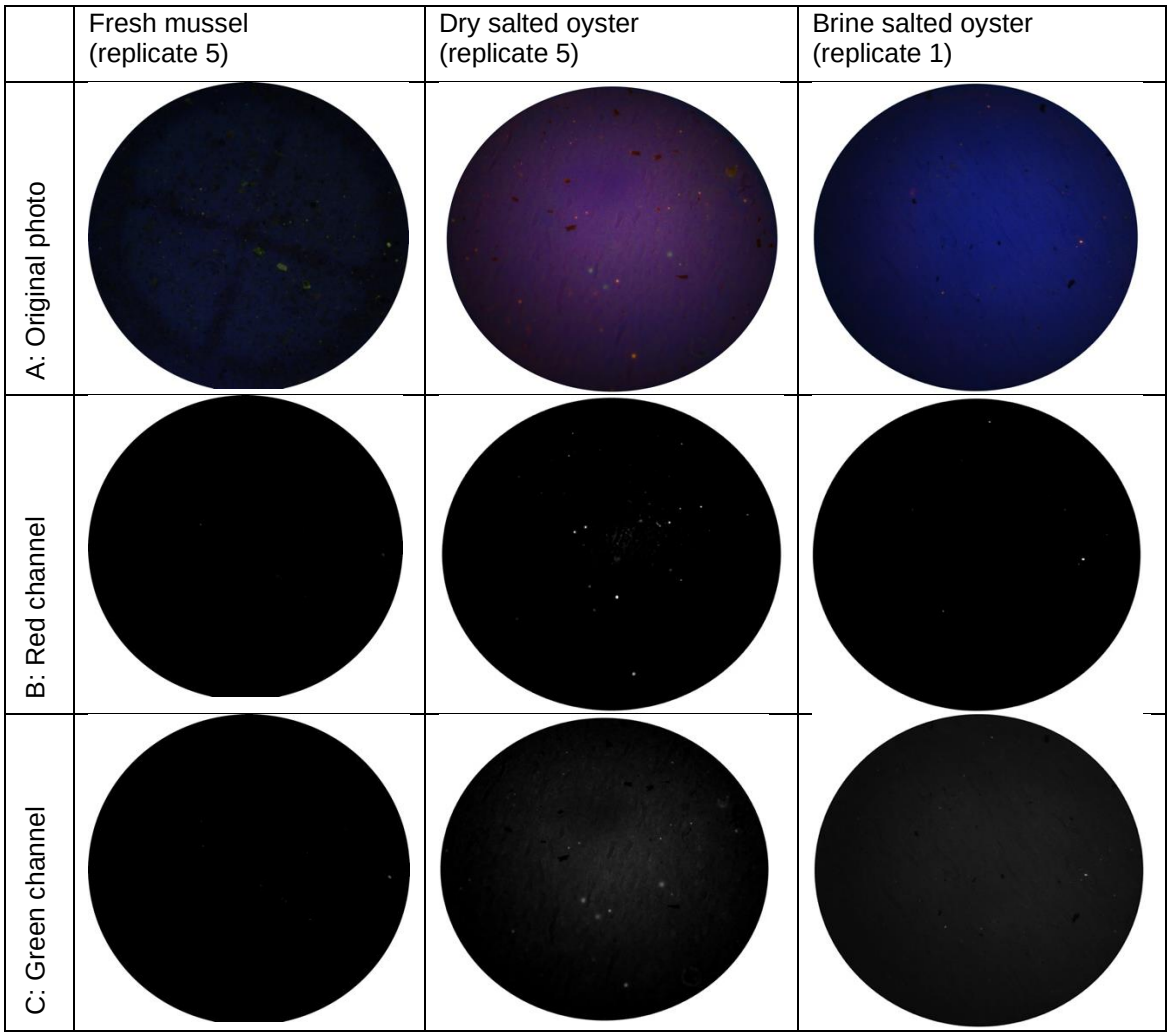

Fig. S1: The first row of images refers to the original photos on the glass-fibre filter containing the digestates of representative samples stained with Nile Red (fresh mussel – replicate 5, dry salted oyster– replicate 5, and brine salted oyster– replicate 1). The second and third rows refer to the same images treated with red and green channels on Image J software, to facilitate the microplastics counting. The choice of the channel used for microplastics quantification was based on the fluorescence emitted by the particles (also considering the contrast with the background emitted by poorly-digested organic matter).

Table S1. Results of independent-samples t-test (TT) and Mann-Whitney non-parametric test (MW) between dry and wet season of oysters and mussels on different variables. Significant results are in bold.

Variable	Test	Test statistic	*p-value*	Pattern
Mercury oysters	TT	3.660	**0.002**	Dry season < Wet season
Mercury mussels	MW	11.000	0.099	-
Microplastics oysters	MW	17.000	**0.040**	Dry season < Wet season
Microplastics mussels	TT	1.563	0.191	-
LPO oysters	TT	-3.065	**0.012**	Wet season < Dry season
LPO mussels	TT	5.536	**0.037**	Dry season < Wet season
Lipids oysters	TT	-8.377	**<0.001**	Wet season < Dry season
Lipids mussels	TT	-5.247	**<0.001**	Wet season < Dry season
Sugars oysters	TT	-5.194	**<0.001**	Wet season < Dry season
Sugars mussels	TT	-3.799	**0.002**	Wet season < Dry season
Proteins oysters	TT	-2.981	**0.008**	Wet season < Dry season
Proteins mussels	TT	-3.209	**0.008**	Wet season < Dry season

Table S2. Influence of each studied variable in PC1 and PC2 regarding oysters PCA aiming to verify which variable contributes the most to data segregation between dry and wet season.

	PC1	PC2
Mercury	0.4323228	0.3331995
Microplastics	0.3025440	-0.5878644
LPO	-0.3656057	-0.3025626
Lipids	-0.4834307	0.2723503
Sugars	-0.4543563	0.3220739
Proteins	-0.3843848	-0.5233957

Table S3. Influence of each studied variable in PC1 and PC2 regarding mussels PCA aiming to verify which variable contributes the most to data segregation between dry and wet season.

	PC1	PC2
Mercury	0.1484189	0.62362155
Microplastics	0.2370488	-0.71101021
LPO	0.5236612	0.11522419
Lipids	-0.5205652	-0.03968438
Sugars	-0.4697846	-0.18744941
Proteins	-0.3948075	0.23573693

Table S4. Results of Kruskal-Wallis non-parametric test (KW) and one-way analysis of variance (ANOVA) among mercury and microplastics contamination and oxidative damage and energy-related biomarkers of fresh oysters and mussels and organisms preserved by different methods and respective multiple comparison patterns. Significant results are in bold. Fresh (FR), Brine salting (BS), Dry salting (DS), Sun-drying (SD), Smoking (S) and Smoking with oil (SO).

Variable	Organism	Comparison	Test	Transformation	Test statistic	*p-value*	Multiple comparison test	Multiple comparison pattern
Mercury	Oyster	Fresh and preserved	KW	-	26.182	**0.000**	Dunn-Bonferroni	FR<BS=DS<SO=S<SD
Mercury	Mussel	Fresh and preserved	ANOVA	Ln	63.522	**0.000**	SNK	FR=BS<DS=S<SO<SD
Microplastics	Oyster	Fresh and preserved	KW	-	17.267	**0.004**	Dunn-Bonferroni	FR<BS=SO=DS=S=SD
Microplastics	Mussel	Fresh and preserved	ANOVA	Sqrt	0.476	0.791	-	-
LPO	Oyster	Fresh vs. t3	ANOVA	Sqrt	37.583	**0.000**	Dunnett	TabS5[1]
		Fresh vs. t15	ANOVA	Sqrt	48.790	**0.000**	Dunnett	TabS5
		t3	ANOVA	Sqrt	24.738	**0.000**	SNK	BS=S<S=DS<SO=SD
		t15	ANOVA	-	20.543	**0.000**	SNK	BS=DS<S=SO<SD
	Mussel	Fresh vs. t3	ANOVA	-	55.530	**0.000**	Dunnett	TabS5
		Fresh vs. t15	ANOVA	Sqrt	85.433	**0.000**	Dunnett	TabS5
		t3	ANOVA	-	18.957	**0.000**	SNK	BS=DS=S=SO<SD
		t15	ANOVA	Sqrt	45.047	**0.000**	SNK	BS<DS=S<SD=SO
Lipids	Oyster	Fresh vs. t3	ANOVA	Sqrt	38.237	**0.000**	Dunnett	TabS5
		Fresh vs. t15	ANOVA	Sqrt	27.539	**0.000**	Dunnett	TabS5
		t3	ANOVA	Sqrt	34.422	**0.000**	SNK	SD=DS=BS=S<SO
		t15	ANOVA	Sqrt	24.927	**0.000**	SNK	SD=DS=BS=S<SO
	Mussel	Fresh vs. t3	KW	-	24.618	**0.000**	Dunn-Bonferroni	TabS5
		Fresh vs. t15	KW	-	23.818	**0.000**	Dunn-Bonferroni	TabS5
		t3	ANOVA	Sqrt	51.194	**0.000**	SNK	BS=SD=DS<DS=S<SO
		t15	ANOVA	-	59.454	**0.000**	SNK	SD=BS=DS<S<SO
Sugars	Oyster	Fresh vs. t3	KW	-	17.459	**0.004**	Dunn-Bonferroni	TabS5
		Fresh vs. t15	KW	-	20.267	**0.001**	Dunn-Bonferroni	TabS5

		t3	KW	-	10.087	**0.039**	Dunn-Bonferroni	SO=BS=S=DS<S=DS=SD
		t15	ANOVA	Ln	6.021	**0.002**	SNK	BS=DS<DS=SO=SD<SO=SD=S
	Mussel	Fresh vs. t3	KW	-	24.695	**0.000**	Dunn-Bonferroni	TabS5
		Fresh vs. t15	KW	-	24.902	**0.000**	Dunn-Bonferroni	TabS5
		t3	ANOVA	-	19.692	**0.000**	SNK	BS=DS<DS=SO=S<SD
		t15	KW	-	19.252	**0.001**	Dunn-Bonferroni	BS<DS<SO=S=SD
Proteins	Oyster	Fresh vs. t3	KW	-	19.844	**0.001**	Dunn-Bonferroni	TabS5
		Fresh vs. t15	KW	-	13.155	**0.022**	Dunn-Bonferroni	TabS5
		t3	KW	-	17.856	**0.001**	Dunn-Bonferroni	SD=SO=S<BS=DS
		t15	KW	-	12.620	**0.013**	Dunn-Bonferroni	SD=S<S=BS=SO=DS
	Mussel	Fresh vs. t3	KW	-	15.168	**0.010**	Dunn-Bonferroni	TabS5
		Fresh vs. t15	KW	-	19.431	**0.002**	Dunn-Bonferroni	TabS5
		t3	KW	-	6.779	0.148	-	-
		t15	ANOVA	Ln	10.105	**0.000**	SNK	S=SO<BS=SD=DS

[1]See Table S5 for the results of Dunnett and Dunn-Bonferroni multiple comparison post hoc tests.

Table S5. Dunnett and Dunn-Bonferroni multiple comparison post hoc tests for differences between fresh and t3 and t15 oysters and mussels preserved by five distinct preservation methods (brine salting, dry salting, sun-drying, smoking and smoking with oil) concerning LPO and nutrient reserves.

				Preservation methods				
Variable	Organism	Control category	Preservation days	Brine salting	Dry salting	Sun-drying	Smoking	Smoking with oil
LPO	Oyster	Fresh	t3	NS[1]	*	*	*	*
			t15	NS	*	*	*	*
	Mussel		t3	*	*	*	*	*
			t15	*	*	*	*	*
Lipids	Oyster		t3	NS	NS	NS	*	*
			t15	NS	NS	NS	*	*
	Mussel		t3	NS	NS	NS	*	*
			t15	NS	NS	NS	*	*
Sugars	Oysters		t3	NS	*	*	NS	NS
			t15	NS	NS	*	*	NS
	Mussel		t3	NS	NS	*	*	NS
			t15	NS	NS	*	*	*
Proteins	Oyster		t3	NS	NS	NS	NS	NS
			t15	NS	NS	NS	NS	NS
	Mussel		t3	NS	NS	NS	*	NS
			t15	NS	*	NS	NS	NS

[1]NS – not significant
*$p < 0.05$

Table S6. Results of independent-samples t-test (TT) and Mann-Whitney non-parametric test (MW) between t3 and t15 oysters' biomarkers preserved by different methods. Significant results are in bold.

Variable		Test	Transformation	Test statistic	*p-value*	Pattern
Brine salting	LPO	TT	-	-0.172	0.871	-
	Lipids	TT	-	-0.123	0.905	-
	Sugars	TT	-	-2.640	**0.033**	t15<t3
	Proteins	MW	-	14.000	0.841	-
Dry salting	LPO	TT	Sqrt	-2.003	0.091	-
	Lipids	TT	Sqrt	-1.095	0.306	-
	Sugars	TT	Ln	-3.309	**0.014**	t15<t3
	Proteins	TT	-	-1.828	0.118	-
Sun-drying	LPO	TT	-	1.843	0.103	-
	Lipids	TT	-	-0.920	0.389	-
	Sugars	TT	-	-0.760	0.477	-
	Proteins	MW	-	9.000	0.548	-
Smoking	LPO	TT	-	3.529	**0.008**	t3<t15
	Lipids	TT	-	-0.136	0.895	-
	Sugars	TT	-	0.676	0.518	-
	Proteins	TT	-	-0.520	0.618	-
Smoking with oil	LPO	TT	-	0.328	0.754	-
	Lipids	TT	-	-2.173	0.064	-
	Sugars	TT	Sqrt	1.796	0.110	-
	Proteins	TT	-	1.916	0.108	-

Table S7. Results of independent-samples t-test (TT) and Mann-Whitney non-parametric test (MW) between t3 and t15 mussels' biomarkers preserved by different methods. Significant results are in bold.

Variable		Test	Transformation	Test statistic	*p-value*	Pattern
Brine salting	LPO	TT	-	-0.508	0.630	-
	Lipids	TT	-	0.880	0.405	-
	Sugars	TT	-	-1.339	0.238	-
	Proteins	TT	-	-0.380	0.721	-
Dry salting	LPO	TT	-	0.570	0.594	-
	Lipids	TT	-	-1.406	0.206	-
	Sugars	TT	-	-0.648	0.538	-
	Proteins	MW	-	21.000	0.095	-
Sun-drying	LPO	TT	-	-1.840	0.119	-
	Lipids	TT	-	-0.512	0.629	-
	Sugars	TT	-	-2.012	0.081	-
	Proteins	TT	-	-1.139	0.288	-
Smoking	LPO	TT	-	1.043	0.351	-
	Lipids	TT	-	3.876	**0.008**	t13<t15
	Sugars	TT	-	-0.692	0.512	-
	Proteins	TT	-	-8.624	**0.000**	t15<t3
Smoking with oil	LPO	TT	-	3.681	**0.007**	t3<t15
	Lipids	TT	-	1.227	0.255	-
	Sugars	MW	-	12.000	1.000	-
	Proteins	TT	-	-3.199	**0.030**	t15<t13

Table S8. Results of Kruskal-Wallis (KW) and Mann-Whitney (MW) non-parametric tests among preserved oysters and mussels' microbial growth in three different media (CCA, TSA and TCBS) and respective comparison patterns. Significant results are in bold. Fresh (FR), Brine salting (BS), Dry salting (DS), Sun-drying (SD), Smoking (S) and Smoking with oil (SO).

Variable		Test	Test statistic	*p-value*	Pattern
Oysters	TSA	KW	6.651	0.084	-
Mussels	CCA	KW	7.533	0.057	-
	TSA	KW	11.538	**0.021**	SO=DS<BS=S<S=SD
	TCBS	MW	4.000	0.333	-

Table S9 - Results of independent-samples t-test (TT) and Mann-Whitney non-parametric test (MW) and respective patterns concerning mussel biometric measurements. Significant results are in bold.

Variable	Test	Test statistic	*p-value*	Pattern
Length t_0	TT	4.968	**<0.001**	Metuge<Mecúfi
Length t_f	TT	-1.675	0.099	-
Length Mecúfi	TT	9.201	**<0.001**	$t_0 < t_f$
Length Metuge	TT	10.844	**<0.001**	$t_0 < t_f$
Width t_0	MW	112.000	**<0.001**	Metuge<Mecúfi
Width t_f	MW	1004.000	0.434	-
Width Mecúfi	MW	164.500	**<0.001**	$t_0 < t_f$
Width Metuge	MW	14.000	**0.000**	$t_0 < t_f$
Height t_0	MW	419.000	**<0.001**	Metuge<Mecúfi
Height t_f	MW	1178.000	0.442	-
Height Mecúfi	MW	121.500	**<0.001**	$t_0 < t_f$
Height Metuge	MW	84.000	**<0.001**	$t_0 < t_f$
Total Weight t_f	TT	-2.566	**0.013**	Mecúfi<Metuge
Total Weight Mecúfi	TT	11.581	**<0.001**	$t_0 < t_f$
Total Weight Metuge	TT	9.212	**<0.001**	$t_0 < t_f$
Edible Weight t_f	TT	-8.581	**<0.001**	Mecúfi<Metuge
Edible Weight Mecúfi	TT	2.423	**0.017**	$t_0 < t_f$
Edible Weight Metuge	TT	9.827	**<0.001**	$t_0 < t_f$
Shell Weight t_f	TT	-2.780	**0.007**	Mecúfi<Metuge
Shell Weight Mecúfi	TT	11.147	**<0.001**	$t_0 < t_f$
Shell Weight Metuge	TT	9.888	**<0.001**	$t_0 < t_f$

Table S10 – Results of one-way analysis of variance (ANOVA) and respective SNK patterns among mussels collected from Mecúfi seacoast in September (t_i) and mussels collected from aquaculture wood-tables in March in Mecúfi (MCt_f) and Metuge (MTt_f) regarding biomarkers analysis and microplastics and mercury contamination. Significant results are in bold.

Variable	Transformation	Z	*p-value*	SNK Pattern
Mercury	Ln	7.146	**0.003**	$MTt_f = MCt_f < t_i$
Microplastics	Sqrt	45.865	**<0.001**	$MTt_f = t_i < MCt_f$
LPO	-	22.067	**<0.001**	$t_i = MCt_f < MTt_f$
Lipids	Ln	15.240	**<0.001**	$MCt_f < t_i = MTt_f$
Sugars	-	72.239	**<0.001**	$MCt_f < MTt_f < t_i$
Proteins	-	5.420	**0.011**	$t_i = MCt_f < MTt_f$